MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: APR 11 1997

FROM: Abraham Karkowsky, M.D., Ph.D. Group Leader HFD-110 Division of Cardio-Renal Drug Products /S/ 4/8/97

SUBJECT: Labeling of Dolasetron (ANZENET) NDA 20-624

TO: Dr. S. Fredd, Director, Division of Gastro-intestinal and Clotting Drugs; HFD-180

THROUGH: Dr. R. Lipicky, Director,Division of Cardio-Renal Drug Products, HFD-110 /S/

This memo is in response to your consult request of 3/18/97.

I 've included under the WARNING section a statement that subjects with underlying cardiac disease (with the specific conditions enumerated) were excluded from clinical protocols. Since DM and DMA are likely sodium channel blockers, with the cardiac adverse event profile potentially different in a patient population with structural heart disease[1]. I think some statement in labeling is appropriate.

I agree with your penciled in comment that the ECG changes should be included under WARNINGS. It seems also appropriate from the flow of the thought processes to link the adverse events presently listed under WARNINGS with the cardiovascular changes listed under PRECAUTIONS. The underlined words are my additions the ~~strikeout words are edited out~~.

WARNINGS:

ANZEMET can cause ECG changes (PR and QTc prolongations, and QRS widening) in healthy volunteers and patients. Patients, however, with underlying cardiac disease such as.(AF, CHF, previous MI???...) were excluded from clinical studies. In patients receiving chemotherapy or undergoing surgery, JT prolongations have also been observed following ANZEMET

[1]Flecainide and Encainide have bad track records particularly in subjects with structural heart disease.

[2]Is this true???

ECG interval changes are related in magnitude and frequency to blood levels of the active metabolite, hydrodolesteron. These changes generally mirror blood levels. Some patients, however, have interval prolongations for 24 hours or longer. Interval prolongations could lead to cardiovascular consequences, including heart block or cardiac arrhythmias. These have been rarely reported in patients receiving ANZEMET.

[4]Severe bradycardia with a brief cardiac pause was observed intra-operatively in a 61 year-old woman who received 200 mg ANZEMET (oral tablet) for the prevention of postoperative nausea and vomiting (PONV). This patient was also taking verapamil. Three PONV patients who received placebo also experienced severe bradycardia with a brief cardiac pause. A 66 year-old man receiving chemotherapy was found dead six hours after receiving 1.8 mg/kg (119 mg) intravenous ANZEMET injections and concomitant anthracycline therapy[5]. Vital signs taken at 1.0 and 4.5 hours after ANZEMET Injection indicated and adequate blood pressure and increased heart rate. This patient had other potential risk factors including substantial exposure to doxorubicin and concomitant cyclophosphamide. There have been no reports of severe bradycardia, heart block or bundle branch block that required a temporary or permanent pacemaker in patients receiving ANZEMET in clinical studies.

Under clinical Pharmacology:
1) I would consider summarizing the animal data. The animal data are suggestive of a effect on depolarization and repolarization.

2nd paragraph 6th line:
2) The magnitude and frequency of the ECG changes increased with dose (related to peak plasma concentrations of hydrodolesteron)[6]

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[3]I don't know if this is true, and if true, at what doses is it true for? And if true and at relevant doses what use is it for the prescriber since normals will not be getting the drug?

[4] I presume these are accurate descriptions of the events, I have not reviewed these events.

[5]Did the sponsor ever do a retrospective analysis of the toxicity with subjects who had high exposures to anthracyclines.

[6] The parent drug is rapidly metabolized to DMA, perhaps by red blood cells and consequently, the effect of the parent drug is only of conjecture. In vitro studies suggest that the parent drug is cardiovascularly active. I would, therefore, be mute on the effect of parent drug on Qtc.

Johnson

MEMORANDUM (Consult Review)

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: JAN 15 1997

FROM: Abraham Karkowsky, M.D., Ph.D. Group Leader HFD-110 Division of Cardio-Renal Drug Products /S/ 1/15/97

SUBJECT: Safety of ANZEMET ® (Dolasetron Mesylate) Tablets ; NDA 20,623; Hoechst Marion Roussel /S/ 1/16/97

TO: Dr. Paula Botstein, Acting Director, Office of Drug Evaluation III
AND: Dr. Stephen Fredd, Director, Division of Gastro-intestinal and Coagulation Drug Products.

Summary: Dolasetron Mesylate[1] [DM], at a dose of 200 mg given once, appears to be active in the prevention of cancer chemotherapy induced nausea and vomiting . There is, however, evidence that DM and/or its major metabolite, MDL 74,156 [DMA], blocks sodium channels as judged by a decrease in the rate of rise of depolarization in guinea pig and dog ex-vivo preparations, as well as by the prolongation of QRS and PR intervals on 12-lead ECGs in normal volunteers and patients. There is some evidence (though not overwhelming), that potassium channels might also be blocked by DM or DMA. The potassium blocking effect, reflected by an increase in QTc and also an increase in JTc, is most prominent at the dose-range planned for preventing cancer chemotherapy induced nausea and vomiting.

