DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date APR - 6 1998

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient:	Ademetionine
Firm:	General Nutrition Corp.
Date Received by FDA:	March 13, 1998
90-day Date:	June 10, 1998

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after June 10, 1998.

Robert J. Moore, Ph.D.

3436 '98 APR -9 P2:18

95S-0316

RPT 26

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

MAR 13 1998

John P. Troup, Ph.D.
Vice President, Scientific Affairs
General Nutrition Corporation
300 Sixth Avenue
Pittsburgh, Pennsylvania 15222

Dear Dr. Troup:

This letter acknowledges receipt by the Food and Drug Administration (FDA) on March 13, 1998 of your notifications, dated March 2, 1998 and March 4, 1998, pursuant to 21 U.S.C. 350b(a)(2) (section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act), providing notice of your intent to introduce, or deliver for introduction into interstate commerce, the new dietary ingredients "ademetionine and katsuobushi oligopeptide.

The date that the agency received your notification submitted under 21 U.S.C. 350b(a), March 13, 1998, is the filing date for the notification. In accordance with the requirements of 21 U.S.C. 350b, for 75 days after the filing date, General Nutrition Corporation shall not introduce, or deliver for introduction, into interstate commerce any dietary supplement that contains either of these new dietary ingredients, ademetionine and katsuobushi oligopeptide.

Please contact us if you have questions concerning this matter.

Sincerely,

Robert J. Moore, Ph.D.
Senior Regulatory Scientist
Division of Programs and Enforcement Policy
Office of Special Nutritionals

cc:
HFS-456 (file)
f/t:rjm:HFS-456:3/13/98:gnc.ack:disc26

GNC LiveWell™

John P. Troup, Ph.D.
Vice President,
Scientific Affairs

March 2, 1998

RECEIVED 3/13/98

Linda S. Kahl, Ph.D.
Office of Special Nutritionals (HFS-450)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street S.W.
Washington, DC 20204

Dear Dr. Kahl:

Pursuant to Section 8 of the Dietary Supplement Health and Education Act of 1994, General Nutrition Corporation located at 300 Sixth Avenue, Pittsburgh, PA 15222 and BASF Corporation, located at 3000 Continental Drive, North, Mount Olive, NJ 07828, wish to notify the Food and Drug Administration that it will market a new dietary ingredient, Ademetionine, synthesized from methionine and ATP. Accordingly, enclosed please find two (2) copies of this notification.

The dietary supplement which contains Ademetionine will consist of five hundred (500) mg of Ademetionine in a tablet or capsule which will be suggested to be taken one (1) time per day.

Attached please find clinical studies and other information which establish that this dietary ingredient, when used under the conditions suggested in the labeling of the dietary supplement, is reasonably expected to be safe. These supporting materials include:

(1) Chemical Pharmaceutical Data

(2) Toxicology (acute, subchronic, testology, chronic, cancerogenicity)

(3) Clinical studies

Very truly yours,

John P. Troup, Ph.D.
Vice President, Scientific Affairs

JPT/jaj

cc: Dan Patriarca

Enclosures

97 APR -9 P2:08

57723

General Nutrition Corporation, 300 Sixth Avenue, Pittsburgh, PA 15222
Tel: (412) 338-8844 Fax: (412) 338-8954

LIST OF ABBREVIATIONS

Ade-SD4	Ademetionine 1,4-butanedisulfonate
Ade-tosylate	Ademetionine sulfate-p-toluenesulfonate
ALT	Alanine aminotransferase
ANIT	α-naphtyl-isothiocyanate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
AUC	Area under the curve
BMTx	Bone marrow transplantation
BRIC	Benign recurrent intrahepatic cholestasis
Cmax	Maximum Concentration
CAS	Chemical Abstract Service
CCl_4	Carbon tetrachloride
CDCA	Chenodeoxycholic acid
CI	Cold ischemia
CLD	Chronic liver disease
CNS	Central Nervous System
co	Crossover
CSF	Cerebrospinal fluid
DMPP	Dimethylphenylpiperazine
DNA	Deoxyribonucleic acid
E	Epinephrine
EO	Ethynyloestradiol
F0	Parent generation
F1	1st generation offspring
F2	2nd generation offspring
GLP	Good laboratory practice
GSH	Glutathione
γ-GT	Gamma-glutamyltransferase

GVHD	Graft verus host disease
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCB	Hexachlorobenzene
HCV	Hepatitis C virus
HeLa	1st continuously cultured human malignant cell (a cervical carcinoma)
HLA	Human leukocyte antigen
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
HRC	Huntingdon Research Centre
IDB	Investigator's Drug Brochure
IgG	Immunoglobulin G
IHC	Intrahepatic cholestasis
INN	International Nonproprietary Name
IU	International units
LD_{50}	Dose letal to 50% of the tested animals
LDH	Lactic dehydrogenase
MAOI	Monoamine oxidase inhibitors
MDA	Malondialdehyde
MPF/WT	Toxicological Department of Knoll AG
mRNA	Messenger ribonucleic acid
MTD	Maximum Tolerated Dose
MTX	Methotrexate
NA	Nicotinic acid
NAG	N-acetyl-β-D-glucosaminidase
NE	Norepinephrine
NMRI mice	Strain of the animal specie: mouse
P_{450}	A cytochrome pigment with the absorption of 450 nm
PBC	Primary biliary cirrhosis
p.c.	Post coitum
PC	Phosphatidylcholine
p.p.	Post partum

R-SV	Rifamycin-SV
RBM	Istituto di Ricerche Biomediche "Antoine Marxer"
RCC	Research & Consulting Company AG
SAP	Serum alkaline phosphatase
SCB	Serum conjugated bilirubin
STB	Serum total bilirubin
STBA	Serum total bile acids
TG	Triacylglycerols
TPN	Total parenteral nutrition
TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
URO-D	Uroporphyrinigen decarboxylase
UW	University of Wisconsin solution
V79	Chinese hamster lung cells
VBDS	Vanishing bile duct syndrome
WI	Warm ischemia

TABLE OF CONTENTS

1. INTRODUCTION

Ademetionine is a naturally occurring molecule found in all living organisms.
In humans, ademetionine is distributed throughout virtually all body tissues and fluids where it acts as a substrate in many biological reactions involving enzymatic transmethylation (the process by which methyl groups are added to compounds), and, through the transsulfuration pathway, it is the precursor of essential amino acids including cysteine, taurine, and glutathione (Friedel et al. 1989).

Ademetionine is synthesized endogenously from methionine and adenosine triphosphate (ATP) in a reaction catalyzed by ademetionine-synthetase. The liver is the major site of ademetionine synthesis (in µg/g amounts), methionine metabolism (as much as 48%), and ademetionine-synthetase activity.

Because ademetionine is a chemically reactive compound, it is very unstable. Instability of ademetionine was largely resolved by preparation of a stable double salt, i.e. Ademetionine sulfate-p-toluenesulfonate (Ade-tosylate) (Figure 1a).
Oral and parenteral Ade-tosylate have been in clinical use for the treatment of different disorders for several years.

Ademetionine 1,4-butanedisulfonate (Ade-SD4) (Figure 1b) is a new more stable salt of ademetionine which is now under development for clinical use.

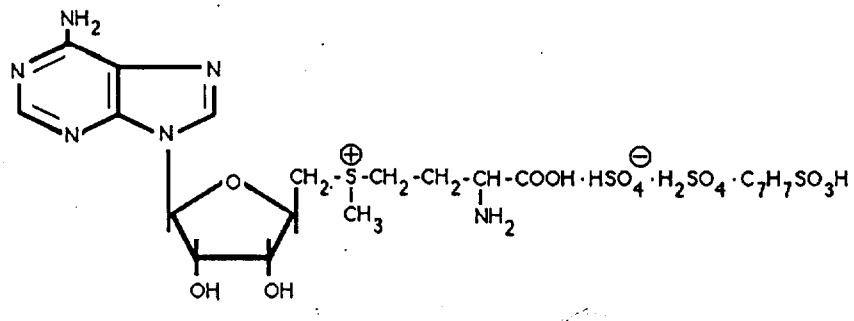


Figure 1a: Ademetionine sulfate p-toluenesulfonate (Ade-tosylate)

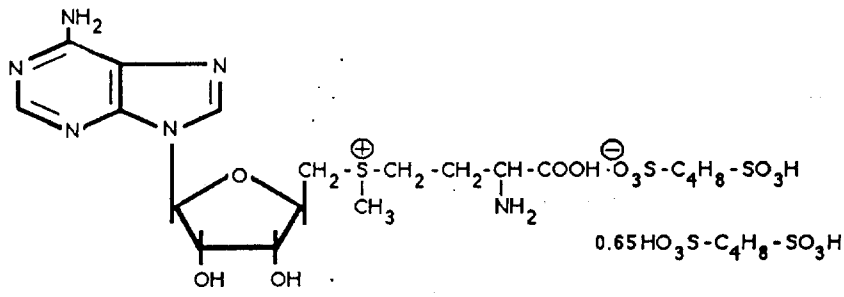


Figure 1b: Ademetionine 1,4-butanedisulfonate (Ade-SD4)

The present IDB focuses on the pharmacological and clinical data pertinent to the use of Ademetionine in liver disorders and updates the contents of the publications on this topic (Lieber & Williams 1990b; Israel et al., 1992; Lieber 1993, 1994; Ortiz et al., 1993; Schenker et al., 1993; Mato et al. 1994).

In particular, Ademetionine has been extensively studied in cholestatic liver disorders both in animal models and in clinical trials. Furthermore, recent experimental settings have provided a sound rationale for the therapeutic use of Ademetionine in alcoholic liver diseases.

Intrahepatic cholestasis

Intrahepatic cholestasis (IHC) is characterized by the accumulation of bile in liver cells and in biliary passages. Functionally, it is defined as a decrease in canalicular bile flow, and, clinically, it appears as the retention in blood of substances normally excreted in the bile (i.e. bilirubin, bile salts, and canalicular enzymes, such as alkaline phosphatase and γ-glutamyltranspeptidase) (Sherlock 1989).

Several mechanisms deserve consideration as to the pathogenesis of IHC. Changes in liver membrane fluidity and related transport activities as well as accumulation of cholestatic metabolites (e.g. nonsulfated bile acids) might be operative in producing IHC (Schreiber & Simon 1983a; Smith & Gordon 1987; Yousef et al. 1987, 1992).

About one third of chronic liver diseases might present with clinical and/or biochemical features of IHC regardless the etiology (Bortolini et al. 1992).

IHC more often appears in the late stages of the disease and is an indicator of liver failure. In fact, hyperbilirubinemia, the biochemical marker of IHC, is regarded as a negative prognostic factor in patients with cirrhosis (Zoli et al. 1991).

IHC contributes to the deterioration of the underlying liver damage. In particular, it has been reported that IHC inhibits by itself normal hepatocyte regeneration (Tracy et al. 1991), and it is able to modulate major histocompatibility complex class I expression in hepatocytes making the liver more vulnerable to immune destruction, phenomenon which is not affected by immunosuppressive treatment (Calmus et al. 1992). Furthermore, the accumulation of bilirubin and bile salts into hepatocytes leads to liver cell degeneration and necrosis associated with focal reactive inflammation and portal and periportal fibrosis (Schaffner 1992).

It deserves, therefore, close monitoring in order to establish adequate therapeutic measures.

The strategies for the treatment of IHC have been recently reviewed and ademetionine has been indicated as an innovative anticholestatic substance (Boyer 1992).

Alcoholic liver diseases

The spectrum of morphological changes which may occur in the liver in response to alcohol includes three, progressively severe, stages: fatty liver, alcoholic hepatitis and cirrhosis. Although these lesions usually occur sequentially, they might coexist in any combination and might be independent entities. Furthermore, several additional patterns of alcohol-induced liver disease have more recently been described including foamy degeneration, perivenular fibrosis, hepatic vein lesions and chronic active hepatitis.

Fatty liver (i.e. accumulation of fat in the hepatocytes) is the earliest and most common lesion induced by alcohol. Although it is commonly regarded as a benign feature, it has been reported that fatty liver might be per se a precirrhotic lesion (Teli et al. 1995; Popper & Lieber 1980; Nakano et al. 1982; Sorensen et al. 1984).

Alcoholic hepatitis is a clinicopathologic syndrome resulting from prolonged

excessive alcohol consumption characterized by an acute or subacute clinical presentation and distinctive histological features. When not coexisting, it is the most important precursor lesion for the development of cirrhosis (Desmet 1986; Marbet et al. 1987; Gluud et al. 1988). It has been estimated to occur in aproximately 40% of chronic alcoholics (Hislop et al. 1983). Acute alcoholic hepatitis may take 1 to 6 months for resolution and up to 60% of severe cases die in the first months of hospital admission (Maddrey et al. 1978; Theodossi et al. 1982). Cirrhosis develops over a 5-10 year period in more than 50% of those patients surviving the acute illness (Sørensen et al. 1984; Bird & Williams 1988).

Alcoholic cirrhosis develops in about 20% of heavy drinkers (Mezey 1982). The overall 5-year survival from diagnosis is about 40% in patients who continue to drink and 63% in those who stop. In patients with decompensated disease the survival rate might decrease to 34% after 5 year (Schenker 1984). The risk of hepatocellular carcinoma as a complication of alcoholic cirrhosis is well established (Naccarato & Farinati 1991).

Three possible not necessarily mutually exclusive mechanisms have been proposed for the pathogenesis of alcohol-induced liver injury.

Oxidative and reductive stresses due to increased production of toxic oxygen radicals during the hepatic metabolism of alcohol. Toxic oxygen radicals are responsible for structural and functional liver cell as well as intracellular organellae (e.g. mitochondria) membranes abnormalities (Castillo et al. 1992; Kato et al. 1990; Shaw & Jayatilleke 1990).

Hepatic metabolism of methionine and related availability of ademetionine are impaired in acute as well as chronic alcohol-induced liver diseases. Hepatic metabolic impairment of methionine leads to an insufficient endogenous free radicals scavenger capacity, i.e. decreased availability of glutathione (Horowitz et al. 1981; Shaw et al. 1981; Speisky et al. 1985; Martin-Duce et al. 1988; Fernandez-Checa et al. 1991; Lieber et al. 1990a; Kamimura et al. 1992; Hirano et al. 1992; Chawla & Jones 1994).

Ethanol increases intestinal permeability to normally nonabsorbed

macromolecules, such as bacterial endotoxins (LPS) and it also impairs their removal by affecting the hepatic reticuloendothelial function. Enhanced exposure to LPS induces hepatotoxic cytokines release, i.e. TNF, IL-1 and IL-6.Il-1 and IL-6 are involved in the induction of the acute symptomatic phase response. TNF, though, has been shown to be toxic to hepatocytes both in vitro and in vivo. The increased TNF levels have clinical relevance correlating with both liver function and acute mortality (Bjarson et al. 1984; Khoruts et al. 1991; Hill et al. 1992; McClain et al. 1993).

No established effective treatment for alcoholic liver diseases is available, so far, (Christensen & Gluud 1995) and liver transplantation is still not regarded as a treatment option (Moss & Siegler 1991).

1.1 Role of ademetionine in cell biochemistry

Ademetionine is the initiator of two important metabolic pathways in humans: transmethylation and transsulfuration (Stramentinoli 1987b) (Figure 2).
The transmethylation pathway involves the transfer of methyl groups (-CH_3) from ademetionine to a broad range of molecules, such as phospholipids, neurotransmitters, nucleic acids, proteins, porphyrins, and a number of drugs, resulting in their biotransformation and enabling their participation in several anabolic or catabolic reactions.
Up to 85% of transmethylation reactions occur in the hepatocyte (Mudd & Poole 1975). One of the most important involves the biosynthesis of phospholipids which play an important role in many intracellular events, such as regulation of sodium/potassium-dependent adenosine triphosphatase (Na^+/K^+-ATPase), ß-adrenergic receptor-adenylate cyclase coupling, secretion of histamine from mast cells and adenylate cyclase activation, by preserving plasma membrane fluidity (Stramentinoli 1987a). In addition to phospholipids, ademetionine methylates membrane proteins. This post-translational methylation of a wide variety of cellular

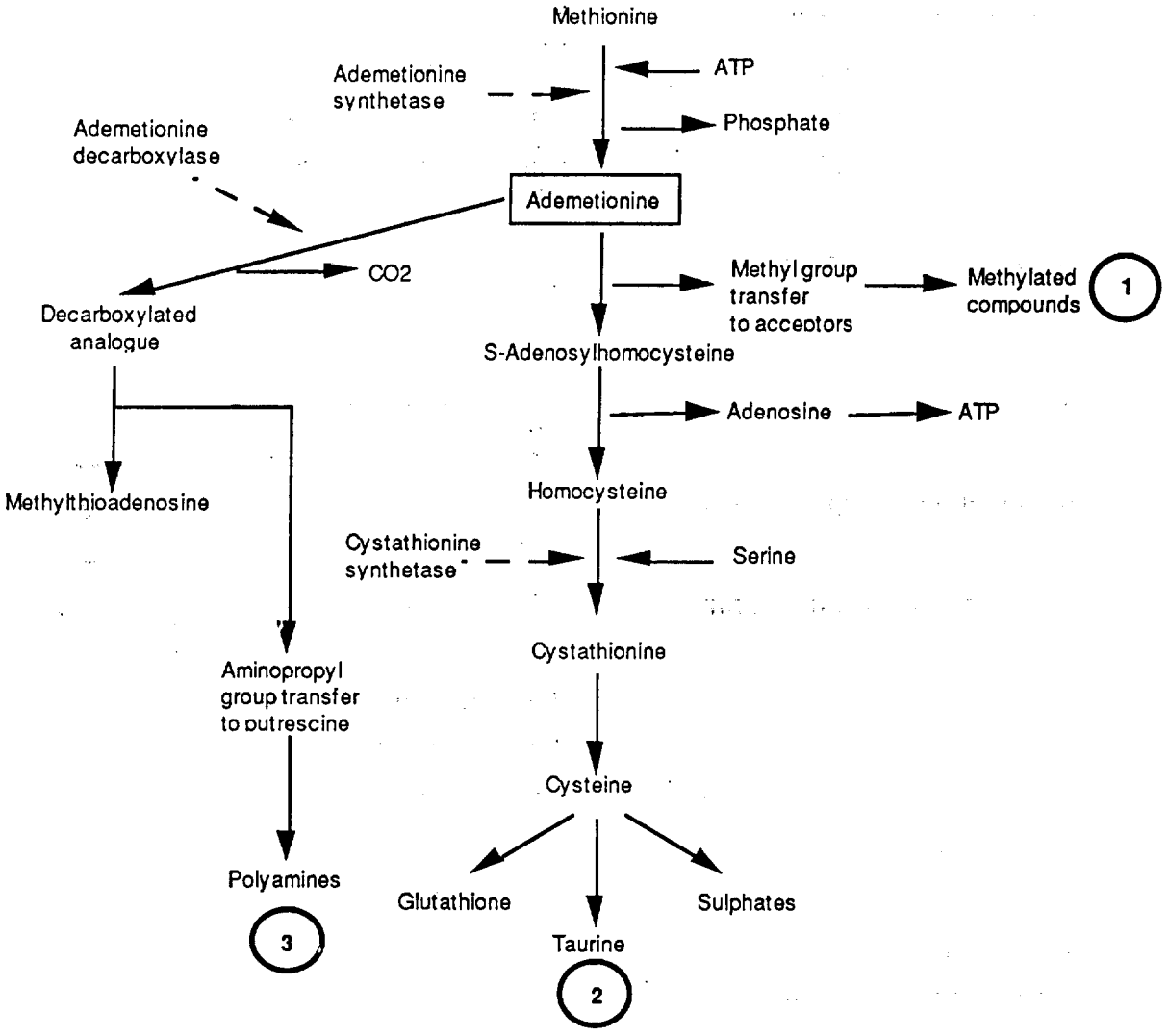


Figure 2: Main metabolic pathways involving ademetionine: (1) transmethylation; (2) transsulfuration; (3) amino propylation (Stramentinoli 1987b)

proteins might affect the activity of certain surface enzymes and might have a role in the repair or degradation of damaged polypeptides (Chawla et al. 1990).

After having given its methyl group, ademetionine is converted to S-Adenosylhomocysteine, thus entering the transsulfuration pathway (Figure 2).

S-Adenosylhomocysteine undergoes hydrolysis to homocysteine and ultimately to cysteine which is the precursor of taurine, sulfates and reduced glutathione.

These pathways play a pivotal role in maintaining the homeostasis of the hepatocyte, and their impairment leads to a derangement of all the subordinate

metabolic processes.
As a consequence of a decreased synthesis of ademetionine, the formation of phosphatidylcholine, the most abundant phospholipid in liver membrane, through transmethylation reactions is impaired resulting in a reduction in membrane fluidity. In this regard, it is well known from experimental studies that changes in hepatocyte plasma membrane fluidity are related to functional activity derangement and bile secretion failure (i.e. cholestasis) (Stramentinoli et al. 1981; Arias 1983; Boelsterli et al. 1983; Schreiber & Simon 1983a).

These membrane alterations are present also in patients with different types of liver injury and are positively correlated with the severity of the liver damage (Schulter et al. 1986). Furthermore, it has recently been reported that small modifications in membrane lipid structure are sufficient to influence in vivo transport activities (Simon et al. 1990). Besides hepatocytes, other cell types, such as erythrocytes, are similarly affected suggesting a widespread occurrence of abnormalities in membrane lipid composition in patients with chronic liver disease (Owen et al. 1982).
These abnormalities have been related to an increase in the cholesterol/phospholopid ratio of plasma membranes (Owen et al. 1982; Pezzoli et al. 1983; Stramentinoli 1986a,b).

An impairment of the transsulfuration pathway leads to cysteine and taurine deficiency which may cause nutritional defects particularly in patients with cirrhosis worsening the prognosis of the liver disease (Pisi & Marchesini 1990). Cysteine is the precursor of glutathione, the main intracellular detoxifying agent (Burk 1981). Hepatic glutathione deficiency results in a reduced protection of liver cells against free radicals and endogenous and exogenous toxic compounds. Furthermore, depletion of hepatic glutathione as a result of liver damage leads to inactivation of ademetionine synthetase, which in turn produces an impairment of the transsulfuration pathway and therefore a further decrease in glutathione (Corrales et al. 1990, 1991a).
Taurine is implicated in the process of bile acids conjugation (Heaton 1985). Since bile acids conjugation with taurine increases their solubility, a reduced availability

of taurine leads to an accumulation of toxic bile acids in the hepatocyte (Hoffmann & Roda 1984; Attili et al. 1986).

Finally, sulfates are the substrate of sulfation reactions which play an important role in detoxifying a number of metabolites, such as bile acids. Sulfated conjugated bile acids might play a protective role during cholestasis either by stimulation of bile flow or by reduction of biliary lipid secretion, thus protecting cell membranes from the detergent properties of high concentrations of non sulphated bile acids (Yousef et al. 1987, 1992).

2. CHEMICAL PHARMACEUTICAL DATA

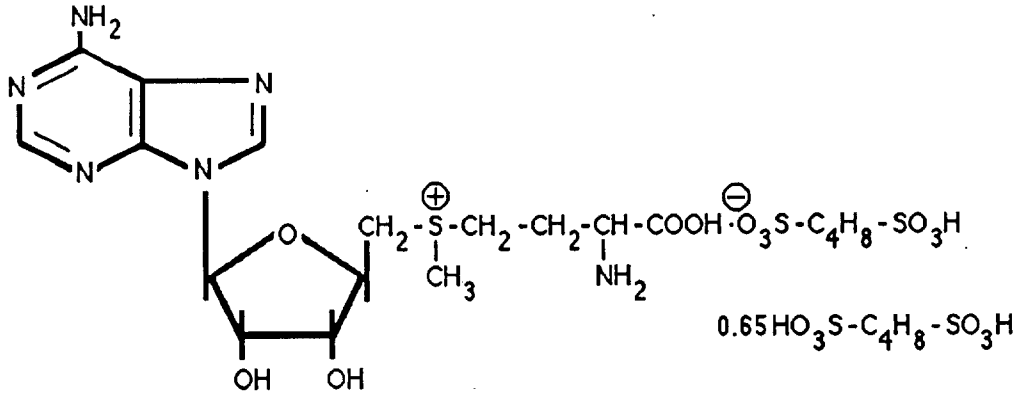
2.1 Physicochemical properties

2.1.1 INN: Ademetionine

CAS registry number: 29908-03-0

2.1.2 Chemical name: Ademetionine 1,4-butanedisulfonate

2.1.3 Structural formula



2.1.4 Molecular formula (as salt):

$C_{15}H_{23}N_6O_5S^+.C_4H_9O_6S_2^-.0{,}65\ C_4H_{10}O_6S_2$

Molecular formula (active cation): $C_{15}H_{23}N_6O_5S^+$

2.1.5 Molecular weight (as salt): 758.55

Molecular weight (active cation): 399.45

2.1.6 Appearance of the raw material:

Ade-SD4 is a white odorless amorphous powder.

2.1.7 Solubility, water

Freely soluble (more than 500 g/l at room temperature as salt).

2.1.8 pH

Water solution 0.4% (weight/volume) has a pH of 2.4

2.1.9 Synonyms

Ademetionine SD4, SAMe SD4, SAMe 1,4-butanedisulfonate, Adomet SD4, active methionine SD4.

2.2 Manufacturing (active ingredient)

Ade-SD4 is produced by fermentation of yeast enriched in ademetionine in the presence of methionine.

At the end of fermentation, ademetionine is extracted by cellular lysis and purified by column chromatography on three different resin types, salified with 1,4-butanedisulfonic acid and obtained as white powder using a spray-dryer.

2.2.1 Principal specification

• Ademetionine cation	48%-52% weight/weight
• 1,4 butanedisulfonic acid	44%-48%
• water	not more than 2.5%
• purity (as salt on dry substance)	not less than 98%

2.2.2 Isomerization

Ade-SD4 is a mixture of two diastereoisomeric forms: (S,S) form and (R,S) form. Isomerization at the sulfur atom occurs spontaneously and is temperature dependent.

The (S,S) isomer content of the final dosage form is between 65-72%.
Both isomers are biologically active (see section 3.3.3) (Dunne et al. 1995).

2.3 Dosage forms

Ade-SD4 is a new more stable salt of Ademetionine and is the only active ingredient present in the injectable and oral formulations.

2.3.1 Injectable form

- 400 mg (as cation) lyophilized in vials
- solvent ampoule containing 5 ml of buffer solution

COMPOSITION:

1 vial contains:

- Ade-SD4 759.6 mg (equivalent to 400 mg cation and 359.6 mg of 1,4-butanedisulfonate)

1 solvent ampoule (5ml) contains:

- l-lysine 342.4 mg
- sodium hydroxide 11.5 mg
- water for injections 4729.6 mg

pH AFTER RECONSTITUTION (VIAL + AMPOULE)

The pH of the solution before injection is 7.5±1.

2.3.2 Oral form

- 400 mg (as cation) gastroresistant white oval shaped tablet in aluminium strip

COMPOSITION

1 gastroresistant tablet contains:

- Ade-SD4 759.6 mg (equivalent to 400 mg cation and 359.6 mg of 1,4-butanedisulfonate)
- non active ingredient 150 mg.

Please note that the dosages reported in this Investigator's Brochure refer to the active cation ademetionine and not to the salt unless stated.

3. PHARMACOLOGY

3.1 General pharmacology

General pharmacodynamic studies with Ade-SD4 were carried out "in vitro" and "in vivo" in anaethetized animals after intravenous administration (Data on Knoll Farmaceutici file; Report no. SPH 01/91).

Effects on the following systems were evaluated:

- cardio-vascular and respiratory systems in cat and in guinea-pig;
- autonomic nervous system in cat;
- neuromuscular transmission in cat;
- visceral motility in cat.

The doses tested "in vivo" were 30, 100, 300 mg/kg in most of the studies by intravenous application. Drug related and dose dependent effects have been registered only in the 100 and 300 mg/kg doses. The main pharmacological effects are the following:

- significant and lasting decrease (more than 1 hour) of systolic and diastolic blood pressure in the cat, not associated with any variations in the contractility index. The effect of the substance is not based on influence on cardiac contractility mechanics (similar to the results of "in vitro" studies on isolated guinea-pig atrium), but could be rated to a substance effect on vascular smooth muscle.

- reduced contraction of the nicitating membrane (maximum decrease by about 30%). A slight dose-dependent increase in the blood pressure response to norepinephrine (NE) and epinephrine (E) might be due to the reduced base level of blood pressure due to bilateral carotid occlusion, was considerably attenuated by Ade-SD4 treatment (-57% at the dose of 100 mg/kg and -79% at the dose of 300 mg/kg). Results obtained seem to low a gangliopegic effect which is also in

accordance with the reduction of pressure response to dimethylphenylpiperazine (DMPP). Furthermore, the inhibition of the carotid reflex suggests a drug effect on central mechanisms.

- a significant recovery after blood pressure reduction, induced by vagal stimulation or injection of acetylcholine, was obtained at the dose of 300 mg/kg i.v. (40% and 20% respectively). A similar trend was observed with the interference of the response to histamine already significant at the dose of 100 mg/kg (anaesthetized cat).

- a dose-dependent transient inhibition of bladder and uterus motility. With 300 mg/kg a total inhibition of contractions (3 - 5 min) followed by a progressive recovery of contractility has been observed.
  In about 50% of the animals tested there was a dose-dependent transient increase in the intestinal (jejunum) motility associated with a slight increase in the basal tonus. Conversely in the other animals there was no effect on intestinal motility.

- a dose-dependent tendency to retention of water and salt during the first hours after oral application.

Regarding the "in vitro" studies, the maximum tested concentration of Ade-SD4 was 10^{-4} M.
At this concentration the following observations have been made:

- no direct effect on contractility and heart rate or coronary resistance (isolated guinea-pig heart; Langendorff's method);

- absence of anticholinergic, antihistaminergic, antiserotoninergic, ganglioplegic and antispastic activities (guinea-pig terminal ileum).

Considering the results obtained from the general pharmacological experiments, it can therefore be concluded that Ade-SD4 seems to be devoid of severe

undesirable effects on the vegetative system, nor does it interfere with the vital functions of the animals tested, except at very high and clinically irrelevant doses.

3.2 Hepatic metabolism of ademetionine in chronic liver disease

In mammals, as much as 48% of methionine is metabolized by the liver (Zeisel & Poole 1979), where about 80% is converted into ademetionine (Mudd & Poole 1975). Having donated its methyl group to a variety of acceptors, ademetionine is converted into S-adenosylhomocysteine and, through the transsulfuration pathway, into cysteine, whose availability is the rate limiting step for the synthesis of glutathione and of sulfurated compounds (Figure 2). Moreover, ademetionine exerts a dose-dependent activating effect on cystathionine synthetase, the rate-limiting enzyme for glutathione biosynthesis, further promoting the formation of sulfurated compounds (Finkelstein et al. 1975; Ortiz et al. 1993; Mato 1994).

Methionine metabolism is impaired in chronic liver disease, as demonstrated by 3 main findings: 1) patients with cirrhosis often show impaired methionine clearance; 2) the activity of ademetionine synthetase is significantly reduced in cirrhotics; 3) the administration of Ade-tosylate to patients with chronic liver disease is followed by increased plasma and biliary concentrations of sulfurated compounds and hepatic glutathione content.

1) Following an oral load of methionine, only 38% of the amino acid is excreted as urinary sulfates within 24 hours in cirrhotic patients in comparison with 71% in healthy controls (Horowitz et al. 1981). The simultaneous delay in methionine plasma clearance and urinary sulfate excretion also supports the evidence for a block in the transsulfuration pathway in cirrhotic patients. As the intermediates homocysteine, cystathionine, homoserine and cysteine do not simultaneously accumulate in plasma or urine, the block appears to occur before homocysteine synthesis. These findings have recently been confirmed by Marchesini et al. (1992a) who also reported a significant correlation of methionine clearance impairment with the severity of liver disease as assessed by galactose elimination and Child-Pugh score.

2) Several studies (Cabrero et al. 1988; Martin Duce et al. 1988) have shown that a marked decrease in activity (about 80%) of the high molecular weight form of ademetionine synthetase, the active form of the enzyme, occurs in cirrhotic liver. This effect is possibly attributable to a decrease in the synthesis of glutathione due to the liver damage and, consequently, impaired protection of the enzyme from oxidizing agents (Corrales et al. 1990, 1991a). In humans, the resulting reduction in ademetionine synthesis appears to be associated with a compensatory reduction in the utilization of hepatic ademetionine (Cabrero et al. 1988).

The administration of Ade-tosylate prevents ademetionine-synthetase inactivation possibly by increasing hepatic glutathione availability (Corrales et al. 1992a; Pajares et al. 1992a, 1992b).

Recently, Loguercio et al. (1994) measured glutathione and cysteine concentrations in erythrocytes of chronic alcohol misuses with (20 subjects) and without liver cirrhosis (20 subjects). All the subjects displayed an impairment of glutathione synthesis as shown by a decrease in erythrocyte glutathione levels and an increase in those of erythrocyte cysteine. The infusion of Ade-tosylate (2 g/daily for 15 days) corrected the erythrocyte thiol alterations in all the cases. The authors suggest that Ademetionine affects the metabolic pathway of erythrocyte glutathione and cysteine by restoring membrane fluidity and deranged amino acid membrane- linked carriers. This would normalise the imput of cysteine in the red blood cells and therefore restore glutathione synthesis.

Studies in patients with pre-cirrhotic chronic liver disease, such as chronic persistent hepatitis and chronic active hepatitis, have revealed very low serum levels of cysteine as compared to normal subjects, suggesting that the impairment of the transsulfuration pathway might be an early event in chronic liver disease (Selhub 1992; Almasio et al. 1994).

A reduction in activity of the enzyme phospholipid methyltransferase has also been demonstrated in patients with cirrhosis (Ortiz et al. 1987; Martin Duce et al. 1988). This enzyme catalyzes the sequential methylation of

phosphatidylethanolamine to phosphatidylcholine, the main membrane phospholipid, through a metabolic pathway which utilizes ademetionine as methyl donor. Membrane phospholipid methyltransferases are highly specific for ademetionine, are not saturated by physiological tissue concentrations of ademetionine and, therefore, are sensitive to exogenously administered ademetionine (Hirata et al. 1978; Hirata & Axelrod 1980; Traver et al. 1984; Osada et al. 1990).

3) Ade-tosylate administration (1200 mg/day i.v. for 3 days followed by 1200 mg/day orally for 30 days) has been demonstrated to increase plasma cystine and taurine levels in patients with severe hepatocellular failure without increasing plasma methionine, which would be potentially harmful in patients prone to hypermethioninaemia (Marchesini et al. 1992b). Furthermore, oral Ade-tosylate (800 mg/day for 60 days) increases hepatic availability of taurine in cirrhotic patients as shown by a rise in biliary taurine concentrations and tauroconjugation of bile salts (mainly chenodeoxycholate) (Angelico et al. 1994).

   Patients with alcoholic cirrhosis treated for 1 month with oral Ade-tosylate 1200 mg/day displayed improved methionine tolerance, as reflected by significantly lower serum methionine levels and improved methionine clearance after an oral load with the amino acid as compared with baseline (Corrales et al. 1991b, 1992b).

   Finally, hepatic glutathione content was restored to nearly normal levels in cirrhotic patients following long term oral Ade-tosylate administration (1200 mg/day for 6 months) (Vendemiale et al. 1989a).

   The results of these studies are further detailed in section 6.3.5.2.

Taken together, these findings show that the administration of ademetionine overcomes the metabolic block due to the reduced ademetionine-synthetase activity, resulting in the restoration of the transmethylation and transsulfuration pathways. This is also supported by the observation that the administration of Ade-tosylate prevents or reverses membrane lipid composition abnormalities and restores ATPase activity of hepatocytes and erythrocytes induced by several

hepatotoxins in animal models or related to chronic liver disease in humans (Boelsterli et al. 1983; Pascale et al. 1989; Osada et al. 1990; Tsuji et al. 1990a; Kakimoto et al. 1992; Muriel & Mourelle 1992; Rafique et al. 1992a, b; Schreiber & Simon 1983a).

3.3 Pharmacodynamics

3.3.1 Experimental cholestasis

The anti-cholestatic activity of Ademetionine (Table I) has been extensively proven in different animal models of cholestasis induced by:

- ethinyloestradiol (Stramentinoli et al. 1981; Boelsterli et al. 1983; Nanno et al. 1987; Fricker et al. 1988).
- hydrophobic bile salts (Schreiber et al. 1983b; Benz et al. 1995; Carubbi et al. 1995).
- α- naphtyl-isothiocyanate (ANIT) (Di Padova et al. 1985; Nanno et al. 1987).
- cyclosporin (Jiménez et al. 1991; Fernadez et al. 1992, 1995; Lucas et al. 1994; Roman et al. 1995).
- total parenteral nutrition (Belli et al. 1994).
- bile ducts ligation (Muriel et al. 1994; Pastor et al. 1996).
- exhaustive exercise (Villa et al. 1993).

Different mechanisms of action by which Ademetionine reverses bile secretion impairment in these models have been postulated. All of them relate to the peculiar biochemical properties of this molecule as methyl donor and precursor of trans-sulphuration products. In particular, through the metylation reactions, Ademetionine might inactivate hepatotoxins such as oestrogens (Stramentinoli et al. 1981; Vore 1987; Larrauri et al. 1992) as well as restore liver cell membrane fluidity and related transport activities (Arias 1986; Fricker et al. 1988; Belli et al. 1994; Muriel et al. 1994; Pastor et al. 1996). Furthermore, Ademetionine promotes the production of glutathione, taurine and sulfates which are known endogenous detoxicating agents (Burk 1981; Chawla et al. 1984; Vendemiale et al. 1989a, 1989b). It provides,

Table I Experimental models of cholestasis and effects of Ademetionine treatment

Reference	Model	Toxic agent	Ademetionine dosage/route	Results
Boesterli et al. 1983	rats	EE 5 mg/kg /d for 3 days p.o.	25 mg/kg t.i.d for 3 days i.m.	↑ Na+/K+-ATPase activity ↑ LPMs fluidity ↓ bile flow impairment
Fricker et al. 1988	isolated membrane vescicles (rats)	EE 5 mg/kg/d for 6 days s.c.	50 μM	↑ PC membrane content ↑ taurocholate transport
Nanno et al. 1987	rats	EE 5 mg/kg/d for 3 days p.o.	25 mg/kg t.i.d for 3 days s.c.	↓ serum total bile acids ↓ serum alkaline phosphatase
Stramentinoli et al. 1981	rats	EE 5 mg/kg/d for 3 days p.o.	25 mg/kg t.i.d. for 3 days i.m.	↑ bile flow ↓ biliary cholesterol molar ratio ↑ methilated EE metabolites in bile
Benz et al. 1995	rat hepatocyte culture	glyco-CDCA 500 μmol/l for 48 h	dose escalating up to > 3,000 μM	500 μM Ade optimal hepato-protective dose (= 100 μM TUDCA). Toxicity at doses > 3,000 μM Ade (vs 500 μM TUDCA)
Carubbi et al. 1995	HepG2 cell line	DCA 350 μM	500 μM	500 μM Ade as protective as TUDCA and UDCA (200 μM). UDCA > 400 μM non-protective. Ade + UDCA > 20% protection vs UDCA alone.
Schreiber et al. 1983b	rats	TLC 0.5 μM/kg i.v.	25 mg/kg/d t.i.d. for 5 days i.m.	↓ no. cholestatic rats (25% vs 90%) ↑ TLC secretory maximum ↑ PC membrane content
Di Padova et al. 1985	rats	ANIT 100 mg/kg once p.o.	25 mg/kg ti.d. for 3 days i.m.	↓ serum bilirubin, ALT, SAP ↑ bile flow

Table I Continue

Nanno et al. 1987	rats	ANIT 100 mg/kg once p.o.	25 mg/kg t.i.d for 3 days s.c.	↓ serum bilirubin, ALT, SAP, GGT ↓ inflammatory infiltration + pericholangial edema at histology.
Fernandez et al. 1992	rats	cyclosporin A 10 mg/kg/d for 2wk i.p.	10 mg/kg t.i.d. for 2 wk s.c.	↓ serum bilirubin ↑ bile flow ↑ biliary secretion of bile salts
Fernandez et al. 1995	rats	cyclosporin A 10 mg/kg/d for 2 wk i.p.	a. 10 mg/kg s.c. 3, 5, 8 h before Cy A b. 10 mg/kg t.i.d. for 2 wk s.c.	a. ↓ bile flow, BA, lipid secretion impairment at 3h > 8 h. b. ↑ bile flow, BA + lipids secretion
Lucas et al. 1994	rats	cyclosporin A 20 mg/kg acute i.v.	20 mg/kg i.p. 1, 3, 5 h before Cy A	↑ bile flow and GSH excretion (3 h dose but not 1 and 5 h)
Jimenez et al. 1991	rats	cyclosporin A 10 mg/kg/d for 2 wk i.p.	25 mg/kg t.i.d. for 2 wk i.p.	↑ bile flow, BA + lipids secretion
Roman et al. 1995	isolated rat hepatocytes	cyclosporin A 75 nM -100 nM	1 mM	protection of pericanalicular cytoskeleton
Belli et al. 1994	rats	TPN (3.4 g aminoacids + 10.2 g dextrose/24 h) for 5 days	75 mg/kg/24 h for 5 days i.v.	↑ bile flow, BA + lipids secretion ↑ Na+/K+-ATPase activity ↓ membrane lipid/protein ratio
Muriel et al. 1994	rats	15-day bile duct ligation	20 mg/kg t.i.d for 15 days i.m.	↓ serum bilirubin, SAP, GGT ↑ Na+/K+-ATPase activity ↓ glycogen depletion and lipid peroxidation levels ↓ perivenular fibrosis, bile canaliculi and mitochondria abnormalities
Pastor et al. 1996	rats	28-day bile duct ligation	10 mg/kg/day for 28 days i.m.	↑ microsomal oxygenases activities ↑ microsomal membrane fluidity no restoration of hepatic GSH and cytochrome P-450 concentrations
Villa et al. 1993	rats	run to exhaustion (152 ± 18 min)	8 mg/kg/ for 10 days s.c.	↑ bile flow + BA secretion ↑ hepatic GSH ↓ lipid peroxidation levels

therefore, the substrates for detoxifyng conjugation processes into the hepatocyte as well as for maintaining the functional integrity of the liver cell (Burk 1981; Hoffmann & Roda 1984; Heaton 1985; Attili et al. 1986; Yousef et al. 1987, 1992). Ade-tosylate treatment also showed to protect the integrity of the pericanalicular cytoskeleton from cyclosporin exposure, as so to mantain canalicular contractions and/or preseve tight-junction function (Roman et al. 1995).