More than 700 subjects/patients have been treated with the proposed or higher doses of DM, including normals, postoperative nausea and vomiting patients and cancer chemotherapy nausea and vomiting patients, with no apparent adverse events[2] that reflect alterations of cardiac conduction or proarrhythmias. Because of the potential for adverse cardiac events, additional safety information would be useful in defining an adequate safety margin.

Since the intended use of this drug is for episodic treatment, given as a single dose in proximity to cancer chemotherapy, the concentration of either Dolasetron Mesylate and a active metabolite(s), MDL 74,156, would depend on both the dose of drug and the efficiency by which DM is metabolized to DMA by the enzyme carbonyl reductase. In the to-be-treated population, DM is evanescent and is rapidly transformed to DMA. Peak electrocardiographic effects i.e. prolongation of QRS, PR, and QTc intervals are correlated with concentrations of DMA. DMA is eliminated both by renal routes and by several CYP450s so it is unlikely that interaction with drugs that also are CYP450 substrates would modify the peak concentrations of DMA and consequently, defining the toxicity of this drug.

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[1]Abbreviations used:
PONV-Post Operative Nausea and Vomiting
CCNV Chemotherapy induced Nausea and Vomiting
DM: Dolasetron Mesylate
DMA: Alcohol Metabolite of DM- Not necessarily a racemic mixture of the R-(+)-isomer and the L-(-)-isomers.

[2]One report of a RBBB occurred in a PONV patient 115 minutes and resolving within 24 hours after DM administration. The subject was also taking verapamil.

Carbonyl reductase activity is dependent on NADPH as a co-factor. NADPH is produced only through the pentose monophosphate shunt and NADPH levels may be compromised in subjects with G-6PD deficiency. Among those who are G-6PD deficient, DM concentrations may reach higher concentrations and persist for longer durations. There are other idiosyncratic factors that may modify both the concentration of DM and DMA. In order to ascertain the electrophysiologic responses in such kinetically marginal individuals, it is necessary to study higher doses of DM in normal subjects. These subjects would generated sufficiently high concentrations so that adequate electrophysiologic measurements can be made at the higher DM and DMA concentrations.

Not only are individuals different in their kinetic handling of DM and DMA, there are also those who at appropriate concentrations of drug and metabolites, have excessive changes in their electrophysiologic parameters. Classically, subjects with hypokalemia, underlying cardiac disease, bradycardia are more sensitive to drug induced prolongation of electrocardiographic intervals. Most such patients were excluded from the clinical study data base. Cardiac damage occurs in patients who have been treated with anthracycline chemotherapy, particularly at high cumulative doses. The ECG effects of such patients would be helpful in defining the extremes in sensitivity to ECG changes at normal doses of DM and normal concentrations of DM and DMA.

I am recommending the following studies to better ascertain the safety profile of DM.

1. An analysis of the data base for those who are G-6PD deficient or alternatively should be a small study looking at the PK and PD of DM /DMA in subjects who are G-6PD deficient.

2. A study of higher single doses of DM in normals. The dose to be studied should be as high as tolerated and should be performed with adequate monitoring with trained personnel available on site, to treat any adverse events.

3. The ECGs of all patients with large cumulative exposures to either daunorubicin or doxorubicin should be analyzed for ECG changes. In the absence of a respectable data base a small study should be considered in those who are receiving high cumulative doses.

4. I presume that present labeling would exclude use of this drug in subjects with underlying cardiac disease concurrent antiarrhythmic therapy or ECG abnormalities.

This memo is in response to your consultation request. I received and reviewed the two binders which contained both Drs. Botstein's and Fredd's memos as well as Dr. Gallo-Torres's medical and Dr. Pradhan's biopharmaceutical reviews. I also received a copy of Dr Ahmad's pharmacology review. Also included in the binders were the analyses of the cardiovascular safety of DM authored by Dr. Craig Pratt's, Baylor School of Medicine and Dr. Claude Benedict, The University of Texas Houston Medical School, both are consultants to Hoechst Marion Roussel.

Drs. Fredd's and Botstein's memos agree that there is adequate efficacy and safety data for the approval of oral DM for the treatment of post-operative nausea and vomiting [PONV] either at a dose of 50 or 100 mg, given within 2 hours of surgery. Efficacy was derived from two placebo-controlled dose ranging (25-200 mg single dose) studies, and safety assured by a data base that contained over 2600 subjects who received 50 mg or higher doses of DM orally or intravenously. There were an additional 1724 subjects who received $\geq$ 1 mg/kg and 1332 who received $\geq$ 1.8 mg/kg doses intravenously (a 50 Kg subject receives a dose of 50 mg at 1 mg/Kg and 90 mg at 1.8 mg/Kg), respectively. Safety at the 50 mg dose was supported, therefore, by experience in > 4300 volunteers/patients. Safety of 100 mg dose by approximately 2900 subjects (see Table 1).

Table 1. Exposure to DM during Clinical Studies (derived from Pages 78-81 of Dr. Pratt's review).

Route of Administration	PBO	25 mg	50 mg	100 mg	150 mg	200 mg	> 250 mg
Healthy Volunteers Single Oral Dose	30	2	14	65	39	242	36
CCNV Single Oral Dose	---	235	243	227	---	238	---
PONV Single Oral Dose	231	235	240	228	---	233	---
PONV IV Single Dose	547	548	551	267	---	---	---
Total	808	1020	1048	787	39	713	36

Table 1B. Intravenous Data (derived from Pages 78-81 of Dr. Pratt's review).

Route of Administration	PBO	≤0.6	1.2	1.8	≥2.4
Healthy Volunteers Single IV Dose	74	160	95	53	145
CCNV Single Dose IV	----	284	251	695	485
Total	74	444	346	748	630

As I understand it, the issue of concern is for the approval of DM as an anti-emetic agent for the prevention of CCNV at a dose of 200 mg. There is little doubt that DM is active in this regard. Three studies, [MCPR0043 (#43), MCPR0048 (#48) an 73147-2-S-087 (#87)] all dose-ranging studies (25-200 mg single dose), with the last study also containing an additional positive control limb (ondansetron at the approved dose of 8 mg administered twice[3]) showed a positive dose-response relationship of DM in preventing CCNV. In study #87, the 200 mg dose of DM was statistically superior to its 100 mg dose and numerically superior to but not statistically different than the positive control ondansetron. In all studies, a single dose of medication was given approximately 1.5 hours before chemotherapy.

Since in all studies DM was administered as a single dose (no studies were done with split doses), titration to benefit not possible. Consequently, the most effective and, therefore, the 200 mg dose seems appropriate for this indication. DM differs from other approved regimens in that it is to be administered only once, before chemotherapy.

There is, however, evidence that DM at the 200 mg dose has modest effects on the cardiovascular system as judged by its effects on ECGs. Listed below is the composite data for the five studies including those of PONV and CCNV with the QT, QTc, PR , QRS and JT_C intervals. ECGs points were measured approximately 1-2 hours[4] after the dose and represent near maximal QTc responses. A positive trend test is indicated by ***bold and italicized*** values. There trend is to modest increases in QTc, PR and QRs intervals associated with increasing doses of DM.

[3]The PDR suggests additional doses on days 2 and 3 post -chemotherapy . These doses were not administered during this study.

[4]Dr. Pratt has made a strong case that maximal effect on QTc prolongation is greatest at 1-2 hours post dose and corresponds to the concentrations of DMA, with these increases disappearing as the concentration of DMA wanes. Dr. Pradhan's review also notes a quantitative relationship between DMA concentrations and QTc. Unfortunately, it is unclear if the effect of QTc is related to an individual optical isomer of the DMA.

Table 2. Change in QT (msec) at approximately 1-2 hours Post Dose Includes Studies of the Prevention of Post-Operative Nausea and Vomiting and Cancer Chemotherapy Nausea and Vomiting:

Study #	Placebo	25 mg	50 mg	100 mg	200 mg
# 43	_____	15.6	12.0	9.3	22.2
# 48	_____	11.6	11.1	14.4	17.2
# 87*	_____	_____	_____	_____	_____
# 95	No	Effect	At	Any	Time
# 292	-0.3	2.3	-2.7	2.0	3.0

* Study had no QT measurements except at 24 hours

Table 3. Change in QTc (msec$^{1/2}$)at approximately 1-2 hours Post Dose Includes Studies of the Prevention of Post-Operative Nausea and Vomiting and Cancer Chemotherapy Nausea and Vomiting:

Study #	Placebo	25 mg	50 mg	100 mg	200 mg
# 43	_____	8.6	4.8	5.9	23
# 48	_____	7.3	7.2	11.5	15.3
# 87*	_____	_____	_____	_____	_____
# 95	No	Effect	At	Any	Time
# 292	3.4	5.0	7.7	7.2	10.7

* Study had no QTc measurements except at 24 hours

Table 4. Change in QRS (msec) at approximately 1-2 hours Post Dose Includes Studies of the Prevention of Post-Operative Nausea and Vomiting and Cancer Chemotherapy Nausea and Vomiting:

Study #	Placebo	25 mg	50 mg	100 mg	200 mg
# 43	_____	2.2	3.1	3.3	5.9
# 48	_____	2.1	2.7	3.3	5.5
# 87*	_____	_____	_____	_____	_____
# 95	No	Effect	At	Any	Time
# 292	-0.3	1.2	0	1.3	3.4