It is interesting to note that Ade-tosylate resulted in being less toxic than TUDCA (Benz et al. 1995) and to enhance by 20% the cytoprotective effect of UDCA (Carubbi et al. 1995) in isolated hepatocytes exposed to hydrophobic bile salts. Furthermore, Ade-tosylate is able to in vitro restore lymphocyte functions affected by CDCA (Filaci et al. 1995).

3.3.2. Effect on alcohol-induced liver injury

The pharmacological effects of Ademetionine on alcohol-induced liver injury have been studied in several in vitro and in vivo experimental settings of acute and chronic exposure to ethanol.

In particular, Ade-tosylate treatment (Table II) proved to:

- Protect the liver cell against mitochondrial damage and dysfunction (Lieber et al. 1990; Devi et al. 1993; Garcia-Ruiz et al. 1995).
- Enhance hepatic cytosolic as well as mitochondrial glutathione content and reduce hepatic and blood acetaldeyde levels (Feo et al. 1986; Pascale et al. 1989; ; Lieber et al. 1990; Battiston et al. 1995; Devi et al. 1993; Garcia-Ruiz et al. 1995).
- Restore the hepatocyte content of depleted endogenous ademetionine as well as Na+/K+-ATPase activity of liver cell membranes (Feo et al. 1986; Pascale et al. 1989).
- Decrease serum TNF concentrations as well as counteract TNF-induced hepatotoxicity (Vara et al. 1994; Chawla et al. 1995; Arias-Diaz et al. 1996 in press)
- Reduce hepatic steatosis, liver cell necrosis, bile flow impairment and prevent liver fibrosis (Pascale et al. 1989; Lieber et al. 1990a; Cutrin et al. 1992a, 1992b; Alvaro et al. 1995).

Table II Experimental models of alcohol-induced liver injury and effects of Ademetionine treatment

Reference	Model	Toxic agent	Ademetionine dosage/route	Results
Alvaro et al. 1995	Isolated perfused rat liver	1% ethanol exposure for 70 min.	25 mg/kg t.i.d for 3 days i.m. before liver removal + 10 μmol/min perfusion	↑ bile flow + BA secretion ↓ AST + LDH
Battiston et al. 1995	rats	ethanol 0.5 g/kg i.p. once	a. pre-treatment, 20 mg/kg/d for 7 days i.m. b. pre-treatment, 20 mg/kg i.m. 14 h	a. b. ↑ hepatic GSH
Cutrin et al.	rats	10% ethanol + CCl4 0.3 ml/kg twice a wk for 1 mo p.o.	25 mg/kg Ade i.p. + 20 mg/kg Nifedipine p.o./d for 1 mo	↓ perivenular fibrosis ↓ LDH
Devi et al. 1993	rat fetal hepatocytes	ethanol 2 mg/ml	pre-treatment 0.1 mmol/L for 24 h	↑ ATP and GSH ↑ cell replication ↓ lipid peroxidation levels
Feo et al. 1986	rats	a. acute ethanol intoxication b. 36% diet as ethanol for 16 d	a. 25 mg/kg every 4 h i.m., 12 h before + 12 h after ethanol b. 25 mg/kg t.i.d for 16 days i.m.	a. ↓ hepatic ademetionine depletion b. ↓ hepatic ademetionine depletion ↓ hepatic fat content and secretion ↓ hepatic GSH and acetaldehyde ↓ plasma acetaldehyde
Garcia-Ruiz et al. 1995	rats	50% diet as ethanol for 4 wk	40-50 ml/d with the diet for 4 wk	↑ cytosol and mitochondrial GSHin PP and PV cells ↑ cellular ATP ↑ mitochondrial membrane potential and uncoupler or ratio of respiration

Table II Continue

Lieber et al. 1990	baboons	50% diet as ethanol for 24 mo	25 mg/kg with the diet for 24 mo	↑ hepatic ademetionine and GSH ↓ plasma GDH and AST ↓ hepatic giant mitochondria and SDH
Pascale et al. 1989	rats	ethanol 5.9 g /kg with the diet for 46 days	94.5 - 189 - 378 μmol/kg/d for 46 days i.m.	↑ Na+/K+-ATPase activity (dose-dependent effect) ↑ hepatic GSH ↓ serum SDH and hepatic fat content + necrosis faster recovery of all the parameters when Ade-tosylate administered after ethanol withdrawal Ade-tosylate better effects than methionine and NAC
Arias-Diaz et al. 1996	isolated rat hepatocytes	TNF 100, 200, 500 ng/ml or IL-1 30, 60, 120 IU/ml up to 24 h	12 μmol/L 24 h pre- and post-culture	↓ LDH release and cellular malondialdehyde, triacylglycerols content ↑ membrane phosphatidylcholine and hepatocyte GSH
Chawla et al. 1995	a. rats b. HepG2 cells	a. choline deficient diet + LPS 2 mg/kg for 2 wk b. ethanol 25 mmol for 10 wk	not reported	a. ↓ serum TNF and ALT b. ↑ intracellular ademetionine ↓ TNF cytotoxicity
Vara et al. 1994	isolated rat hepatocytes	TNF 100 ng/ml or IL-1 30 IU/ml overnight	5 μg/ml	↓ LDH release, lipid peroxidation levels, TG synthesis ↑ membrane phosphatidylcholine and hepatocyte GSH

Most of these effects have been related to the high glutathione pool maintained by Ade-tosylate treatment since they are not observed after administration of glutathione depleting agents (Pascale et al. 1989).

3.3.3. Liver fibrosis

Previous "in vitro" observations showed that the addition of Ade-tosylate at the concentrations greater than 0.05 nmoles/ml to monolayers of normal human dermal fibroblasts resulted in a significant decrease of collagen production without affecting their proliferation and viability (Casini et al. 1989).
Following these findings, the potential antifibrotic activity of ademetionine was also investigated in animal models.

The intraperitoneal administration of carbon tetrachloride (CCl_4) for 9 weeks to rats resulted in hepatic fibrosis, increase in prolyl hydroxylase activity, marked reduction of total ademetionine-synthetase activity, and depletion of hepatic glutathione content as compared to a control group (Corrales et al. 1992a; Caballeria et al. 1994b). The treatment with Ade-tosylate (3 mg/kg/day i.m.) combined to CCl_4 administration in a third group of animals significantly reduced liver collagen deposition and prolyl hydroxylase activity. This effect was associated with a decrease in the number of rats developing liver cirrhosis (3 out of 6 in the CCl_4 group and 1 out of 7 in Ade-tosylate-treated rats). Furthermore, Ade-tosylate treatment corrected the reduction of ademetionine-synthetase activity without affecting mRNA levels. This finding suggests that Ade-tosylate administration restores the activity of the enzyme, rather than inducing its synthesis. Finally, liver glutathione content was also restored to control values by Ade-tosylate administration.
To confirm this results, a second study was performed aimed at investigating whether the effects of Ade-tosylate were similar in the same experimental models once CCl_4 liver damage had been initiated (Caballeria et al. 1994a; Gassò et al. 1994).
This study has shown that Ade-tosylate (10 mg/kg i.m. daily) administered for 6 weeks from the 3rd week after the first CCl_4 injection significantly ($p<0.05$) reduced hepatic collagen content, prolyl hydroxylase-activity and increased ademetionine

synthetase activity and hepatic glutathion concentrations as compared to the control groups (CCl_4 alone). Furthermore, only 1 rat out of 6 receiving Ade-tosylate for 6 weeks developed cirrhosis whereas all rats from the control group had cirrhosis at the end of the study.

Rats receiving Ade-tosylate for only 3 weeks from the 6th week after the first injection of CCl_4, did not differ from the control animals as for the hepatic parameters evaluated as well as the frequency of cirrhosis possibly because of the short treatment period in relation with the severe damage already established.

To test whether changes in the metabolism of methionine are induced directly by CCl_4 administration and precede the pathological changes produced by this agent, an additional experimental setting was performed (Varela-Moreiras et al. 1995). In this study, CCl_4 was administered to rats for only 3 weeks as so not to induce fibrotic changes and resulted in a marked hypomethylation of hepatic DNA, reduction of hepatic ademetionine/S-adenosylhomocysteine ratio (i.e. the methylation ratio), depletion of hepatic folate and massive increase in serum homocysteine. These results confirm that methionine metabolism is already impaired in the early stages of the exposure to the toxic agent. They also indicate that the reduction in hepatic ademetionine-synthetase and the depletion of hepatic GSH seen after 9 weeks of CCl_4 treatment are most likely sequelae of the early alterations of methionine metabolism. The concomitant treatment with Ademetionine (10 mg/kg/day, intramuscularly) corrected all these abnormalities by resetting the methylation ratio.

Recently, in a further experimental model of hepatic cirrhosis induced in rats by the administration of CCl_4 and ethanol the effect of Ade-tosylate (25 mg/kg every other day by intraperitoneal injections) in combination with a calcium antagonist blocker (i.e. Nifedipine 20 mg/kg every other day by intragastric administration) on liver fibrosis was investigated (Cutrin et al. 1992a, 1992b; Barrio et al. 1993). These experiments showed that perivenular fibrosis (an early histological marker of progression toward cirrhosis) was significantly reduced in the animals treated with the combination Ade-tosylate/Nifedipine for one month in comparison with the group receiving only CCl_4 and ethanol.

Finally, the administration of Ade-tosylate (200 mg/kg bwt/day intraperitoneally for

54 weeks) to Long-Evans rats (animal model of spontaneous liver cancer) significantly reduced the incidence of cholangiofibrosis (6% vs 57% in the control group) (Kokuryu et al. 1992).
According to the present experimental data, ademetionine not only is active as anticholestatic agent but, as reported by preliminary evidences, it may also have antifibrotic potential. Since cholestasis "per se" might induce hepatic fibrosis (Schaffner 1992), the two effects of ademetionine may operate synergically in improving the outcome of cholestatic chronic liver diseases.

3.3.4 Animal models of organ transplant

Pharmacological activity of Ade-SD4 has recently been demonstrated in three animal models of liver ischaemia, namely normal perfusate flow hypoxia, stop-flow "warm" and stop-flow "cold" ischaemia.

Normal-flow hypoxia of rat liver, which also resembles to a certain extent low-flow liver ischaemia (Jaeschke & Mitchell, 1990), resulted in hepatocellular multilobular necrosis and massive bile flow reduction (Pezzoli et al. 1991; Thom et al. 1992).
Furthermore, Chawla et al. (1994) observed that in rats maintained under physiologic hypoxia (10% oxygen) for 9 days have a significantly subnormal hepatic ademetionine concentration and a significant decrease in expression of mRNA and activity of ademetionine synthetase. These results suggest that altered 1-carbon metabolism due to decreased ademetionine may contribute to altered hepatic function during pathophysiologic conditions involving hypoxia.
Application of 100 µM Ade-SD4 to the perfusion medium prevented liver cell necrosis as assessed by an improvement in histological features and by a decrease in the release of cytosolic enzymes (Pezzoli et al. 1991; Thom et al. 1992). Ade-SD4 application also significantly enhanced bile flow and restored the levels of cellular ATP and glutathione which were reduced by the effect of hypoxia. These preliminary findings may have clinical relevance for the preservation of organ function before and during transplantation. In fact, Ade-SD4 administration not only improved liver histology but also restored bile flow, an important predictive parameter of liver graft function (Starzl et al. 1989), and hepatic ATP and glutathione levels, markers of cellular energy state and detoxifying potential,

respectively.
In order to better clarify these results, further experiments have been performed in the model of stop-flow ischaemia which resemble more closely the situation during liver transplantation.

Accordingly, stop-flow ischaemia of rat liver and subsequent reperfusion was performed in two experimental sets at different temperatures ("CI - cold ischaemia" at 4 °C for 1 hour and "WI - warm ischaemia" at 37 °C for 1 hour) (Dunne et al. 1993a, 1993b, 1994).
CI caused hepatocytes injury, monitored by the release of aspartate aminotransferase (AST) into the perfusion medium soon after reperfusion.
WI produced more severe injury than CI as shown by greater initial increase in perfusate AST, glucose and oxygen extraction ratio as well as an impairment in the initial (15 min) mean blood flow and bile flow.

Ade-SD4 treatment of the donor (125 µmol/kg bwt s.c.) 16-18 hours prior to hepatectomy and inclusion in UW and as a bolus in the perfusate just before reperfusion (100 µmol, respectively) restored blood flow and oxygen delivery, consumption and extraction ratio towards normal in all the experiments.
Furthermore, bile production to 15 min was increased 5-fold by Ade-SD4 and rose progressively towards control values at 3 hours. Ade-SD4 substantially decreased both glucose release and acid production over 3 hours. Ade-SD4 had no benefit to parenchimal or endothelial cell damage as judged by perfusate levels of AST and purine nucleoside phosphorylase.

Subsequent experiments demonstrated that benefits with Ade-SD4 was derived from each of the three treatment stages.
These results suggest that Ade-SD4 is a novel potent agent for the improvement of liver function after cold preservation as well as warm ischaemic injury and that benefit is achieved both by improving hepatocellular metabolic function and correcting haemodynamic abnormalities.
Additional experiments in the same model, were aimed at elucidating the mechanism of action of Ademetionine by using its diastereomers (Dunne et al.

1995). Both the endogeneous (S, S') and the synthetic (R,S') isomers of Ademetionine proved to be biologically active with the former showing greater choleretic activitiy and the latter greater hemodynamic effects.

Recently, Vara et al. (1994) suggested a protective effect of ademetionine (5 µg/ml) against the toxic effects of cytokines in isolated hepatocytes. In this model cytokines significantly increased hepatocyte LDH release, MDA content and TG synthesis. None of these effects was observed in the presence of Ade-tosylate. In addition, Ade-tosylate was able to prevent the lipid peroxidation, the decrease in PC synthesis and the decrease in GSH induced by TNFα. Throughout these modifications ademetionine has a protective action against some effects of cytokines.

3.3.5 Effect on liver damage induced by drugs or hepatoxins

The protecive effects of Ademetionine against liver damage caused by various cholestatic agents have already been described in the previous sections.

In addition, pharmacodynamic activities of Ademetionine have also been tested in in vitro and in vivo models (Table III) after exposure to known hepatotoxic drugs like paracetamol, cyclosporin, heroin, and methadone (Stramentinoli et al. 1979b; Bray et al. 1991b, 1992; Jimenez et al. 1991; Ponsoda et al. 1991; Fernandez et al. 1992, 1995; Jover et al. 1992; Lucas et al. 1994; Roman et al. 1995;) as well as to galactosamine (Stramentinoli et al. 1978; Wu et al. 1996), bromobenzene (Wu et al. 1996), CCl_4 (Tsuji et al. 1990a, b; Corrales et al. 1992a; Muriel & Mourelle 1992; Caballeria et al. 1994a, b; Gassò et al. 1994), thioacetamide (Osada et al. 1986) and lead (Paredes et al. 1985).

In these settings, Ade-tosylate administration reduced the leakage of intracellular AST and ALT and liver necrosis as well as it restored the hepatic glutathione content and the biliary glutathione excretion. Furthermore, it reduced mortality in animals receiving large doses of paracetamol (Bray et al. 1991b, 1992; Stramentinoli et al. 1979b) or CCl_4 (Muriel & Mourelle 1992).

Table III Experimental models of drug/hepatotoxins-induced liver injury and effects of Ademetionine treatment

Reference	Model	Toxic agent	Ademetionine dosage/route	Results
Bray et al. 1991b, 1992	mice	paracetamol 300 - 500 mg/kg single dose	0.5 - 2.5 mmol/kg 0 or 2 or 5 h after challenge i.p.	↓ mortality (0-9.5% vs 26.5%) at any administration time ↓ AST and liver necrosis ↑ plasma GSH lowest dose not effective Ade-tosylate equally effective on mortality as NAC (equimolar doses) but better in preventing GSH depletion
Jover et al. 1992 Ponsoda et al. 1991	isolated rat hepatocytes isolated human hepatocytes	• sulphur-deficient medium • heroin 0.25, 0.5, 1, 2 mM/3 h • methadone 0.05, 0.1, 0.2, 0.5 mM/6 h • paracetamol 0.35, 0.8, 2, 4 mM/6 h • ethanol 50, 100, 150 mM/48 h	10, 30, 100, 400 μmol/L throughout 24 h before drug challenge	dose-dependent ↑ hepatocytes GSH (max concentrations at 30-100 μmol/L) time-dependent ↑ hepatocytes GSH (max concentrations at 20-24 h) ↓ cytotoxicity
Stramentinoli et al. 1979b	mice	paracetamol 710 mg/kg i.p. once	10, 20 mg/kg i.m 5 min before and 20 min after drug challenge	↓ mortality (8.3 7.9%% vs 43%) ↓ AST and liver necrosis ↑ hepatic GSH ↓ radiolabelled paracetamol binding to microsomal proteins

Table III Continue

Wu et al. 1996	isolated rat hepatocytes	D- galactosamine 25-50 mmol/L 24 h Bromobenzene 1.6 mmol/L 2 h	0.5-3 mmol/L	↓ LDH cellular leakage ↑ cellular GSH content
Stramentinoli et al. 1978	rats	D-galactosamine 400 mg/kg i.p. twice	10, 20 mg/kg i.m. t.i.d. for 5 days	↓ AST, ALT and liver necrosis ↑ hepatic ademetionine content and adomeli synthetase activity dose-dependent effects with 60 mg/kg/d Ade-tosylate equally effective to 100 mg/kg/d prednisolone
Fernandez et al. 1992	rats	cyclosporin A 10 mg/kg/d for 2wk i.p.	10 mg/kg t.i.d. for 2 wk s.c.	↓ serum bilirubin ↑ bile flow ↑ biliary secretion of bile salts
Fernandez et al. 1995	rats	cyclosporin A 10 mg/kg/d for 2 wk i.p.	a. 10 mg/kg s.c. 3, 5, 8 h before Cy A b. 10 mg/kg t.i.d. for 2 wk s.c.	a. ↓ bile flow, BA, lipid secretion impairment at 3h > 8 h. b. ↑ bile flow, BA + lipids secretion
Lucas et al. 1994	rats	cyclosporin A 20 mg/kg acute i.v.	20 mg/kg i.p. 1, 3, 5 h before Cy A	↑ bile flow and GSH excretion (3 h dose but not 1 and 5 h)
Jimenez et al. 1991	rats	cyclosporin A 10 mg/kg/d for 2 wk i.p.	25 mg/kg t.i.d. for 2 wk i.p.	↑ bile flow, BA + lipids secretion
Roman et al. 1995	isolated rat hepatocytes	cyclosporin A 75 nM -100 nM	1 mM	protection of pericanalicular cytoskeleton
Caballeria et al. 1994a,b Gassò et al. 1994	rats	CCl_4 0.5 ml twice a week for 9 wk i.p	10 mg/kg/d i.m. from wk 3 to 9 (6 wk) or from wk 6 to 9 (3 wk)	↑ hepatic ademetionine synthetase activity (6 wk > 3 wk) ↑ hepatic GSH (both 6 and 3 wk) ↓ hepatic collagen and prolyl-hydroxilase activity (6 wk > 3 wk) and lipid peroxidation levels ↓ no. cirrhotic rats (6 wk > 3 wk)

Table III Continue

Corrales et al. 1992a	rats	CCl_4 0.5 ml twice a week for 9 wk i.p.	3 mk/kg/d for 9 wk i.m.	↑ hepatic ademetionine synthetase activity and GSH ↓ hepatic collagen and prolyl-hydroxilase activity ↓ no. cirrhotic rats
Muriel & Mourelle 1992	rats	CCl_4 0.4 g/kg p.o. for 8 wk	20 mg/kg i.m. t.i.d. for 8 wk	↓ mortality (20% vs 40%) ↓ membrane cholesterol/phospholipid ratio ↑ membrane Na+/K+-ATPase activity
Tsuji et al. 1990	isolated rat hepatocytes	CCl_4 5 mM/24 h	1.3 mM/24 h	↓ AST, ALT leakage ↑ no. of viable cells and preservation of morphology
Varela-Morales et al. 1995	rats	CCl_4 0.5 ml/kg twice a week for 3 wk i.p	10 mg/kg/d i.m. for 3 wk	restoration of methyl groups incorporation into hepatocyte DNA ↑ hepatic ademetionine and folate
Osada et al. 1986	rats	thioacetamide 50 mg/kg /d i.p. for 8 days	2, 200 mg/kg/d i.p. for 3 days	↓ AST, ALT and liver necrosis 2 mg/kg/d equally effective to 200 mg/kg/d
Paredes et al. 1985	mice	lead 4 mg/kg/d i.p. every 2 days (acute) lead 10 mg/ml p.o. for 26 days (chronic)	20 mg/kg/d s.c. for 22 days	↑ plasma and hepatic GSH ↓ plasma, liver, kidney lead concentrations ↑ plasma, liver, kidney, spleen, brain ALA-D activity

Again, these effects have been explained by the improvement of methylations of liver cell membrane components and the enhancement of thiol concentrations via the transsulfuration pathway. In fact, a pretreatment with inhibitor of methionine synthesis abolished the protective effect of Ade-tosylate (Bray et al. 1991b, 1992).

3.3.6 Effect on experimental porphyria

Experimental hexachlorobenzene (HCB)-induced porphyria, which is characterized by accumulation of porphyrins in the liver due to inhibition of uroporphyrinogen decarboxylase (San Martin de Viale et al. 1977), closely resembles human porphyria cutanea tarda (Ockner & Schmid 1961). In rats with HCB-induced porphyria, Ade-tosylate reduced hepatic porphyrin levels and hyperbilirubinaemia, but did not affect HCB-induced inhibition of uroporphyrinogen decarboxylase activity (Cantoni et. al 1990) or normalize biliary function (apart from the suppression of cholesterol secretion) or liver morphology (Cuomo et al. 1991). It is feasible that Ade-tosylate may mobilize porphyrins from the liver or interfere with their hepatic biosynthesis (Cantoni et al. 1990).

4. PRECLINICAL PHARMACOKINETICS

4.1 Absorption

Studies performed in rats, hamsters and mice, treated with Ade-SD4 (Data on Knoll Farmaceutici file) by oral route, showed a low bioavilability of ademetionine (1%), probably due to an extensive 1st-pass effect occurring upon the absorption of the drug (Stramentinoli et al. 1979a). Intramuscular absorption was studied with Adetosylate and bioavailabilities higher than 80% were found in rats and rabbits (Stramentinoli et al. 1976).

4.2 Distribution

Plasma protein binding of Ade-tosylate was found to be negligible in rats and dogs. Tissue distribution studied performed in mice and rats with [methyl-^{14}C] labelled ademetionine, as tosylate salt, showed that the highest radioactive concentrations were achieved in kidneys. Liver and adrenals also showed high radioactivity contents, though lower than kidneys. The blood-brain-barrier seemed to be crossed slowly by the compound (Placidi et al. 1977).

Studies in dogs showed that the intravenous application of Ade-tosylate induces significant increases in the cisternal fluid concentrations of the substance (Data on Knoll Farmaceutici file; Report no. PK CSF-D-82).

Autoradiographic studies in pregnant mice receiving [methyl-^{14}C]-ademetionine as tosylate salt, showed that the drug passes the placental barrier only to a very limited degree (Placidi et al. 1979).

4.3 Metabolism

Metabolic studies performed in rats treated with Ade-tosylate labelled at sulfur, methyl group and side-chain carbon atoms, showed that exogenous ademetionine moieties are incorporated into transmethylation- and transsulfuration-derived metabolites, e.g. creatine, phospholipids, and sulfates. Oxidation of the side-chain carbon atoms and decarboxylation were also observed. These reactions, known to occur with the endogenous compound, thus seem to be shared by exogenous ademetionine.

4.4 Elimination

Ademetionine is eliminated from the body through the above described metaboic reactions as well as by renal excretion.
Negligible biliary excretion (<2%) was observed in rats and in isolated perfused rat liver using labelled ademetionine, as tosylate salt (Data on Knoll Farmaceutici file; Report no. ANNT 0179).

Plasma half-lives of ademetionine were estimated in different animal species after intravenous administration of Ade-tosylate and Ade-SD4 and values ranging from 15 minutes in hamsters to 40 minutes in dogs were found, as compared to a mean half-life of 91 minutes observed in man.

Urinary excretion and overall metabolism of the substance were studied in rats, hamsters and mice, after intravenous administration of 10 and 200 mg/kg of [carboxyl-^{14}C] Ade-SD4. A sharp difference in the urinary excretion was seen between rats and the other two species at the lower dose: rats excreted 45, while hamsters and mice excreted 45 and 67% of the i.v. applied Ade-SD4, respectively.

These results were in agreement with the finding that rats metabolized to CO_2 66% of i.v. applied [carboxyl-^{14}C] Ade-SD4 as compared to 11 and 12% observed in hamsters and mice, respectively.
Since decarboxylation at the amino acidic site occurs in every metabolic pathway of ademetionine, exhaled CO_2 represents the amount of substance that is metabolized by each animal species.

When a 200 mg/kg dose was administered, rat urinary excretions increased to 50% and the overall metabolism decreased to 22% of the applied ademetionine, thus suggesting a saturation of the metabolism and/or of the tubular reabsorption of the compound (Data on Knoll Farmaceutici file; Report no. ANPK 01/02 91).
Among the studied species, rat resulted to be the most different from man, with respects to the urinary excretion of ademetionine, since in human, after i.v. injection of Ade-SD4 (400 mg ≅6 mg/kg), 65% of the applied drug was recovered in urine.

5. TOXICOLOGY

The toxicological studies on Ade-SD4 by parenteral and oral administration (Table IV) were performed in different animal species legally recommended and commonly utilised in pre-clinical research laboratories and include mutagenicity investigations as well. All these studies were performed in compliance with GLP.

The groups and the number of animals per group were sufficient to allow a complete statistical evaluation of the results obtained through assessment of the toxic effects.

Table IV Summary of toxicological studies on Ade-SD4

Type of study	Species	Route	Duration of treatment	Doses administered as ademetionine (mg/kg bwt/day)
Acute toxicity	Mouse	oral	1 day (single dose)	0-4640
Acute toxicity	Mouse	i.v.	1 day (single dose)	0-464-681-825-908-1000
Acute toxicity	Rat	oral	1 day (single dose)	0-4640
Acute toxicity	Rat	i.v.	1 day (single dose)	0-921-960-1000-1041-1085-1130
Acute toxicity	Rat	i.m.	1 day (single dose)	0-600
Subchronic toxicity	Mouse	oral	13 weeks	0-400-900-2000 (MTD)
Subchronic toxicity	Rat	oral	13 weeks	0-400-900-2000 (MTD)
Subchronic toxicity	Rat	i.v.	13 weeks	0-7-21-63-190
Subchronic toxicity	Rat	i.m.	4 weeks	0-50-100-200
Subchronic toxicity	Dog	oral	13 weeks	0-250-500-1000
Subchronic toxicity	Dog	i.v.	13 weeks	0-50-120-300
Chronic toxicity	Rat	s.c.	26 weeks	0-50-100-200
Chronic toxicity	Rat	oral	52 weeks	0-440-1000-2000 (2x1000)
Chronic toxicity	Dog	oral	52 weeks	0-200-400-800
Chronic toxicity	Dog	s.c.	26 weeks	0-50-100-200

Table IV Continue

Type of study	Species	Route	Duration of treatment	Doses (mg/kg bwt/day) administered as ademetionine
Fertility	Rat	oral	F0 generation: males 17 weeks females 5 and 8 weeks resp. F1 generation: males 15 weeks females 18 weeks	0-2x440-2x663-2x1000
Fertility	Rat	s.c.	males 18 weeks females 11 weeks	0-100-200-400
Embryotoxicity	Rat	oral	day 6-15	0-120-548-2500
Embryotoxicity	Rat	i.v.	day 6-15	0-100-200-400
Embryotoxicity	Rabbit	oral	day 6-18	0-100-223.6-500
Embryotoxicity	Rabbit	s.c.	day 6-18	0-25-50-100
Peri-post natal toxicity	Rat	oral	day 15 p.c. up to day 20 p.p.	0-2x440-2x663-2x1000
Peri-post natal toxicity	Rat	i.v.	day 15 p.c. up to day 22 p.p.	0-100-200-400
Carcinogenicity	Mouse	oral	78 weeks	0-500-1000-2000
Carcinogenicity - study I	Rat	oral	104 weeks	0-400 (200)-750 (440)-1400 (1000) *
Carcinogenicity - study II	Rat	oral	104 weeks	0-50-100-200 (+187 SD4)
Mutagenicity				
-"in vivo" studies: micronucleus test	Mouse	oral	1 day (single dose)	0-400-900-2000
micronucleus test	Rat	i.m.	1 day (single dose)	600
- "in vitro" studies:	- Ames test (up to 5000 µg/plate) - HPRT-test (up to 5000 µg/ml) - Chromosomal aberration in human lymphocytes (up to 5000 µg/ml) - Unscheduled DNA synthesis in HeLa cells culture (up to 5000 µg/ml)			

i.v. = intravenous; = i.m.: intramuscular; s.c.: = subcutaneous; p.c. = post coitum; p.p. = post partum

* = dose reduction from day 51 onward

5.1 Single Dose Toxicity

The single dose toxicity studies were performed in mice and rats, both sexes. The administration routes were: intravenous, intramuscular and oral. Changes related to sex were not observed.

Overview:

Animal species	Route of administration	LD_{50} (mg/kg) male + female
Mouse	oral	>4640
Mouse	i.v.	908
Rat	oral	>4640
Rat	i.v.	1071 (1028 - 1115)
Rat	i.m.	> 600 (Limit test)

5.1.1 Oral administration/male and female mice

Knoll AG Study Code: MPF/WT 9223 (Data on Knoll AG file).

Clinical symptoms were forced respiration, clonic convulsions, partial palpebral closure and diarrhoea.
Body weight gain in the treatment group did not differ from that in the control group.
No substance-related organ alterations were observed.

The approximate LD_{50} values were estimated for a 14-day recovery period to be higher than 4640 mg/kg bwt for males and females as well.

5.1.2 Intravenous administration/male and female mice

Knoll AG Study Code: MPF/WT 9224 (Data on Knoll AG file).

The main clinical signs in mice were: forced respiration, dyspnoea, prone position, clonic convulsions, running and jumping fits, partial palpebral closure. The onset of the above signs was 3 to 30 minutes after administration and lasted up to 5 minutes to 1 hour. A dose of 825 mg/kg bwt/day was well tolerated without lethality by males and females.

The patho-anatomical examination of sacrificed animals revealed no substance-related organ changes.

The approximate LD_{50} was calculated to be 908 mg/kg bwt for males and females.

5.1.3 Oral administration/male and female rats

Knoll AG Study Code: MPF/WT 9222 (Data on Knoll AG file).

No other symptoms with the exception of diarrhoea in one case only occurred and no lethal effect was observed up to the dose of 4640 mg/kg.
Patho-anatomical investigations revealed no substance-related organ changes.

The approximate LD_{50} values were estimated for a 14-day recovery period to be higher than 4640 mg/kg bwt for males and females as well.

5.1.4 Intravenous administration/male and female rats

RBM Study Code: 890567 (Data on Knoll AG file).

The LD_{50} was calculated to be 1071 mg/kg, with 95% confidence limits of 1028 - 1115 mg/kg bwt.

The main clinical signs in rats were: muscular hypotonia and ataxia (frequently), shallow breathing, tachypnoea, vasodilatation, piloerection, vocalization during injection, depression of CNS functions, respiratory distress, cyanosis, and clonic (asphyxial) convulsions. The onset of the above signs was within 1 minute of administration and lasted up to 30 mins - 2 hours.

All the surviving rats appeared normal between 30 mins and 4 hours after treatment.

The observation performed on the days following treatment, showed a moderate transient decrease of body weight (Day 3) which returned to normal values (Day 14), although some animals did not show this trend.

The gross pathology examination performed on animals of all dosage groups that died, showed a high occurrence of changes in the lung (mainly congestion and oedema).

Death was recorded within a few minutes of administration of the test article.

5.1.5 Intramuscular administration/male and female rats

RBM Study Code: 890567 (Data on Knoll AG file).

No mortality occurred in rats treated at the dosage of 600 mg/kg bwt (limit test); therefore, the LD_{50} was considered to be higher than 600 mg/kg.
In all the rats vocalization appeared during the injection and lameness lasting for a few minutes just after treatment.

The general clinical signs observed in the animals treated by intravenous route were also noted with this route. The above signs presented a delayed onset (30 mins after administration) and were of long duration (up to Day 6). These signs regressed until their complete disappearance on Day 7.

The body weight decrease showed the same trend as after the intravenous administration.

At autopsy no changes were found either in the organs or at the injection sites.

5.2 Repeated Dose Toxicity

5.2.1 Subchronic toxicity

5.2.1.1 Oral administration/male and female mice/13 weeks (MTD study)

RCC Study Code: 280675 (MPF/WT 9075E) (Data on RCC file).

Males and females NMRI mice were administered orally, by gavage, with doses of 0, 400, 900, and 2000 mg/kg bwt/day over a period of 13 weeks.
The treatment with Ade-SD4 did not effect survival. The water consumption was higher in all treated males with respect to the control.

In males and females the absolute and relative organ weights, for example of the liver and heart, were only marginally influenced (as a slight reduction) by the test article at mid or high dose.
There were no treatment-related changes in the macroscopic and microscopic

appearance of any organ or tissue.

5.2.1.2 Oral administration/male and female rats/13 weeks (MTD study)

RCC Study Code: 280697 (MPF/WT 9074E) (Data on RCC file).

The test substance was administered orally, by gavage, to Sprague-Dawley rats at the doses of 0, 400, 900 and 2000 mg/kg bwt/day. There was no dose-related effect on survival.

At 2000 mg/kg food consumption was lower than in controls in both sexes throughout the study. There was only a lower body weight gain at 2000 mg/kg in males and at 900 and 2000 mg/kg water consumption was increased in males dose-related, but only temporarily (week 3-5) increased in females at 2000 mg/kg.

No treatment-related effects on organ weights occurred in males nor in females at low dose (400 mg/kg). In females only, at mid and high dose the relative kidney weights were slightly increased.

With the exception of the kidney no histopathologic alterations were diagnosed. Only at 2000 mg/kg in the kidney tubular necrosis of the outer medulla, associated with mineralization, hyaline casts and vacuolisation of cortical tubules, was seen in 3 out of 20 surviving animals. These morphological changes corresponded to those ones of the prematurely dead rats.

5.2.1.3 Intravenous administration/male and female rats/13 weeks

RCC Study Code: 296594 (MPF/WT 9120 E) (Data on RCC file).

Sprague-Dawley rats received doses of 0, 7, 21, 63 and 190 mg/kg bwt/day.
In the high dose of 190 mg/kg the urinary volume was increased in both sexes, but the urinary osmolality was reduced in female rats only. Indicators of nephropathy as urinary enzymes (i.e. γ-GT) were significantly changed in the two high dose groups (63 and 190 mg/kg).

Kidney weights were increased for both sexes at 190 mg/kg bwt.

Evidence of nephropathy at 21, 63 and 190 mg/kg was revealed by histopathology (i.e. tubular necrosis, tubular vacuolisation and/or dilatation). Tubular necrosis, more prominent in males than in females, was seen, especially at 63 and 190 mg/kg bwt. Renal tubular regeneration was observed in most of the animals at 21, 63 and 190 mg/kg bwt.

A "no observable effect level" was established at 7 mg/kg bwt/day (equivalent to 13.6 mg Ade-SD4/kg bwt/day).

5.2.1.4 Intramuscular administration/male and female rats/4 weeks

Knoll Farmaceutici Study Code: 06/87 (Data on Knoll Farmaceutici file).

The test substance was administered to rats by intramuscular route over a 4-week period, at doses of 0, 50, 100 and 200 mg/kg bwt/day.
No animal died during the study.

No abnormal clinical signs were noted during the treatment period, but most animals moaned immediately after the injection of the test substance.

At macroscopic examination, dose-related haemorrhages were found at the injection sites.

5.2.1.5 Oral administration/male and female dogs/13 weeks

RCC Study Code: 337050 (MPF/WT 9251 E) (Data on RCC file).

Pure-bred Beagle dogs are given oral doses (by capsule) of 0, 250, 500 and 1000 mg/kg bwt/day.

Food intake, body weight gain as well as clinical biochemistry parameters were unaffected.
Treatment with the test article at 500 or 1000 mg/kg bwt/day was associated with an increased incidence of diarrhoea in all dogs.

There were no substance-related organ weight changes or pathomorphological organ lesions.

5.2.1.6 Intravenous administration/male and female dogs/13 weeks

RCC Study Code: 289618 (MPF/WT 9097 E) (Data on RCC file).

Pure-bred Beagle dogs were given doses of 0, 50, 120 and 300 mg/kg bwt/day.
Retching and vomiting was observed clinically in dogs administered 120 and 300 mg/kg bwt.

At 300 mg/kg bwt a moderate increase in kidney weight was recorded in female dogs and only a slight increase in the severity and incidence of renal tubular dilatation was seen in both sexes of this high group, but no necrosis of the kidney epithelium was seen.
A dose of 120 mg/kg bwt/day (equivalent to 232.6 mg Ade-SD4/kg bwt/day) was regarded as the "no observable toxic effect level".

5.2.2 Chronic Toxicity

5.2.2.1 Subcutaneous administration/male and female rats/26 weeks

Knoll Farmaceutici Study Code: 01/88 (Data on Knoll Farmaceutici file).

The test substance was administered to rats by subcutaneous route over a period of 26 weeks, at the doses of 0, 50, 100 and 200 mg/kg bwt/day.
No substance-related death occurred.

Evident toxicological effects were swelling of the injection site followed by a dose-related thickening of skin: at the injection site dose-related hemorrhages were noted.
The necropsy examinations did not show any alterations related to the administration of the test article.

5.2.2.2 Oral administration/male and female rats/52 weeks

RCC Study Code: 311545 (MPF/WT 9215 E) (Data on RCC file).

The test substance was given orally, by gavage, at doses of 0, 440, 1000 mg/kg bwt/day and b.i.d. 1000 mg/kg bwt/day respectively.
Survival was not affected by treatment with the test article.

During the study decreased food consumption, decreased body weight and diarrhea (soft/fluid stool) were noted in animals at 1000 and 2 x 1000 mg kg/bw/day.

This is compatible with the necropsy finding of dilated cecum with liquid contents mainly recorded in animals a these doses.
Hematology data are pointing at a mild anemia in animals at mid and high dose as indicated by a decrease in erythrocyte count and an increase in reticulocytes. These changes were found to be reversed after the recovery period.

Early in the study at high dose, urinary enzymes were increased, but later even lower urinary excretion of some enzymes (alkaline phopshatase, leucine aminopeptidase, gamma-glutamyltransferase) was noted for animals at the high dose when compared with the controls.
Other changes in urinary parameters of the high dose group like the decrease in creatinine excretion and clearance, the presence of blood and protein indicated an impaired renal function.

At the end of treatment the relative kidney weight was increased (at high dose level) and histopathology showed a dose-related increase of renal tubular vacuolation and dilatation. Both clinical laboratory and morphological kidney findings were almost completely reversed after the 8-week recovery period, and therefore are considered to be functional adaptive responses possibly due to an osmotic effect of the test article.

In this study the "no-toxic-effect level (NOTEL)" is considered to be 440 mg/kg bw/day.

5.2.2.3 Oral administration/male and female dogs/52 weeks

RCC Study Code: 344856 (MPF/WT 9344 E) (Data on RCC file).

Pure-bred beagle dogs were administered doses of 0, 200, 400 and 800 mg/kg bw/day.
There were no unscheduled deaths.
Under the conditions of this study, the remarkable changes of body weight

depression, loose stool, elevated liver enzymes, equivocal red blood cell values and urinary pH findings were considered to represent physiologic rather than toxicologic findings in the absence of electrolyte imbalance and histopathologic changes.
Therefore, the no-toxic-effect-level (NOTEL) was considered to be ≥ 800 mg SAMe kg/day.

5.2.2.4 Subcutaneous administration/male and female dogs/26 weeks

RBM Study Code: 870421 (Data on Knoll AG file).

In the dogs the test-substance was administered subcutaneously at doses of 0, 50, 100 and 200 mg/kg bwt/day for 26 weeks. Also in this study, a dose-related local reaction was seen with the same symptomatology as noted in the rat.

The histological examination showed inflammation and fibrosis in the subcutis at the injection sites.
No mortality occurred.
No systemic substance-related toxicological findings were demonstrable in this species.

5.3 Reproduction Toxicity

5.3.1 Fertility and Reproduction

5.3.1.1 Oral administration/male and female rats

RCC Study Code: 345734 (MPF/WT 9323 E) (Data on RCC file).

Male and female Sprague-Dawley rats (F0 generation) are treated orally, by gavage, twice daily (time interval 6 h) at doses of 0, 440, 663 and 1000 mg/kg bwt, corresponding to total daily doses of 0, 880, 1326 and 2000 mg/kg.

The males are dosed throughout the pre-mating and mating period until necropsy; the females are dosed throughout the pre-mating, mating, gestation and lactation periods.

One half of the mated females are sacrificed on day 21 p.c. and the foetuses removed by Caesarean section and the other half of the mated females are allowed to give birth to and rear their young - F1 generation.

Ade-SD4 given twice daily displayed no influence on the reproduction capacity of treated F0 parents and their untreated progeny. Ade-SD4 influenced food and water consumption of the F0 parents. Foetotoxic effects on the F1 foetuses were observed at the intermediate and high dose. Pup toxicity (F1 pups) occurred only at the high dose. A latent toxic effect was observed in F1 parents of F0 animals treated with 2000 mg/kg daily.

5.3.1.2 Subcutaneous administration/male and female rats

HRC Study Code: BOR 7/90234 (Data on HRC file).

Male and female rats were treated with 0, 100, 200 and 400 mg/kg bwt/day subcutaneously.
The treatment of males commenced 9 weeks prior to mating and was continued for another 9 weeks after mating; for females the treatment started 2 weeks prior to mating and lasted up to 6 weeks after giving birth, incl. interim kill of females at Day 20 p.c.

During the first two treatment weeks, males of the F0 generation in the intermediate and high dose group showed only loss of body tone, prostration, body tremors and rapid respiration. No similar observation was made in the females.

Other parameters modified by the treatment were: increase of water consumption in all dose groups, severity and duration of this effect was sex-dependent; decreased food consumption, observed only in males, during weeks 1 and 9 of the treatment; sex and time dependent retarded increase of body weight.

The macroscopic analysis showed changes around the injection site, in the form of hemorrhages and thickening of the skin (dose-dependent, but also present in controls). The same findings were also seen in the chronic toxicity study in rats.
They are most probably attributable to the puncturing of the skin, the applied volume, the pH value and/or the concentration of the test article solution.

Furthermore, a dose-related cortical scarring of the kidneys was found in all treated groups with slightly higher intensity in males.

The kidney weight of males and females was significantly increased at 200 and 400 mg/kg bwt, however, after the treatment with 100 mg/kg bwt, the kidney weight was increased in males only.

Concerning the litter of the F0-generation, a minor effect on implantation and litter size at the highest dose (400 mg/kg bwt) was observed, however, it was not considered to be of relevance.

No teratogenic effects were seen in their offspring. The total development of the F1-generation was not influenced by the treatment of Ade-SD4.

5.3.2 Embryotoxicity

5.3.2.1 Oral administration/female rats

Knoll AG Study Code: MPF/WT 9145 (Data on Knoll AG file).

The substance was administered orally by gavage at doses of 0, 120, 548 and 2500 mg/kg bwt/day, commenced on day 6 of pregnancy and continued up to and including day 15 of pregnancy. Hysterectomy was performed on day 20 p.c.

The general condition of the treated dams was not impaired. Food consumption and body weight gain of the dams from the low (120 mg/kg bwt) and mid dose group (548 mg/kg bwt) did not show substance-related effects.

At the high dose (2500 mg/kg bwt) food consumption was statistically significantly reduced from days 6-15 p.c. and body weight gain from days 9-11 p.c. as well as on days 14 and 15 p.c. (maternal-toxic effect).

The absolute kidney weight of the dams from each drug-treated group was not influenced, but the histopathological examination of this organ revealed minimal to marked renal tubular alterations only at the mid and high dose group (548 and 2500 mg/kg bwt respectively), namely degeneration and regeneration, dilatation, hyperplasia or interstitial fibrosis.

A nephrotoxic effect on the foetuses was not ascertained.

No effect on embryonal or foetal development was seen in drug-treated groups. Substance-related, macroscopically visible organ alterations or skeletal anomalies of the foetuses - teratogenic effects - were not observed in the treated groups.

5.3.2.2 Intravenous administration/female rats

HRC Study Code: BOR 9/891822 (Data on HRC file).

The substance was administered intravenously at doses of 0, 100, 200 and 400 mg/kg bwt/day, commenced on Day 6 of pregnancy and continued up to and including Day 15 of pregnancy.
Clinical signs such as loss of body tone, prostration, dark eyes, lacrimation and lethargy were observed only from the first to the third day of treatment.

Following the initiation of treatment there was a rapid transient increase in water consumption in all treated groups; from Day 12 onwards the water consumption rapidly declined and intake was essentially comparable with controls.

At the higher dosages a reduction of food intake was noted during the first 4 days of treatment and thereafter it returned to control values.
The body weight showed the following trend: an initial dose-related retardation in all treated animals.
A brief recovery was noted during the following days of treatment and at the end of dosing period the body weight gains returned to control values.
The autopsies revealed no gross macroscopic changes attributable to the treatment.
The only finding was a slight but significant increase in kidney weight among treated groups.
The analysis of litter parameters did not show significant differences among controls and treated groups.
The tendency for treated groups to have slightly lower litter sizes, compared with controls is attributed to variation in ovulation rate and pre-implantation losses. Since these events occurred prior to initiation of treatment they are most probably not due to a test article-related effect.

Slight differences were observed concerning embryofetal development such as litter weight (significantly lower at 200 and 400 mg/kg bwt), mean foetal weight (significantly lower than in controls, but not dose-related) and the rate of skeletal ossification (i.e. incomplete ossification of post lumbar vertebral centra).
The effects were so minor, therefore they could not be attributed to the test article.
No teratogenic effects of Ade-SD4 were observed.

5.3.2.3 Oral administration/female rabbits

Knoll AG Study Code: MPF/WT 9112 (Data on Knoll AG file).

The substance was administered orally by gavage at doses of 0, 100, 223.6 and 500 mg/kg bwt/day, started on day 6 and continued up to and including day 18 p.c.
The dams were hysterectomized on day 29 p.c.
At the high dose of 500 mg/kg maternal toxicity was obvious in form of diarrhoea and decreased body weight gain.
Morphologically no substance-related renal changes were observed at this dose.

At 223.6 and 500 mg/kg embryotoxicity, i.e. statistically significant higher resorption rates compared with controls, were observed.

There were no foetotoxic and teratogenic effects.

5.3.2.4 Subcutaneous administration/female rabbits

RBM Study Code: 870284 (Data on Knoll AG file).

In the rabbit the substance was administered by subcutaneous route at doses of 0, 25, 50 and 100 mg/kg bwt from Day 6 to Day 18 of pregnancy.
No drug-related clinical signs were observed in any group.

At the two highest doses a decrease of body weight, compared to controls, was observed.
In the group treated with the highest dosage (100 mg/kg bwt) three does aborted and died. This could be related to a maternal toxic effect of the test article. These three animals showed a body weight decrease in the days preceding the abortion.
Toxic effects such as an increase of post-implantation losses and of skeletal anomalies (i.e. unossified 5th sternebra) were observed at the highest dosage

only.
No malformed foetuses were observed even at the dosages toxic for the does.

5.3.3 Peri/Postnatal Toxicity

5.3.3.1 Oral administration/female rats

RCC Study Code: 344531 (MPF/WT 9322 E) (Data on RCC file).

Sprague-Dawley mated female rats are treated orally, by gavage, from day 15 p.c. until day 20 p.p. (last administration) at daily doses of 2x440, 2x663 and 2x1000 mg/kg (time interval 6 h).

The purpose of this study is to assess the effects of Ade-SD4 upon late foetal development, labour, delivery, lactation, neonatal viability, growth and behaviour of the pups.

No test article-related macroscopical findings were noted in parent females treated with Ade-SD4. Reproduction parameters (duration of gestation, mean number of implantations, mean post implantation loss, litter data, e.g. litter size and developmental indices, and number of females rearing the offspring to termination) were unaffected by treatment. No abnormal findings were noted in any F1 pup of any group at birth, during rearing or during necropsy.

5.3.3.2 Intravenous administration/female rats

HRC Study Code: BOR 8-R/891155 (Data on HRC file).

This study was carried out in rats according to International Standard. The animals were treated with 0, 100, 200 and 400 mg/kg bwt/day by intravenous route, commencing on Day 15 of pregnancy and continued daily up to sacrifice of the dams on Day 22 p.p. (a total of 30 days).

Regarding kind, time of onset and frequency, clinical signs were comparable to those observed in the previous rat studies, and from the ninth treatment onwards these post-dosing reactions were not seen any longer.
As in the previous studies changes of the following parameters were observed: initial increase of water consumption and delayed increase of body weight.

These effects were seen in all treated groups, but they were more evident at the 400 mg/kg bwt regarding the water consumption.

Kidney lesions were not seen and no other undesirable effects were observed in the offspring.

Consequently, the "no observable effect level" for the offspring was determined to be 400 mg/kg bwt/day.

5.4 Mutagenic toxicity

The mutagenic potential of Ade-SD4 has been studied both "in vitro" in presence and in absence of metabolic activation using prokaryotes and mammalian cells and "in vivo" using two rodent species (mouse and rat).

5.4.1 "In Vitro" Studies

5.4.1.1 Ames Test/Salmonella typhimurium

RBM Study Code: 870244 (Data on Knoll AG file).

Mutagenicity test with Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 was performed with 1, 10, 100, 1000 and 5000 µg/plate of Ade-SD4, in a duplicate experiment.
The test article did not induce a significant increase in the number of revertants both in the absence and in the presence of metabolic activation up to the concentration of 5000 µg/plate.

5.4.1.2 Chromosome aberrations/Human lymphocytes culture

Knoll AG Study Code: MPF/WT 9131 (Data on Knoll AG file).

Chromosomal aberrations in human lymphocytes cultured "in vitro" were tested with 160, 500, 1600, 2500 and 5000 µg/ml of Ade-SD4.
The test compound did not induce numerical aberrations in this test system.
However, Ade-SD4 induced a slight, significant increase in the rate of structural chromosomal aberrations when given at concentrations where cytotoxicity begins

(5000 µg/ml with and 2500 µg/ml without metabolic activation).

Thus "in vitro" a clastogenic effect of the test compound when given at extremely high concentrations cannot be ruled out.

5.4.1.3 Unscheduled DNA synthesis/HeLa cells culture

RBM Study Code: 89A625 (Data on Knoll AG file).

Unscheduled DNA synthesis in cultured HeLa Cells 'in vitro" was tested with 10, 100, 1000 and 5000 µg/ml of Ade-SD4, in a duplicate experiment.

The results of the study showed that the test article up to the dosage of 5000 µg/ml, both in the presence and in the absence of metabolic activation, did not induce statistically significant increases in incorporation of tritiated thymidine in presence of hydroxyurea in cultured HeLa cells.

5.4.1.4 HPRT-Test

RBM Study Code: 89B625 (Data on Knoll AG file).

Gene mutation in V79 Chinese hamster cells was tested with 1, 10, 100 and 1000 µg/ml and with 10, 30, 100, 1000 and 5000 µg/ml of Ade-SD4 in a duplicate experiment.

The results of this assay indicated absence of mutagenic activity of the test article in V79 cells, up to the dosage level of 5000 µg/ml, both in the absence and in the presence of hepatic microsomal enzymes.

5.4.2 "In Vivo" Studies

5.4.2.1 Micronucleus test/oral administration/mouse bone marrow

Knoll AG Study Code: MPF/WT 9130 (Data on Knoll AG file).

In the micronucleus test on bone marrow of NMRI mice the induction of structural and numeric chromosomal aberrations were investigated.

Doses of 0, 400, 900 and 2000 mg/kg of the test article were applied orally

including two positive controls (cyclophosphamide and vincristine).

Under the test conditions given the test article did not induce micronuclei as well as numeric chromosomal aberrations.
There is no clastogenic potential of the test article.

5.4.2.2 Micronucleus Test/intramuscular administration/rat bone marrow

RBM Study Code: 89D625 (Data on Knoll AG file).

The micronucleus induction in bone marrow cells of rats treated intramuscularly with 600 mg/kg of Ade-SD4 was performed using Mitomycin C as the positive control.

The results of this study indicated that the test article did not induce any statistically significant increase in the frequency of micronucleated cells in the bone marrow after 18, 42 and 66 hours from the administration.
The results of the positive controls proved the sensitivity of the employed system.

5.4.3 Conclusion

The results of all five test systems indicate no relevant genotoxic risk.

5.5 Carcinogenicity Studies

The subject of these studies is the investigation of the tumorigenic potential of Ade-SD4. As usual both the rodent species, mouse and rat were employed.

5.5.1 Oral administration/male and female mice/78 weeks

RCC Study code: 280686 (MPF/WT 9116 E) (Data on RCC file)

The test compound was administered to mice by gavage over a period of 78 weeks, at doses of 0, 500, 1000 and 2000 mg/kg bwt/day.
There were two control groups and three treatment groups of 50 males/50 females each.

Concerning mortality, survival was only adversely affected in males at 2000 mg/kg bwt/day. Whereas in the male control group 8% of the animals died spontaneously and further 8% were killed in extremis the respective percentage in males at 2000 mg/kg were 14% and 16%. There were no changes neither of clinical signs, food intake, haematology nor organ weights. At 2000 mg/kg only a slight decrease in mean body weight was noted for males.

Kidney lesions consisting of dilatation of cortical tubules, tubular vacuolisation as well as cortical tubular necrosis in some animals were observed at 1000 and 2000 mg/kg. These changes may be attributed to an increased excretory load on the nephron. In the liver, the hepatocellular hypertrophy seen in all treated groups is considered to be an adaptive response to the increased demand on the functional capability of this organ.

Regarding incidence and type of tumours there was no difference between control and treated animals. The daily administration of the test article did not reveal an oncogenic effect.

5.5.2 Oral administration/male and female rats/104 weeks - study I

RCC Study code: 280708 (MPF/WT 9115 E) (Data on RCC file)

The test substance was administered to Sprague-Dawley rats by gavage over a period of 104 weeks at doses of 0, 400, 750 and 1400 mg/kg bwt/day (from day 51 onwards the doses were reduced to 200, 440 and 1000 mg/kg bwt/day).

The groups (including two control groups) consisted of 50 males and 50 females each. At the end of treatment survival rats were in the same range for treated groups (20-33%) and controls (26-36%) for both sexes. There were no relevant changes neither of clinical signs, food consumption, body weight and haematology (with the exception of marked decrease of body weight and soft/fluid stool in males at the highest dose tested).

Histopathologically an increased incidence and/or severity of tubular alterations (vacuolisation, dilatation, necrosis and regeneration) was observed in the kidneys at all dose levels. The renal changes which predominantly occurred in intercurrent

deaths may be explained by inadvertent pulmonary exposure and/or an increase excretory load imposed on the kidneys, the effects of which being largely reversible even if treatment is continued.

Furthermore, the effect on the gastrointestinal tract (soft/fluid stool) observed clinically at the high dose as well as liquid contents in the cecum of males at necropsy at the mid and high dose may be attributed to an osmotic imbalance due to the hypertonic dosing solutions.

Neoplastic findings diagnosed in the treated rats are considered to be incidental and not to differ from those of the control animals. Therefore the daily administration of doses up to 1400/1000 mg/kg bwt/day to rats by gavage for a period of two years did not result in oncogenic effects.

5.5.3 Oral administration/male and female rats/104 weeks - study II

RCC Study code: 311567 (MPF/WT 9176 E) (Data on RCC file)

The test substance was administered by gavage to Sprague-Dawley rats over a period of 104 weeks at daily doses of 0, 50, 100 and 200 mg/kg bwt/day.
An additional group was treated with 187 mg SD4/kg bwt/day to guarantee an exposure to SD4 identical to that of treatment with the top dose of Ade-SD4. The study comprised six groups (including 2 control groups) of 55 males and females each. Concerning mortality, survival was not affected by treatment. There were no relevant changes for clinical signs, food consumption, body weight, haematology, organ weights and macroscopical findings.

Histopathologically, minor degrees of renal tubular vacuolation in the kidneys, seen in all groups with Ade or SD4 treatment, was the only treatment related alteration observed. This finding may represent a transitory osmotic effect of the salt.
Regarding to the incidence and type of neoplastic findings there was no evidence of any oncogenic effect of Ade-SD4 and SD4 in the rat for the doses tested.

6. CLINICAL PHARMACOLOGY

6.1 Clinical Pharmacokinetics

6.1.1 Absorption

Orally administered ademetionine, given as SD4 salt, is absorbed from the gastrointestinal tract and a mean peak plasma concentration (Cmax) of 0.7 mg/l was found in healthy volunteers after a single administration of 400 mg of the drug as an enteric-coated tablet.
Peak plasma concentrations occurred at 2-6 hours after the administration, possibly because of the transit time of the gastroresistant tablet through the stomach.

The oral bioavailability of Ade-SD4 resulted to be about 5% (Data on Knoll Farmaceutici file; Report no. PK-03-91), probably due to an extensive presystemic metabolism, as previously shown in animals (Stramentinoli et al. 1979a).
The occurrence of such an elevated metabolism was confirmed by studies in humans receiving orally 200 mg of [methyl -^{14}C]ademetionine. In this study, 24% of the applied radioactivity was eliminated with the faeces and 16% was excreted in the urine. Thus, 60% of the applied radioactivity was incorporated into stable pools, in accordance with the findings obtained in animals (Stramentinoli et al. 1987b).

After intramuscular administration, an almost complete (95%) bioavailability of the drug was obtained (Data on Knoll Farmaceutici file; Report no. ENG-88).

6.1.2 Distribution

Protein binding of Ade-tosylate in human serum is negligible.

The administration of Ade-tosylate induced significant increases in the cerebrospinal fluid (CSF) concentration both by intravenous and oral route (Bottiglieri et al. 1990).

6.1.3 Plasma kinetics

Plasma kinetics was studied after single intravenous bolus administration of 400 mg Ade-SD4 to healthy volunteers.
A biexponential decay of plasma concentrations was observed with terminal half-life of 91±7 min (mean ± S.D. n=18). Apparent volume of distribution, estimated on

the basis of an open two-compartment model was 21±31.

Plasma half-life did not change after a repeated administration of the substance, 800 mg/day by intravenous 2-hour infusion for three days (88±7 min, n=12 at day 1 and 3, respectively) (Data on Knoll Farmaceutici file; Report no. A-001-91).

6.1.4 Metabolism and excretion

Metabolic studies performed in animals with labelled ademetionine as tosylate salt showed that administered ademetionine can share the metabolic pathways of the endogenous compound, and leads to the formation of trans-methylation metabolites, like creatine and phospholipids as well as transsulfuration-derived compounds like sulfates.

After intravenous bolus administration of 400 mg Ade-SD4 to healthy volunteers, urinary excretion accounted for 65±10% (n=8) of the applied dose (Data on Knoll Farmaceutici file; Report no. ENG-88).

6.1.5 Kinetics in patients

Preliminary studies performed in patients with chronic liver disease (n=11) and in controls with normal hepatic functions (n=9) (Kaye et al. 1990) receiving 400 mg of Ade-tosylate by intravenous route, showed only slight effects of the disease on the kinetics of the exogenously applied ademetionine: plasma half-life increased from 96 min in controls to 114 min in patients, and clearances in the two groups were 1.9 and 1.4 ml/min/kg, respectively.

6.2 Safety studies in humans

A cross-over placebo-controlled study was performed in 12 healthy volunteers in order to evaluate safety and tolerability of Ade-SD4. The substance was given for three days at a daily dose of 800 mg by 2-hour intravenous infusions (500 ml). The substance was well tolerated and no substance-related side effects were seen either clinically or in the biochemical parameters, as well as on urinary enzymes and proteins [NAG (N-acetyl-β-D-glucosminidase), α1 microglobulin, IgG, albumin] and creatinine/urea clearances, taken as nephrotoxicity markers (Data on Knoll Farmaceutici file; Report no. A-001-91).

6.3 Clinical trials

6.3.1 Intrahepatic cholestasis: Introduction

The efficacy of ademetionine in the treatment of patients with intrahepatic cholestasis complicating mainly chronic liver diseases of different etiology or occurring during pregnancy has been studied in several trials (Table V).
Due to the scant availability of alternative effective and safe therapies, the comparative trials were performed almost exclusively with placebo.

The parameters of efficacy considered in these studies include both subjective symptoms (i.e. pruritus, fatigue, and general discomfort) and biochemical serum markers of cholestasis and cytolysis [i.e. total and conjugated bilirubin, alkaline phosphatase, γ-glutamyltranspeptidase (γ-GT), total bile salts, and aminotransferases]. In particular, serum bilirubin was regarded as the primary efficacy parameter since it is commonly accepted as adequate endpoint to assess efficacy of anticholestatic drugs. Serum bilirubin is, indeed, a prognostic indicator of the outcome of the underlying liver disease, whereas other markers such as alkaline phosphatase and transaminase, have no prognostic significance (Reichen 1993).

Subjective symptoms were evaluated by descriptive severity scores or visual analogue scales.

6.3.2 Intrahepatic cholestasis: studies with parenteral administration

6.3.2.1 Dose ranging trials

In a single-blind placebo-controlled trial, 18 women with intrahepatic cholestasis of pregnancy (ICP) were randomly allocated to three groups of treatment (Frezza et al. 1984). Six patients received Ade-tosylate at the daily dosage of 200 mg dissolved in 500 ml saline intravenously for 20 days; 6 patients received 800 mg/day Ade-tosylate according to the same treatment schedule; 6 patients were given 500 ml saline alone and served as controls.

The group treated with the highest dose of Ade-tosylate showed a significant ($p<0.01$) improvement of serum conjugated bilirubin, serum total bile salts,

Table V Summary of the main quoted trials with Ade-tosylate and Ade-SD4 in the treatment of patients with intrahepatic cholestasis of pregnancy or complicating CLD.

Reference	Diagnosis	Study design	No. of pts	Daily dosage (mg) route of administration	Duration of the study	AST, ALT	γ-GT	Total and/or conjugated bilirubin	Alkaline phosphatase	Total bile acids	Other Indice
Dose ranging trials with i.v. Ade-tosylate											
Frezza et al. (1984)	Cholestasis of pregnancy	r, sb, pg	6 6 6	Ade-tosylate 200 i.v. Ade-tosylate 800 i.v. Placebo	20 days	NS ↓ ↑	NE NE NE	NS ↓ ↑	NS ↑* NS	NS ↓ ↑	NS pruritus ↓ pruritus NS pruritus
Placebo-controlled trials with i.v. Ade-tosylate											
Giannuoli et al. (1986) §	CLD with pruritus	s	20	Placebo Ade-tosylate 800 i.v.	1 week 2 weeks	NE NE	NE NE	NE NE	NE NE	NE NE	NS pruritus ↓ pruritus + scratching
Cacciatore et al. (1989) §	CLD with pruritus	s	18	Placebo Ade-tosylate 800 i.v.	1 week 2 weeks	NE NE	NE NE	NE NE	NE NE	NE NE	NS pruritus ↓ pruritus + scratching
Frezza et al. (1990a)	Cholestasis of pregnancy	r, sb, pg	15 15	Ade-tosylate 800 i.v. Placebo	18 days (mean)	↓ NS	NE NE	↓ NS	NS NS	↓ ↑	↓ pruritus ↑ pruritus
Ribalta et al. (1991)	Cholestasis of pregnancy	r, db, pg	9 9	Ade-tosylate 800 i.v. Placebo	20 days	NS NS	NE NE	NS NS	NS NS	NS NS	NS pruritus NS pruritus
Manzillo et al. (1992)	Intrahepatic cholestasis of CLD	r, db, pg	129 127	Ade-tosylate 800 i.v. Placebo	2 weeks	↓ NS	↓ NS	↓ NS	NS NS	NE NE	↓ pruritus NS pruritus
Uncontrolled trials with i.v. Ade-tosylate											
Caro et al. (1987) §	Primary biliary cirrhosis	nc	19	Ade-tosylate 800 i.v.	10 days	NE	NE	NE	NE	NE	↓ pruritus
Jorge et al. (1987) §	Cholestasis (various)	nc	33	Ade-tosylate 200 - 1200 i.v.	2 weeks	NE	NE	NE	NE	NE	↓ pruritus
Bonfirraro et al. (1990)	Cholestasis of pregnancy	nc	9	Ade-tosylate 800 i.v.	2 weeks	↓	NE	NS	NS	↓	↓ pruritus
Catalino et al. (1992)	Cholestasis of pregnancy	nc	55	Ade-tosylate 800 i.v.	1-4 weeks	↓	NE	↓**	↓	↓	↓ pruritus

transminases, and pruritus. These beneficial effects were recorded already after 10 days of therapy but disappeared soon after treatment withdrawal. On the contrary, a general worsening of all the parameters was recorded in the placebo group during the treatment period, whereas the group receiving the lowest dose of Ade-tosylate experienced neither improvement or worsening of both pruritus and liver biochemistry.

On the basis of these findings, the daily dose of 800 mg by intravenous route was regarded as the effective dosage and selected for the following i.v. trials.

6.3.2.2 Comparative trials

PLACEBO-CONTROLLED TRIALS

Eighteen and twenty patients with chronic hepatitis or cirrhosis complaining about persistent pruritus (1-84 months) resistant to conventional symptomatic treatment, were enrolled in two single-blind placebo-controlled trials (sequential design), respectively (Cacciatore et al. 1989; Giannuoli et al. 1986), In both studies; Adetosylate therapy (800 mg/day i.v.) induced a significant ($p<0.01$) improvement of pruritus and scratching lesions after 2 weeks of treatment. The beneficial effect was recorded as early as the first week of therapy. On the other hand, itching did not improve during the 3 week-washout- period with placebo preceding Ade-tosylate treatment.

These findings were confirmed by a large double-blind placebo-controlled trial carried out in 420 patients of both sexes with liver disease at different stages (52% cirrhosis, 24% chronic hepatitis, 16% acute hepatitis, 11% substance-induced cholestasis) and of different etiology (viral, alcoholic, cryptogenic, substance induced) (Manzillo et al. 1992).

The patients were randomly assigned to treatment with Ade-tosylate (800 mg/day i.v.) or indistinguishable placebo for 2 weeks. The treatment groups were comparable for demographic characteristics and basal biochemical and clinical parameters. The patients did not receive any other symptomatic treatment for pruritus or cholestasis during the study period.

Three hundred and eight one patients completed the 2 week-trial: of these 343 (180 treated with Ade-tosylate and 163 with placebo) were suitable for statistical analysis.

The analysis of variance showed a significant treatment to time to disease (acute

Table V Continue

Reference	Diagnosis	Study design	No. of pts	Daily dosage (mg) route of administration	Duration of the study	AST, ALT	γ-GT	Total and/or conjugated bilirubin	Alkaline phosphatase	Total bile acids	Other Indices
Placebo-controlled trials with i.v. and i.m. Ade-SD4											
Mascio et al. (1991)	Intrahepatic cholestasis of CLD	r, db, pg	20 20 20	Ade-SD4 800 i.v. Ade-SD4 800 i.m. Placebo i.m.	2 weeks	↓ ↓ NS	↓ ↓ NS	↓ ↓ NS	↓ ↓ NS	NE NE NE	↓ pruritus ↓ pruritus NS pruritus
Dose ranging trials with oral Ade-tosylate											
Lafuenti et al. (1988)	Cholestasis of pregnancy	nc	10 7	Ade-tosylate 1800 p.o. Ade-tosylate 600 p.o.	2 weeks	↓ NS	NE NE	↓ ↓	NS NS	↓ NS	↓ pruritus ↓ pruritus
Comparative trials with oral Ade-tosylate											
Frezza et al. (1990b)	Intrahepatic cholestasis of CLD	r, db, pg	110 110	Ade-tosylate 1600 p.o. Placebo	2 weeks	↓ NS	↓ NS	↓ NS	↓ NS	NE NE	↓ pruritus, fatigue and discomfort NS pruritus, fatigue and discomfort
Bray et al. (1991a)	Primary biliary cirrhosis	co, r, s,	8 8 8	Ade-tosylate 2400 p.o. R 300 p.o. UDCA 800-1200 p.o.	2 months	NS NS NS	NS NS ↓	↓ ↑ ↓	NS NS NS	NE NE NE	↓ pruritus ↓ pruritus ↓ pruritus
Manzillo et al. (1992)	Responders to i.v. treatment CLD @	r, db, pg	34 34	Ade-tosylate 1600 p.o. Placebo	2 months	NE NE	NE NE	↓ ↑	↓ ↑	NE NE	NE NE

* = The increase of alkaline phosphatase might reflect improvement in the functional state of placenta associated with a concomintant improvement of parameters of cholestasis.
** = considering only the cases with abnormal levels at baseline.
§ = primary objective of the study: improvement of pruritus.
@ = normalisation or ↓ 50% in total bilirubin or conjugated bilirubin or alkaline phosphatase.

Abbreviations: CLD = chronic liver disease; r = randomised; sb = single-blind; db = double-blind; co = crossover; s = sequential; pg = parallel groups; nc = non-comparative versus placebo; i.v. = intravenous; p.o. = orally; i.m. = intramuscular; R = Rifampicin; UDCA = Ursodeoxycholic acid; AST = aspartate amino transferase; ALT = alanine aminotransferase; γ-GT = γ-glutamyl transpeptidase; ↓ = decrease in level or severity; ↑ = increase in level or severity; NS = not significant; NE = not evaluated.

hepatitis/chronic liver disease) interaction for all the considered parameters, indicating that the response to the treatment was differently affected by the underlying liver disease. Accordingly, a separate analysis was carried out for patients with acute hepatitis (total number 87; 51 treated with Ade-tosylate, and 36 with placebo) and with chronic liver disease (total number 256; 129 in the Ade-tosylate group and 127 in the placebo one). The results observed in patients with acute hepatitis are reported in the section 6.3.5.5.

With regard to the patients with chronic liver disease, a significant treatment to time interaction was observed for serum total and conjugated bilirubin, aminotransferases (ALT, AST) and γ-GT indicating that Ade-tosylate is more effective than placebo in improving these biochemical parameters (Table VI).

Table VI Serum liver biochemistry (mean and Δ%) before (B) and after (A) a 2 week intravenous treatment with Ade-tosylate (800 mg/day) and placebo in patients with intrahepatic cholestasis of chronic liver disease

	ADE-TOSYLATE			PLACEBO		
	B	A	Δ%	B	A	Δ%
STB (μmol/l)**	44.3	24.3	-45%	36.3	27.4	-24%
SCB (μmol/l)**	17.0	8.9	-48%	16.3	11.4	-30%
SAP (μkat/l)	4.82	3.86	-20%	4.93	3.97	-19%
γ-GT (μkat/l)*	1.90	1.18	-38%	1.83	1.31	-28%
AST (μkat/l)*	1.38	0.94	-32%	1.33	1.04	-22%
ALT (μkat/l)**	1.24	0.79	-36%	1.21	1.01	-16%

* = $p<0.05$ treatment-to-time interaction in the Ade-tosylate group; ** = $p<0.01$ treatment-to-time interaction in the Ade-tosylate group; Δ% = percent decrease as compared to baseline
STB = serum total bilirubin; SCB = serum conjugated bilirubin; SAP = serum alkaline phosphatase; γ-GT = γ-glutamyltranspeptidase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
Normal values: STB ≤ 17 μmol/l; SCB ≤ 4 μmol/l; SAP < 2.0 μkat/l; γ-GT < 0.5 μkat/l; AST < 0.58 μkat/l; ALT < 0.58 μkat/l.

On the other hand, serum alkaline phosphatase levels did not change significantly in either group. At baseline, 63 patients in the Ade-tosylate group and 42 receiving placebo complained of pruritus. At the end of the treatment period, pruritus was completely relieved in 54 patients treated with Ade-tosylate and in only 16 in the placebo group ($p<0.001$).

Patients with chronic liver disease who responded to intravenous Ade-tosylate therapy in terms of normalization of or at least a 50% decrease in serum bilirubin or alkaline phosphatase levels, were randomized to receive oral treatment with Ade-tosylate or placebo up to 2 months. The results of this follow-up are discussed in the section 6.3.3.2.

The efficacy of ademetionine treatment in intrahepatic cholestasis of pregnancy

was assessed in two placebo-controlled studies (Frezza et al. 1984, 1990a).
The first study (Frezza et al. 1984) has been discussed in the section 6.3.2.1. "Dose ranging trials" relative to parenteral treatment.

In a more recent study by Frezza et al. (1990a), 30 women with intrahepatic cholestasis of pregnancy in the last trimester of pregnancy were randomly assigned to receive Ade-tosylate (800 mg/day i.v.) or placebo until delivery for a mean period of 18 days.
After Ade-tosylate therapy, the patients exhibited significantly ($p<0.01$) lower serum levels of conjugated bilirubin, total bile salts, and aminotransferases as compared with baseline as well as with placebo group. Furthermore, Ade-tosylate therapy significantly ($p<0.01$) reduced pruritus whereas placebo was ineffective. The follow-up of these cases showed a lower incidence of premature labour in the Ade-tosylate group (2/15) than in patients receiving placebo (5/15).

In contrast to these findings, a placebo-controlled trial failed to demonstrate any significant beneficial effect of intravenous Ade-tosylate 800 mg/day on liver biochemistry and pruritus in 9 Chilean women with relatively severe intrahepatic cholestasis of pregnancy (Ribalta et al. 1991). The lack of efficacy in this study might be partly attributed to differences in the clinical features and in the ethnic origin of the two populations (pure Caucasian in the studies by Frezza et al., Caucasoid in the study by Ribalta et al.) as well as to the delay elapsed between the onset of pruritus and the initiation of the treatment in the Chilean patients.

6.3.2.3 Uncontrolled trials

The results of the controlled trials are supported by two open studies carried out respectively in 19 patients with primary biliary cirrhosis and 33 patients with intrahepatic cholestasis complicating acute or chronic hepatitis or cirrhosis (Caro et al. 1987; Jorge et al. 1987), and by two open studies carried out in 9 and 55 women respectively, with intrahepatic cholestasis of pregnancy (Bonfirraro et al. 1990; Catalino et al. 1992).

The main target of the first two trials was the relief of pruritus. The patients received Ade-tosylate i.v. at the daily dosage of 800 mg (Caro et al. 1987) or 200 to 1200 mg according to an escalating schedule (Jorge et al. 1987). Considering the two studies together, pruritus was completely relieved in 30 out of 52 cases (58%) and significantly improved in the remaining 22 patients (42%) as early as the first 2-5

days of therapy.
The two open trials in intrahepatic cholestasis of pregnancy contributed a total number of 64 cases treated with Ade-tosylate 800 mg/day i.v. (Bonfirraro et al. 1990; Catalino et al. 1992). The treatment started as early as cholestasis was diagnosed (onset of pruritus and abnormal liver biochemistry) and lasted up to delivery (1-4 weeks). A significant ($p<0.01$) improvement of total bile salts and aminotransferases was recorded after treatment in both studies. In these series, most of the patients showed normal serum levels of bilirubin at baseline possibly because of the early diagnosis of the disease. When the cases with abnormal basal bilirubin levels were evaluated separately, it was observed that Ade-tosylate treatment significantly reduced this parameter as compared with baseline. Furthermore, all the patients experienced a significant ($p<0.01$) amelioration of pruritus that was totally relieved in 45 out of 64 cases (70%) and significantly improved in the remaining 19 patients (30%) at the end of the treatment.

6.3.2.4 Trials with Ade-SD4

Sixty patients with viral, alcoholic, or cryptogenic cirrhosis and intrahepatic cholestasis were randomly allocated to 3 groups of treatment in a single-blind placebo-controlled trial (Mascio et al. 1991). Upon entry, 43 patients suffered from pruritus.

Ade-SD4 was administered for 15 days at the daily dosage of 800 mg intramuscularly (i.m.) in 20 patients (group I) and by intravenous infusion (i.v.) in additional 20 patients (group II). A third group of 20 patients received 800 mg of indistinguishable placebo i.m. for 15 days.

The short-term treatment with either i.m. or i.v. Ade-SD4 proved significantly ($p<0.01$) more effective than placebo in improving liver biochemistry (i.e. serum total and conjugated bilirubin, alkaline phosphatase, γ-GT and aminotransferases) as well as in alleviating pruritus.

Short-term (10 days) treatment with intravenous Ade-SD4 at the daily dosage of 1200 mg resulted in a significant improvement in the hepatic handling of organic anions, as assessed by a reduction in Rifamycin-SV-induced hyperbilirubinaemia and the plasma elimination half-life of Rifamycin-SV in cirrhotic patients (Persico et al. 1990b).
This study will be further discussed in the section 6.3.5.1.

6.3.3 Intrahepatic cholestasis: studies with oral administration

6.3.3.1 Dose ranging trials

The efficacy of different doses of Ade-tosylate administered by oral route, was tested in an open study carried out in 17 women with intrahepatic cholestasis of pregnancy (Lafuenti et al. 1988).

The patients were allocated to receive 600 mg (7 pts) or 1800 mg (10 pts) daily of Ade-tosylate for 15 days.
The lowest dose proved effective in significantly ($p<0.01$) reducing serum conjugated bilirubin and pruritus, whereas a trend towards improvement was recorded for serum total bile salts and aminotransferases. Serum total bile salts and aminotransferases were significantly reduced in the group receiving the highest dose.

6.3.3.2 Comparative trials

PLACEBO-CONTROLLED TRIALS

Sixty eight out of 78 patients with chronic liver disease who experienced a normalization or at least a 50% decrease in serum bilirubin or alkaline phosphatase after a 2-week-treatment with i.v. Ade-tosylate, were randomized to receive oral Ade-tosylate (34 patients) (1600 mg/day) or indistinguishable placebo (34 patients) for 2 months (Manzillo et al. 1992).
The groups did not significantly differ at baseline (i.e. at the end of the i.v. treatment). After 2 months of oral treatment, a further improvement of serum total and conjugated bilirubin and alkaline phosphatase or their preservation into the normal range were found in patients treated with Ade-tosylate (significant treatment-to-time interaction; $p<0.001$). On the other hand, patients who received placebo, showed a significant ($p<0.01$) worsening of these biochemical parameters. This suggests that the continuation of the treatment with oral Ade-tosylate not only maintains the effects obtained by an acute parenteral course but it can also further improve them. Therefore, a treatment longer than 2 weeks may be necessary in some patients to achieve a "full" anticholestatic effect.

In a double-blind placebo-controlled trial, 220 inpatients (26% chronic active hepatitis, 68% cirrhosis, 6% primary biliary cirrhosis) with stable (1 month or more) at least two fold increase in serum total and conjugated bilirubin and alkaline phosphatase were randomly allocated to oral Ade-tosylate (1600 mg/day in two

administrations) or indistinguishable placebo (Frezza et al. 1990b). The patients received the treatment for two weeks.

Serum markers of cholestasis (total and conjugated bilirubin, alkaline phosphatase) and of cytolysis (alanine aminotransferase) significantly ($p<0.01$) decreased after Ade-tosylate treatment as compared with placebo post-treatment values (Table VII).

Furthermore, Ade-tosylate therapy significantly ($p<0.01$) improved clinical symptoms (pruritus, fatigue, general discomfort) whereas placebo was ineffective (Table VIII).

When the effects of Ade-tosylate administration were analysed separately in patients with chronic active hepatitis and in those with cirrhosis, similar improvements in liver biochemistry and in clinical symptoms were found.
A rebound of both the biochemical parameters and the clinical symptoms to pre-treatment values was recorded 30 days after active treatment withdrawal.

ADEMETIONINE VS OTHER ACTIVE TREATMENTS

In a 3-phase-cross-over trial, 12 women with a diagnosis of primary biliary cirrhosis (stage III-IV) were enrolled and randomized in a sequential design to receive oral Ade-tosylate (2.4 g/day), Rifampicin (0.3 g/day), and ursodeoxycholic acid (UDCA) (0.8-1.2 g/day) for 2 months alternated with 1 month of washout with placebo (Bray et al. 1991a).

Eight patients completed the 8-month trial. The comparison of serum total bilirubin levels at the end of each treatment showed significantly ($p<0.05$) lower values after Ade-tosylate therapy as compared with the other 2 treatments.
UDCA was significantly more effective than Ade-tosylate and Rifampicin with regard to γ-GT. Ade-tosylate significantly reduced serum total protein as compared with UDCA and Rifampicin without affecting albumin levels.
All the treatments were equally effective in alleviating pruritus.

Table VII Comparison of biochemical values (mean and Δ %) between treatment groups during the trial

	Baseline		1st week of treatment				2nd week of treatment			
	Ade-tosylate	Placebo	Ade-tosylate	Δ %	Placebo	Δ %	Ade-tosylate	Δ %	Placebo	Δ %
STB (µmol/l)	76.7	77.2	51.3 *	33 %	66.3	11 %	37.6 **	51 %	57.9	25 %
SCB (µmol/l)	38.6	37.1	29.4	24 %	35.9	3 %	22.5 *	42 %	32.4	13 %
SAP (µkat/l)	4.5	4.7	3.7 *	18 %	4.6	2 %	3.2 **	29 %	4.4	4 %
γ-GT (µkat/l)	2.5	2.2	1.9	24 %	1.9	14 %	1.5	40 %	1.7	23 %
AST (µkat/l)	2.4	2.1	1.8	25 %	1.8	14 %	1.0	58 %	1.7	19 %
ALT (µkat/l)	3.3	2.8	2.3	30 %	2.4	14 %	1.5 *	55 %	2.2	21 %

Ade-tosylate = 110 patients; placebo = 110 patients; Δ % = percent decrease as compared to baseline.
STB = serum total bilirubin; SCB = serum conjugated bilirubin; SAP = serum alkaline phosphatase; γ-GT = γ-glutamyltranspeptidase; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

* = $p < 0.05$; ** = $p < 0.01$ vs. corresponding placebo value.

Normal values: STB ≤ 17 µmol/l; SCB ≤ 4 µmol/l; SAP < 2.0 µkat/l; γ-GT < 0.5 µkat/l; AST < 0.58 µkat/l; ALT < 0.58 µkat/l.

Table VIII Comparison of subjective symptoms (mean and Δ %) between treatment groups during the trial

	Baseline		1st week of treatment				2nd week of treatment			
	Ade-tosylate	Placebo	Ade-tosylate	Δ %	Placebo	Δ %	Ade-tosylate	Δ %	Placebo	Δ %
Pruritus (cm)	5.3	5.3	3.5 *	34 %	4.8	9 %	2.7 *	49 %	4.1	23 %
Fatigue (cm)	5.5	5.3	3.5 *	36 %	5.0	6 %	2.6 *	53 %	4.8	9 %
General discomfort (score 0-4)	1.8	1.9	1.0 *	44 %	1.8	5 %	0.4 *	78 %	1.4	26 %

* = $p < 0.01$ vs corresponding placebo value
Δ % = percent decrease as compared to baseline

6.3.4 Alcohol related liver disorders

In a study of 6 healthy volunteers given a sequence of injections at 3-day intervals of absolute ethanol (0.5 g/kg) or ethanol plus Ade-tosylate (15 mg/kg) with additional pretreatment with Ade-tosylate on the day prior to the challenge, both ethanol and acetaldehyde plasma concentrations were significantly lower when Ade-tosylate was administered (Di Padova et al. 1984b). This observation was attributed to accelerated synthesis of membrane phospholipids and thiol compounds capable of combining with ethanol and acetaldehyde, rather than the interference with the main route of alcohol metabolism (Table IX).

A treatment with Ade-tosylate at the daily dose of 1,200 mg p.o. for 1 to 6 months showed to improve methionine clearance and to increase cysteine, taurine and glutathione in plasma, erythrocytes and liver of patients with alcoholic pre-cirrhotic or cirrhotic liver diseases (Corrales et al. 1992; Loguercio et al. 1994; Marchesini et al. 1992a, 1992b; Vendemiale et al. 1989b). For further details see sections 6.3.5.1.3. and 6.3.5.2.

As ademetionine enhances the liver detoxification processes via the transsulfuration pathway, its possible use in reducing ethanol abuse in the outpatient treatment of alcoholic patients was studied by Cibin et al. (1988).

Sixty four alcoholics without evidence of liver cirrhosis were treated with Ade-tosylate (200 mg daily intramuscularly) or placebo for 30 days in a double-blind, randomized, parallel groups study. Ade-tosylate therapy reduced serum γ-GT and transaminases, improved clinical symptoms such as fatigue, anorexia, insomnia, anxiety and depression ($p<0.05$) and significantly reduced blood alcohol levels ($p<0.01$) as compared to placebo.

Moreover, only 1 patient dropped out of the active treatment group compared with 8 taking placebo ($p<0.05$). These findings are encouraging and deserve further study. One hundred thirteen patients with alcoholic-related liver disorders were randomized in a prospective comparative trial to receive Ade-tosylate 1200 mg/day or UDCA 600 mg/day or a combination of the two drugs at the same dosages orally for 12 months (Trespi et al. 1995). Patients had to have histologically proven diagnosis of non-cirrhotic alcoholic liver disease and to be actual drinkers (≥ 50 g/day). Thirtynine patients dropped out during the study because withdrew drinking

Table IX Summary of the main quoted clinical studies with Ade-tosylate in the treatment of alcoholic liver disorders

Reference	Design	Population	No. of cases	Daily dose Route	Duration	Outcome
Di Padova et al. 1984	OP challenge	Healthy subjects	6	15 mg/kg b.w. i.v.	3 days	↓ plasma alcohol + acetaldehyde
Corrales et al. 1992	DB, R vs P	alcoholic cirrhosis	10 vs 8	1,2 g p.o.	60 days	↑ methionine clearance
Vendemiale et al. 1989	OP, R vs P	alcoholic cirrhosis	9 vs 8	1,2 g p.o.	6 mo.	↑ hepatic GSH
Caballeria & Moreno 1990	OP	alcoholic steatosis	30	100 mg i.m.	2 mo.	↓ hepatic steatosis + ALT
Marchesini et al. 1992	OP, R vs P	alcoholic cirrhosis	8 vs 8	1,2 g p.o.	30 days	↑ plasma cysteine + taurine
Loguercioet al. 1994	OP, R vs P	alcoholic cirrhosis alcoholics no LD	10 vs 10 10 vs 10	2 g i.v.	15 days	↑ erythrocyte GSH
Trespi et al. 1995	OP	non-cirrhotic ALD	37	1.2 g p.o.	12 mo	↓ ALT, GGT; ↑ GEC, A1
Knoll 1996	DB vs P + O	ALD vs non-ALD	282 vs 1370	0.5g i.m. 0.8 g i.v. 1.6 g p.o.	2 wk up to 2 mo	ALD vs non-ALD $p < 0.01$ Odds ratio 1.61

or because not compliant. Seventy-four patients completed the 12 months follow up and entered the final analysis. Patients treated with Ade-tosylate showed a significant improvement of ALT, GGT, and serum total bile acids levels after 8 and 12 months of treatments as compared to baseline. The hepatic galactose clearance was also measured and progressively improved from the fourth month of treatment with Ade-tosylate. Patients who received UDCA alone only showed an improvement of GGT and SAP. Interestingly, the combination of the two substances led to a sinergistic effect with improvement of all the aforementioned parameter as well as increase in serum Apolipoprotein A1, a marker of progression of liver fibrosis in alcohol drinkers (Mathurin et al. 1996; Poynard et al. 1986, 1991), which was not affected by the single treatments. All the three regimens were well tolerated and no untowards effects were recorded.

The effect of Ade-tosylate treatment of fatty liver lesions in alcoholic patients has also been observed (Caballeria & Moreno 1990). For further details see section 6.3.5.6.

In order to identify the factors influencing the response to Ademetionine treatment for cholestasis, a systematic overview using individual patient data from recent clinical studies was conducted and multivariate logistic regression analysis was used (Di Padova et al. 1996). A total of 1,652 patients with cholestatic chronic liver disease of different stages and etiologies underwent this analysis. The results of this investigation not only confirmed a significant better effect of Ade-tosylate than placebo on biochemical markers of cholestasis, but also identified the subgroup of patients with alcoholic liver disease as that which better benefits from the active treatment.

6.3.5 Supportive trials

6.3.5.1 Clinical pharmacology studies in challenge models

The two main putative activities of ademetionine as anticholestatic agent, i.e. improvement of cholephilic anion transport and methionine metabolism, have been further elucidated in clinical pharmacology studies performed in challenge models.

6.3.5.1.1 Ethinyloestradiol challenge

Ethinyloestradiol (EO) is a synthetic oestrogen found in many oral contraceptives. It causes a predictable and reversible reduction in hepatic excretory function when administered to susceptible women or in large doses. Hepatic excretory function abnormality after EO treatment is associated with decreased membrane lipid mobility, possibly due to an increased content of cholesterol in liver surface membrane (Simon 1978).

EO-induced cholestasis is regarded as a "pure" cholestasis as the damage involves only the liver plasma membranes in the absence of hepatocyte necrosis and inflammation. It therefore represents a good clinical model for investigation of substances whose mechanism of action involves the liver plasma membrane.

Six women with a past history of intrahepatic cholestasis of pregnancy volunteered to receive an oral load of EO (0.1 mg/day) for one week and, after 3 months, the same dose of EO plus oral Ade-tosylate (800 mg/day) for one week (Frezza et al. 1988). EO alone significantly increased serum levels of conjugated bilirubin, aminotransferases, and total bile salts with respect to basal values. After the rechallenge with EO plus Ade-tosylate, the liver function tests did not change significantly compared to baseline and were significantly lower than those obtained after the challenge with EO alone.

6.3.5.1.2 Organic anions transport: challenge with Nicotinic Acid and Rifamycin-SV

Nicotinic acid (NA) and Rifamycin-SV (R-SV) are two organic anions which compete with bilirubin for the uptake by a common liver membrane carrier (Gentile et al. 1984).
In subjects showing an impairment of bilirubin uptake (i.e. subjects with Gilbert's syndrome, elderly subjects, and cirrhotics), an intravenous load with NA or R-SV leads to a transient increase in serum bilirubin levels (Gentile et al. 1984, 1990; Persico et al. 1990a; 1994). This hyperbilirubinaemic effect is exploited to estimate the bilirubin uptake capability of liver cells.

The effects on the metabolization rate of bilirubin and NA of two dosages (200 mg and 800 mg/day, respectively) of Ade-tosylate, were evaluated in 10 males with Gilbert's syndrome (Gentile et al. 1988). In this trial, each patient received both intravenous Ade-tosylate (200 mg and 800 mg/day) and placebo according to a

sequential design including a one-week wash-out course after each treatment period lasting 10 days.

Serum unconjugated bilirubin levels were significantly lower ($p<0.01$) after 800 mg of Ade-tosylate than after placebo as well as after the lower dose of Ade-tosylate. Accordingly, the bilirubin time concentration curve, expressed as area under the curve (AUC), was significantly ($p<0.01$) reduced after the higher dosage of Ade-tosylate as compared with the values obtained after placebo as well as after 200 mg of Ade-tosylate. Plasma NA half-life was also significantly reduced ($p<0.01$) by Ade-tosylate 800 mg/day and not by placebo or the lower dosage.

In an additional clinical trial, NA half-life and serum bilirubin pharmacokinetics after NA intravenous load were studied in 10 healthy young males and in 10 healthy elderly males before and after Ade-tosylate administration (800 mg/day i.v. for 10 days) (Gentile et al. 1990). At baseline, the mean serum bilirubin time-concentration curve after NA load, expressed as AUC, was significantly higher in the elderly subjects than in the young ones, confirming an impairment in liver plasma membrane mobility occurring with age. Ade-tosylate treatment produced a significant decrease ($p<0.01$) of bilirubin AUC after NA load in elderly subjects as well as a significant shortening ($p<0.01$) of NA half-life in both groups.

Finally, the aim of a third challenge study was to investigate the effect of ademetionine therapy on hepatic handling of organic anions (half-life and hyperbilirubinaemic effect of R-SV) in patients with liver cirrhosis (Persico et al. 1990b). Forty eight cirrhotic patients (stage A-C) were enrolled in this trial and randomized to receive Ade-SD4 (1200 mg/day i.v.) or placebo for ten days. After treatment, the patients who received Ade-SD4 showed a significant ($p<0.05$) decrease in R-SV half-life and R-SV-induced hyperbilirubinaemia as compared with baseline as well as with post-treatment placebo values. In this regard, it should be underlined that hyperbilirubinaemia after R-SV load significantly relates with the degree of liver function as assessed by the Child-Pugh criteria as well as by antipiryne clearance (Persico et al. 1994). Furthermore, a significant ($p<0.001$) improvement of aminotransferases and alkaline phosphatase was observed after Ade-SD4, whereas placebo was ineffective.

6.3.5.1.3 Methionine challenge

Hepatic methionine metabolism and, as a consequence, the transsulfuration

pathway are impaired in cirrhotic patients (Horowitz et al. 1981, Marchesini et al. 1992a). The defective metabolic step is located at the site of ademetionine synthesis from methionine and ATP because of reduction in the activity of the enzyme ademetionine synthetase (Carbrero et al. 1988, Martin Duce et al. 1988). Accordingly, reduced ademetionine synthesis and utilization might result in decreased formation of secondary sulfur amino acids (cystine, taurine), with nutritional defects as well as reduced glutathione availability with potential substance hepatotoxicity (Chawla et al. 1984).

In a double-blind randomized trial 16 alcoholic cirrhotic males (8 in each group) were treated for 1 month with Ade-tosylate (1200 mg/day orally) or placebo (Corrales et al. 1991b. 1992b). At the end of treatment the degree of methionine intolerance was investigated after an oral methionine load (50 mg/kg bwt). The maximum values of plasma methionine were reached about one hour after the methionine overload and they were significantly ($p<0.05$) lower in the group receiving Ade-tosylate than in the placebo group. Furthermore, the average half-life of methionine was also significantly ($p<0.05$) lower after Ade-tosylate therapy than after placebo. Sulfate excretion in 24 hours urine collection was similar in both groups. These results indicate that ademetionine treatment improves methionine tolerance in patients with liver cirrhosis.

6.3.5.2 Methionine metabolism impairment

The effects of ademetionine administration on the metabolism of methionine and on the availability of sulfated compounds in patients with chronic liver disease have been elucidated in the following studies.

One-month treatment with Ade-tosylate (1200 mg/day orally) improved methionine tolerance in patients with liver cirrhosis (Corrales et al. 1991b, 1992b.). This study has been discussed in section 6.3.5.1.3.

In placebo-controlled trial, the effect of a treatment with Ade-tosylate on hepatic glutathione content in patients with liver cirrhosis was investigated (Vendemiale et al. 1989b). Twenty-four patients with alcoholic or non-alcoholic cirrhosis were allocated to receive Ade-tosylate (1200 mg/day) orally or placebo for 6 months. In addition, 15 normal subjects served as controls. Pre-treatment levels of hepatic glutathione were significantly lower in cirrhotic patients than in normal controls. Ade-tosylate therapy resulted in a significant increase in hepatic glutathione

content both in patients with alcoholic cirrhosis and in those with non-alcoholic liver disease as compared with placebo-treated patients.

Sixteen patients with liver cirrhosis were enrolled in a placebo-controlled study and distributed in two groups carefully matched as to age, sex, aetiology and severity of the liver disease receiving Ade-tosylate (1200 mg/day i.v. for 3 days followed by 1200 mg/day orally for 30 days) or placebo (Marchesini et al. 1992b). In patients given Ade-tosylate, long-term treatment doubled cystine and taurine plasma levels, which were low to normal at baseline, without any change in the concentration of methionine, neutral amino acids, and polyamine. No changes in plasma amino acid pattern were observed in the control group.

Taurine is a sulfur-containing amino acid produced by the transsulfuration pathway of methionine. The best known, and possibly the most important, function of taurine in humans is bile acid amidation in the liver. Bile acid conjugation with taurine plays an important role as a detoxifying process during cholestasis (Heaton 1985). As the proportion of taurine conjugation is closely correlated with the hepatic availability of taurine (Hardison 1978), a decrease in its synthesis, due to transsulfuration derangement, might lead to an impairment of bile acids amidation.

In order to investigate the effect of Ade-tosylate on the levels of sulfur amino acids in bile and on bile acids amidation with taurine, ten patients with cirrhosis were enrolled in an open study and treated with Ade-tosylate (800 mg/day orally) for two months (Gandin et al. 1992). Bile was obtained before and after treatment using a string-test device after gallbladder contraction with caerulein. An increase in bile concentration of glutamine and taurine was recorded after therapy. Bile salts analysis showed a trend towards increased tauroconjugation for all individual bile salts with significant ($p<0.05$) increase in taurochenodeoxycholate and a drop in glycocholate biliary concentration. These data suggest that, in cirrhotic livers, exogenous ademetionine is partially metabolized to taurine, which is used for bile acids amidation.

6.3.5.3 Primary biliary cirrhosis (PBC)

The efficacy of a 2-month oral treatment with Ade-tosylate (2400 mg/day) on pruritus and liver biochemistry in 8 patients with PBC stage III-IV has been reported in section 6.3.3.2 "Ademetionine vs other active treatments" (Bray et al. 1991a).

These results were also confirmed in a multicentre trial in patients with intrahepatic cholestasis complicating chronic liver disease, to which 32 patients with PBC (stage I-III) took part (Manzillo et al. 1992; Giudici et al. 1993).
Patients were randomized to receive intravenous Ade-tosylate (800 mg/day) or placebo for 2 weeks. Ade-treated patients showing a decrease ≥50% vs baseline or normalization of at least one of the markers of intrahepatic cholestasis (i.e. serum total and conjugated bilirubin or alkaline phosphatase) were subsequently randomized to oral Ade-tosylate (1600 mg/day) or placebo for 8 weeks.

Ade-tosylate treatment was significantly more effective than placebo in improving serum total and conjugated bilirubin, ALT and γ-GT.
Pruritus was relieved in 5 out of 8 patients (62%) on i.v. Ade-tosylate and in 4 out of 11 (36%) patients on placebo. Oral Ade-tosylate treatment was significantly superior to placebo in further decreasing serum total and conjugated bilirubin.

The anticholestatic activity of Ade-treatment in PBC might also be explained by its capability to decrease toxic endogenous bile acid pool as shown by a significant decrease in biliary deoxycholic acid paralleled by an increase in cholic as well as chenodeoxycholic acid and an improvement of liver biochemistry and pruritus in 5 patients with PBC (stage I-III) receiving Ade-tosylate at the dose of 800 mg/day p.o. for 2 months (Roda et al. 1992). This effect has also been shown when Ade was combined to UDCA treatment as compared to UDCA alone in patients with PBC (Roda et al. 1993).

6.3.5.4 Prevention of substance-induced hepatotoxicity

6.3.5.4.1 Oestrogens

Eighteen healthy women on oral contraceptives, who developed liver biochemistry abnormalities after 2 cycles of treatment, showed a normalization of serum aminotransferases, serum bile salts, and bile cholesterol saturation index when they were given oral Ade-tosylate (600 mg/day) during the 2 following cycles of oral contraceptives (Frezza et al. 1987; Di Padova et al. 1984a).

The activity of Ade-tosylate in counteracting oestraogen -induced hepatotoxicity was also proven in high risk subjects, such as women with a past history of intrahepatic cholestasis of pregnancy (Frezza et al. 1988). This study has been discussed in section 6.3.5.1.

Furthermore, in an additional experiment carried out in 7 women with a past history of intrahepatic cholestasis of pregnancy who exhibited bile cholesterol supersaturation, oral Ade-tosylate (800 mg/day) for 2 weeks decreased bile cholesterol saturation index to normal values (Frezza et al. 1988).

These findings indicate a possible use of ademetionine in allowing women susceptible to oestrogen-induced hepatotoxicity to continue using oral contraceptives.

6.3.5.4.2 Androgens

See case reported on section 6.3.5.8.

6.3.5.4.3 Paracetamol

Paracetamol (acetaminophen) is inactivated via 2 main pathways resulting in the formation of sulfate and glucuronide derivatives.

Reduced levels of sulfur compounds and glutathione make patients with cirrhosis more susceptible to the hepatotoxic effects of this substance (Lauterburg & Velez 1988). In a study of 6 cirrhotic patients, 1 week of intravenous administration of Ade-tosylate

800 mg/day significantly enhanced the formation of sulfate and glucuronide conjugates of paracetamol given at an oral dose of 1.0 g.

In addition, the urinary excretion of paracetamol mercapturate, a highly reactive hepatotoxic intermediate of paracetamol metabolism, was decreased (Vendemiale et al. 1989a).

6.3.5.4.4 Psychoactive agents and anticonvulsants

Protection by ademetionine against drug-induced liver dysfunction was also investigated by Torta et al. (1988) in a large group of patients receiving long term treatment with various psychoactive agents and anticonvulsants.

This study included 5 groups: (a) monoamine oxidase inhibitors (MAOI; 60 patients); (b) tricyclic antidepressants (350 patients); (c) benzodiazepines (500 patients); (d) anticonvulsants (445 patients); (e) alcoholics taking antidepressants or anticonvulsants (18 patients). In the first 4 groups half of the remainder, plus those in group 5, received the substance only when serum γ-GT, a sensitive indicator of hepatotoxicity, became elevated. Patients receiving treatment with MAOIs or with anticonvulsants (phenytoin or phenobarbitone) showed abnormal liver enzyme activity which was reversed or prevented by concomitant Ade-tosylate treatment, while those

receiving tricyclic antidepressants or benzodiazepines showed no enzyme abnormality either with or without Ade-tosylate. Alcoholics receiving Ade-tosylate in addition to substance therapy also showed a normalization of liver γ-GT. Thus, Ade-tosylate appears to be capable of antagonizing or preventing substance-induced hepatotoxicity under the conditions of this study.

6.3.5.5 Acute hepatitis

Twenty six patients of both sexes affected by intrahepatic cholestasis complicating acute hepatitis in the majority of the cases, or chronic liver disease, were allocated to three groups (Adachi et al. 1986). Nine were treated with 600 mg/day of Ade-tosylate i.v., nine received prednisolone in decreasing doses of 30 to 10 mg/day orally, and eight patients served as a control group receiving no treatment. After 3 weeks of therapy, a significant ($p<0.01$) decreased in serum bilirubin levels was observed in both Ade-tosylate and prednisolone groups, a trend which was already evident after the first week of therapy in both treatment groups. On the contrary , no improvement in liver biochemistry was recorded in the control group.

In a double-blind placebo controlled study, patients were stratified according to diagnosis (acute hepatitis/chronic liver disease) and a separate statistical analysis were performed at the end of the trial (Manzillo et al. 1992).

Eighty seven patients with acute viral or alcoholic hepatitis were randomly allocated to receive Ade-tosylate 800 mg/day intravenously (51 patients) or placebo (36 patients) for 2 weeks. A significant treatment-to-time interaction was found for serum total and conjugated bilirubin, alkaline phosphatase, γ-GT, and aminotransferases (AFT, AST). Therefore, Ade-tosylate treatment induced a greater improvement of liver biochemistry than placebo. At baseline, 16 patients receiving Ade-tosylate and 7 patients treated with placebo complained of pruritus. After therapy the symptom was totally relieved in all the patients receiving Ade-tosylate and in none in the placebo group ($p<0.001$).

In a placebo-controlled trial, 28 patients with acute viral hepatitis due to HAV, HBV, or HCV were enrolled and randomized to receive Ade-tosylate (500 mg/day i.v.) or placebo for 10 days (Botero & Del Gado 1991). All the patients had evidence of acute hepatitis lasting less than 15 days and were followed at least 10 days, 20 days, 1 month and 3 months intervals. After treatment, a significant ($p<0.01$) decrease in aminotransferases plasma values was observed in the group treated with Ade-

tosylate as compared with placebo. The assessment of aminotransferases levels during the follow-up, showed that patients who received Ade-tosylate therapy experienced a more rapid improvement of these parameters as compared with the placebo group. Furthermore, Ade-tosylate treatment was well tolerated with no adverse effects.

Forty seven hospitalized patients with acute type B hepatitis were enrolled in a double-blind, randomized, placebo-controlled study (Di Nola - Data on Knoll Farmaceutici file). The patients were consecutively randomized to receive Ade-tosylate 1200 mg/day orally (24 patients) or placebo (23 patients) for 30 days and were followed at 10, 20 and 30 days after starting the treatment. The two study groups were well matched with the exception of higher basal levels of serum aminotransferases and bilirubin in the group receiving Ade-tosylate.

A significant treatment-to-time interaction for aminotransferases as well as for serum total and conjugated bilirubin ($p<0.05$) was observed in the patients treated with Ade-tosylate. The results were confirmed by covariance analysis to correct for systematic bias such as difference between groups at baseline. Furthermore, a significant difference between groups was observed for aminotransferases from day 10 but not for serum total and conjugated bilirubin.

Recovery, meant as normalization or reduction to a value below the double of the upper normal level for transaminases or <50% for bilirubin, occurred significantly earlier in patients receiving Ade-tosylate than in the placebo group, i.e. 21 days vs 30 days for aminotransferases and 8 days vs 13.5 days for bilirubin, respectively ($p<0.05$).

Concomitantly, prothrombin activity increased to a greater extent after Ade-tosylate therapy than placebo. Furthermore, 33% of the patients treated with Ade-tosylate became HBsAg negative during treatment and 28% developed HBsAb as compared to 18% and 14% in the placebo group. Although this difference was not statistically significant, the title of serum HBsAg was significantly decreased in the Ade-tosylate group ($p<0.01$).

Finally, the treatments proved to be safe.

6.3.5.6 Fatty liver degeneration

Patients with chronic active hepatitis treated with steroids often develop fatty liver degeneration. Twenty patients receiving prednisone (20 mg/day) with or without azathioprine (100 mg/day) received 200 mg/day Ade-tosylate intravenously for 20 days, then 100 mg 3 times weekly intramuscularly for 9 months (Piccinino et al. 1982). At the time of the first observation, 3 patients showed fatty degeneration and 17 no fatty changes at liver histology. Liver biopsies performed at the end of treatment showed the absence of fatty degeneration in 15 patients, steatosis developed in 2 patients, and a reduction of pre-existing fatty degeneration was observed in the remaining 3. In a control group of 40 patients receiving prednisone with and without azathioprine (at the same doses as the study group), steatosis was more severe in 3 out of 7 patients showing fatty liver degeneration at baseline and develops in 9 out of the remaining 33 who had no fatty changes at baseline.

In a recent open uncontrolled trial, 30 patients with hepatic fatty degeneration due to alcoholism or hyperlipidaemia were treated with i.m. Ade-tosylate for 2 months (Caballeria & Moreno 1990). After one month of dietary therapy to achieve a steady baseline status, the patients received Ade-tosylate by i.m. route at the dosage of 100 mg/day for one month and 100 mg every other day during the second month.

At the end of treatment, a significant ($p<0.001$) decrease in hepatic fatty deposition assessed by echography and graded according to a severity score was recorded. Furthermore, γ-GT serum levels fell significantly ($p<0.01$) after Ade-tosylate therapy and in the subgroups of alcoholics, who showed increased levels of aminotransferases at baseline, a significant ($p<0.05$) reduction of these enzymes was also found.

Ade-tosylate administered orally (600 mg/day) for 1 month was also proven to be effective in significantly reducing hepatic fatty degeneration histologically assessed in patients with cirrhosis or chronic hepatitis mostly due to alcohol abuse as compared with placebo (Micali et al. 1983). Histological features with regard to non-steatosis alterations, i.e. cloudy swelling, necrobiotic foci, and fibrotic component, were also improved after Ade-tosylate therapy whereas no changes were observed in patients receiving placebo.
Furthermore, oral Ade-tosylate significantly reduced AST, ALT and γ-GT serum levels and improved bromosulphthalein retention as compared with placebo.

6.3.5.7 Congenital disorders of bilirubin and porphyrin metabolism

Gilbert's syndrome, a familial defect of bilirubin metabolism, may lead to symptoms of fatigue, anorexia, general malaise and anxiety often associated with jaundice. The possible use of ademetionine to reduce associated hyperbilirubinaemia has been addressed in 2 preliminary studies. Bombardieri et al. (1985) treated 14 patients with a 10-day course of Ade-tosylate (200 mg daily intravenously) and found a significant decrease ($p<0.002$) in levels of unconjugated and total bilirubin and a concomitant increase in urinary excretion of D-glucaric acid, an indicator of liver microsomal enzyme activity. Bilirubin levels in 9 of the patients fell further after additional treatment with oral Ade-tosylate (1200 mg daily) for 10 days following a 3-month washout period.

Gentile et al. (1988) treated 10 patients with a daily intravenous infusion of Ade-tosylate 800 mg or 200 mg or placebo for 10 days, each with a week's washout between the various courses of treatment. Unconjugated bilirubin values after i.v. nicotinic acid load were significantly lowered only by the 800 mg dose of Ade-tosylate. The delayed elimination of nicotinic acid, observed in Gilbert's syndrome, also tended to be normalized. This study has been discussed in section 6.3.5.1.2.

The mechanism of action of ademetionine in producing the noted improvements could be through its contribution to phospholipid synthesis and liver cell membrane turnover (Bombardieri et al. 1985) or an action at the level of hepatocyte uptake where bilirubin, nicotinic acid and rifamycin-SV share a common membrane carrier protein (Gentile et al. 1984).

Ade-tosylate has also been used in the treatment of 2 cases of infantile porphyria cutanea tarda characterized by skin lesions associated with porphyrin overproduction and excretion (Batlle et al. 1987). The disorder is due to either a hereditary or an acquired deficiency in hepatic uroporphyrinogen decarboxylase (URO-D), and treatment is aimed at restoring hepatic levels of iron and porphyrin to normal. Ade-tosylate administration (200 mg/day orally) in combination with chloroquine resulted in complete clinical and biochemical remission. In these cases, enhanced glutathione availability may have prevented inhibition of URO-D by iron but the exact mechanism of action is not known. Adult patients treated with oral Ade-tosylate alone for 3 weeks (15 to 45 mg/kg/day), followed by a second course after 2 months, experienced improvement in the clinical or biochemical parameters (Batlle et al. 1987).

6.3.5.8 Case reports

Benign recurrent intrahepatic cholestasis (BRIC) is a rare, inherited disorder of unknown aetiology characterized by relapsing episodes of jaundice and pruritus. The prolonged cholestasis is often accompanied by fat malabsorption and weight loss.

In a recent case report (Everson et al. 1989) intravenous Ade-tosylate (800 mg/day) was reported to be ineffective in the short-term (9 days) treatment of 4 cases of BRIC, and possibly hepatotoxic due to an elevation in serum aminotransferases. However, the same group recently reported that one of the 2 patients studied had progressed to cirrhosis 6 years later, which suggests that his condition was not BRIC (Everson & Krawitt 1991).

Opposite results were found in one case of BRIC on a long-term (11 weeks), varied regimen of oral (1600 mg/day) and intravenous (800 mg/day) Ade-tosylate therapy (Rafique et al. 1991, 1992a). After treatment, amelioration of pruritus as well as a parallel decrease in total bilirubin, alkaline phosphatase and erythrocyte membrane cholesterol/phospholipid molar ratio were found. Aminotransferases remained normal, suggesting the absence of hepatotoxic effect. The patient's previous episode of BRIC lasted 15 months, suggesting that the recover of the present, lasting only 3 months, might be due to ademetionine treatment rather than to spontaneous reversion.

Intrahepatic cholestasis is a common complication **of total parenteral nutrition (TPN)**, occurring in 30 to 60% of treated patients.
A patient suffering from ulcerative cholitis in active phase and treated with TPN for 29 days who benefited from intravenous Ade-tosylate therapy (800 mg/day), has recently been reported (Caballero Plasencia et al. 1991). Intrahepatic cholestasis developed on the thirteenth day of TPN. Intravenous Ade-tosylate was administered from then until to the end of the TPN therapy (16 days), which continued unmodified. By the end of the first week of Ade-tosylate treatment, a marked decrease in all abnormal biochemical parameters (i.e. total and conjugated bilirubin, alkaline phosphatase and γ-GT) was observed and complete normalisation was recorded at the end of TPN and Ade-tosylate therapy.

Several cases of **cholestasis induced by the C17 alkylated anabolic steroid danazol** have been reported.

Although liver function returned to normal after the substance withdrawal, this can take as long as 3 months even in the absence of jaundice. Treatment to accelerate normalization of liver function might therefore be of particular benefit in cases in which severe symptoms occur.

A 60-year old male, with a 2-years history of chronic autoimmune haemolytic anaemia treated with prednisolone, azathioprine and splenectomy, was started on danazol 200 mg three times daily because of his refractary anaemia.
Thirty-two days later, he complained of tiredness, nausea, left upper quadrant abdominal discomfort, pruritus, dark urine, pale stools and deep jaundice. Serum bilirubin rase up to 936 μmol/l (n.v. 0-35). Danazol was discontinued and he was referred to the hospital.
Histological examination 24 days after stopping danazol showed features of substance-induced cholestasis with mild cholangiolytic changes and focal perivenular cell loss.

Ade-tosylate by intravenous infusion was commenced at a dose of 800 mg twice daily of 10 days reducing to 800 mg daily for a further 10 days. This resulted in a prompt decrease of serum bilirubin from 476 to 137 μmol/l, and an improvement of renal function (serum creatinine form 227 μmol/l to within the normal value of 84 μmol/l). The patients was discharged home on oral Ade-tosylate (2400 mg/daily) for a further 6 weeks. At this time, his serum bilirubin had returned to that observed prior to danazol (44 μmol/l) and he was symptomatic well (Bray et al. 1993).

Non specific chronic hepatitis following graft versus host disease (GVHD) after allogenic bone marrow transplantation (BMTx) can progress to **Vanishing Bile Duct Syndrome (VBDS) and secondary biliary cirrhosis.** Steroids and/or UDCA are administered in the early stages of disease, but they may have no effect once cirrhosis is established.

A 17-year old boy with chronic myeloid leukemia underwent to BMTx from an HLA identical mixed lymphocytes culture non reactive brother. Despite methotrexate (MTX) and methylprednisolone treatment, 60 days after BMTx he developed an acute GVHD with cholestatic features. MTX was replaced with Azathioprine, but 29 months after BMTx liver biopsy showed chronic active hepatitis and initial VBDS. Stage III Hodgkin's disease was diagnosed 3.5 years later and polychemotherapy

was instituted with complete remission. After 5.5 years a cholestatic syndrome developed with severe pruritus and serum total bilirubin (STB = 446 µmol/l), serum total bile acids (STBA = 150µmol/L) and ALP = 336 U/l. The patient was treated with i.v. Ade-SD4 800 mg/day. After 15 days a marked reduction of STB = 213 µmol/l, STBA = 45µmol/l, a decrease of ALP = 292 U/l and a complete remission of pruritus were observed. Discontinuation of Ade-SD4 was followed by reappearance of pruritus and increase of serum markers of cholestasis. A resumption of i.v. Ade-SD4 led again to an improvement and its tolerability was good so that a maintenance i.v. treatment was established (Iemmolo et al. 1993).

6.3.6 Tolerability

Adverse effects noted with Ade-tosylate therapy in all clinical trials to date have generally been mild and transient with no serious adverse reactions observed. Furthermore, during all these trials, there were no dropouts due to side effects.

These findings are mainly supported by the evaluation of the two large multicenter studies.

In the first (Manzillo et al. 1992), 180 patients were treated by intravenous treatment with Ade-tosylate and 163 with placebo and were evaluated for tolerability. No statistically significant difference between the two groups in terms of tolerability was reported, as illustrated in Table X.

Table X Profile of Ade-tosylate tolerability (800 mg/day i.v.). Study with double blind assessment versus placebo.

SIDE EFFECTS	ADE-TOSYLATE (NO. 180)	PLACEBO (NO. 163)
Transient insomnia	5	2
Nausea	3	4
Sweating	1	0
Superficial phlebitis	7	3
Rash	0	1
Total	16 (8.8%)	10 (6.1%)

There were no dropouts for side effects, whereas 17 patients (2 in the Ade-tosylate group and 15 in the placebo group) withdrew from the study due to the inefficacy of the treatment and in 12 patients on Ade-tosylate and 10 on placebo for reduced compliance.

Regarding the oral form, tolerability was more or less the same as that reported in the previous study (Table XI). The overall incidence of adverse effects did not differ from that reported in the placebo group. No dropouts due to side effects were recorded, whereas 2 patients in the Ade-tosylate group and 9 controls refused to continue the treatment because of inefficacy (Frezza et al. 1990b).

Table XI Profile of Ade-tosylate tolerability (1600 mg/day orally). Study with double blind assessment versus placebo.

SIDE EFFECTS	ADE-TOSYLATE (NO. 108)	PLACEBO (NO. 101)
Transient insomnia	3	0
Nausea	7	6
Headache	1	1
Heartburn	2	1
Diarrhoea	2	3
Total	15 (13.8%)	11 (10.8%)

Furthermore, it should be underlined that high doses of Ade-tosylate (3 g/day) intravenously infused over 24 hours for 1 week in patients with coma due to renal failure or acute drug intoxication were well tolerated and did not produce any untoward effects (Capogrossi et al. 1983).

Finally, although in a different indication, the results of extensive clinical trials, which have enrolled about 22,000 patients, further support the safety of ademetionine therapy (Di Padova 1987).

As far as Ade-SD4 treatment is concerned, it has been found to be well tolerated and safe both when administered intramuscularly and intravenously (Mascio et al. 1991).

6.3.7 Aspect related the safety of the substance

LONG-TERM TREATMENT

In a long-term treatment study with oral Ade-tosylate (1200 mg/day) administered for 6 months to cirrhotic patients, no side effects were reported (Vendemiale et al. 1989b).

EFFECT ON AMMONIA, METHIONINE AND MERCAPTANS METABOLISM

Abnormal metabolism or increased concentrations of ammonia, methionine and mercaptans (i.e. methanethiol), have been suggested to be involved in the biochemical mechanism of hepatic encephalopathy (Cooper 1983; Phear et al.

1956; Zieve 1981).
Most of the ammonia is produced in the colon and comes from bacterial degradation of urea and other nitrogenous substances like intraluminal amino acids.
Ademetionine molecule has two amino groups which are not substrates of the enzymes catalyzing the formation of ammonia (Schlenk & Zydek 1968). Therefore, the nitrogenous moiety of ademetionine does not enter the metabolic pathways producing ammonia.

Experimental data show that intravenous Ade-tosylate improves cerebral ammonia detoxication process in dogs with hyperammonemia (Benzi et al. 1977). In addition, both parenteral and oral Ade-tosylate therapy does not affect serum ammonia levels in cirrhotic patients (De Caprio et al. 1980; Micali et al. 1983).

Methionine can be degraded in the liver via two pathways: transsulfuration and transamination. Only the latter pathway includes methanethiol but not ademetionine as intermediate (Benevenga 1984).

In healthy subjects, the transamination pathway is not of quantitative significance. However, in patients with ademetionine-synthetase deficiency, this pathway might slightly contribute to methionine degradation (Gahl et al. 1988).

The administration of ademetionine to cirrhotic patients overcomes the metabolic block due to a reduced activity of ademetionine-synthetase as shown by an increase in plasma cystine and taurine (Marchesini et al. 1992b) as well as in hepatic glutathione content (Vendemiale et al. 1989a). This effect which seems mainly to be referable to the restoration of ademetionine-synthetase activity induced by exogenous ademetionine (Corrales et al. 1992a), in turn improves methionine tolerance as shown in cirrhotic patients challenged with methionine (Corrales et al. 1991b, 1992b). Accordingly, after exogenous ademetionine, the utilization of dietary methionine improves and proceeds in the direction of the transsulfuration rather than the transamination pathway.
Furthermore, ademetionine is able to activate the enzyme cystathionine-synthetase (Finkelstein et al. 1975) and to inactivate betaine-homocysteine methyltransferase (Finkelstein & Martin 1984), promoting the utilization of homocysteine in the irreversible transsulfuration sequence and, therefore, limiting the resynthesis of methionine via the methylation of homocysteine.

These assumptions are further supported by the evidence that long-term oral ademetionine does not increase plasma methionine levels (Marchesini et al. 1992b).
Methanethiol might be also formed from methionine by the intestinal flora (Conn & Lieberthal 1979).

Both oral methionine (Adibi & Gray 1967) and oral ademetionine (Bombardieri et al. 1983, Knoll Farmaceutici file) are absorbed very fast by the small intestine. Although methionine clearance is strongly impaired in cirrhotic patients and its intestinal absorption is quite efficient, no increase in methionine plasma concentration was seen in patients treated with oral Ade-tosylate (1200 mg/day) for 30 days (Marchesini et al. 1992b). Furthermore, the intraintestinal application of ademetionine does not increase methionine levels in the mesenteric blood (Bombardieri et al. 1983). These findings would exclude a significant formation of methionine from ademetionine in the intestine during the preabsorption phase. As methionine is the precursor of mercaptans, it seems therefore unlikely that methanethiol can be produced in the intestine starting from ademetionine.

Furthermore, mercaptans are metabolized to less toxic metabolites through S-methylation by thiol S-methyltransferase, an enzyme mainly present in the microsomes of cecal and colonic mucosa and in the liver, using ademetionine as a substrate (Weisiger et al. 1980). Ademetionine might, therefore, be involved in the detoxication processes of mercaptans in the large intestine and in the liver.

MODIFICATIONS OF THE HAEMATOLOGIC AND OTHER PARAMETERS

In the studies including the trials with Ade-SD4 (Mascio et al. 1991, Persico et al. 1990b) no adverse effects of the drug were reported in terms of red blood cell count, blood and urinary parameters abnormalities. The only laboratory parameters which exhibited marked changes were the biochemical markers of cholestasis and liver cell damage.

CONCOMITANT DISEASES

Therapeutic doses of Ade-tosylate were administered to patients affected by various concomitant diseases. In patients with arterial hypertension no interference of Ade-tosylate with blood pressure control was observed (Data on Knoll Farmaceutici file). The administration of Ade-tosylate in patients with type II diabetes did not modify the glycemic values (Data on Knoll Farmaceutici file).

However, in a clinical trial carried out in patients with liver disease, a decrease of fasting plasma glucose levels was reported in two patients with type II diabetes after 2 weeks of Ade-tosylate administration (600 mg/day i.v.) (Adachi et al. 1986). No adverse reactions were also reported in patients treated for heart failure (Data on Knoll Farmaceutici file) and rheumatic diseases (Data on Knoll Farmaceutici file).

USE DURING PREGNANCY

The use of high doses of Ade-tosylate in women developing intrahepatic cholestasis in the last three months of pregnancy did not lead to any side effect either in mother or fetus or difference in the Apgar score as compared to those treated with placebo, whereas beneficial effects were noted on laboratory markers of intrahepatic cholestasis (Bonfirraro et al. 1990; Catalino et al. 1992; Frezza et al. 1984, 1990a; Lafuenti 1988).

TREATMENT WITHDRAWAL

Treatment withdrawal does not induce rebound effects. However, it has been shown that the suspension of Ademetionine treatment may negate previously achieved therapeutic actions (Frezza et al. 1984, 1990b; Manzillo et al. 1992).

CONTRAINDICATIONS

No form of hypersensitivity towards the product has been reported.

As there are no experiences in the first two trimesters of pregnancy, it is recommended not to prescribe the substance to pregnant women in this period.

7. HANDLING AND DISPENSING

7.1 Stability

Vials can be kept at room temperature (≤25°C).
The solution of Ade-SD4 after reconstitution with the solvent is stable for 6 hours at room temperature.

Ade-SD4 tablets, stored in aluminum strips, are stable for 2 years, according to accelerated stability test date.
The tablets can be kept at room temperature (≤25°C).

We recommend opening the aluminum strip only immediately before use.

7.2 Recommendation for clinical use

7.2.1 Dosage instructions

On the basis of the available clinical data the recommended doses are the following:

- 800 mg/day intravenously and intramuscularly. The lyophilized compound has to be reconstituted in its appropriate solvent before intramuscular injections. When the substance is administered intravenously, after reconstitution of the lyophilized compound in its appropriate solvent, the total dosage has to be further diluted in 250 cc 5% dextrose or saline solution. The solution should be prepared fresh daily and administered immediately.
- Oral administration: daily 800 mg bid.

Additional dose finding studies should better define the therapeutic window.

7.3 Spills and waste disposal

Pick up by mechanical means and rinse with water.
Contact with skin: rinse with plenty of water

Contact with eyes:	rinse with plenty of water and seek medical advice against acid solution
Water solution disposal:	according to domestic law on pharmaceutical products disposal

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