* Study had no QRS measurements except at 24 hours

Table 5. Change in PR (msec) at approximately 1-2 hours Post Dose Includes Studies of the Prevention of Post-Operative Nausea and Vomiting and Cancer Chemotherapy Nausea and Vomiting:

Study #	Placebo	25 mg	50 mg	100 mg	200 mg
# 43	_____	6.3	5.7	10.8	13.2
# 48	_____	5.4	6.2	6.7	14.6
# 87*	_____	_____	_____	_____	_____
# 95	No	Effect	At	Any	Time
# 292	0.7	4.2	2.3	4.0	7.5

* Study had no PR measurements except at 24 hours

Table 6. Change in JTc [5](msec$^{-1/2}$) at approximately 1-2 hours Post Dose Includes Studies of the Prevention of Post-Operative Nausea and Vomiting and Cancer Chemotherapy Nausea and Vomiting [6]:

Study #	Placebo	25 mg	50 mg	100 mg	200 mg
# 43	_____	8.7	3.1	3.7	16.9
# 48	_____	6.4	8.6	8.7	9.6
# 87*	_____	_____	_____	_____	_____
# 95	No	Data	Available	At Any	Time
# 292	2.8	3.1	5.4	4.7	5.4

* Study had no JT measurements except at 24 hours

Both PR-interval and QRS-intervals are unquestionably increased with DM; QTc-intervals are also increased. The increase in QTc, as stated by the sponsor's consultants, is largely a result of the prolongation of the QRS complex. JT- interval changes, that is the QT-interval minus the QRS-interval, did not show a dose-effect in any of the studies. However, heart-rate correcting the JT interval i.e. JTc trends to higher values as the dose of dolasetron approaches 200 mg[3] .

Based on the above effects on ECG (i.e. prolongation of the QRS and PR intervals), DM and/or one or several of its metabolite(s) likely blocks sodium channels. In *ex-vivo* or *in vitro* animal preparations, cardiac conduction are altered at concentrations of DM or DMA at 1-10 uM. These concentrations are not that dissimilar from the C_{max} observed in individuals treated with a dose of 200 mg[7].

I've tabulated below the electrophysiologic effects of DM and/or its metabolites in intact animals, *ex vivo* and *in vitro* as summarized in Dr. Ahmad's review. The 5' and 6'-hydroxy derivatives of DM had no significant electrophysiologic effects on guinea pig papillary muscle.

Table 5. Pre-Clinical Studies on the Electrophysiologic effects of Dolasetron Mesylate (MDL 73,147EF), its major metabolite and some additional metabolites.

Study System	MDL 73,147 EF parent Drug	MDL 74,156 Alcohol Metabolite Racemate?	MDL 73,405 (+)-enantiomer of MDL 74,156	MDL 73,349 (-)-enantiomer of MDL 74,156	MDL 73,902 (epimer of MDL73,147 ?)
Perfused Guinea Pig Heart	at 1 uM, increased absolute refractory period by 29%	at 1 uM, increased absolute refractory period by 17%			at 1 uM, increased absolute refractory period by 45%

APPEARS THIS WAY
ON ORIGINAL

[5]In order to rate correct the JT, I multiplied the JT interval by QTc /QT. This multiplication is equivalent to dividing the JT by (R-R)1/2

[6]Since I did not have the raw data I could not calculate the trend statistics for JTc.

[7]C_{max} for a 200 mg dose of Dolasetron Mesylate are generally around 600 ng/ml. Given the MW of 438 , peak concentrations are approximately 1.5 uM. Free concentrations are somewhat lower because of protein binding.

Action Potential Guinea Pig Papillary Muscle	at 10 uM, APD not changed. Vmax- dV/dt decreased 33-42%		at 1 uM, Vmax (dV/dt) was inhibited to 18%. Class Ic anti-arrhythmic activity. No statement re APD.	at 1 uM , Vmax (dV/dt) was inhibited to 50%. Class Ic anti-arrhythmic activity. No statement re APD.	
Dog Purkinje Fibers	Same as with guinea pig papillary muscles				
Cloned and expressed in Xenopus Oocytes alpha-subunit human heart sodium channel.	Kd 50 values of 1.1 mM		Kd Value of 0.567 mM	Kd Value of 1.2 mM	

Neither DM or DMA binds strongly to the cloned and expressed alpha-subunit of the sodium channel of the human heart. Although this result appears inconsistent with the effect of dolasetron mesylate or its metabolites on ECGs (i.e. prolonging the QRS- and PR-intervals), the interaction of Dm or DMA may be with another subunit of the sodium channel (i.e. the $beta_1$ subunit). Alternatively, it is also possible that the cloned subunit structure differs from the native structure by post-transcriptional processes such as glycosylation.

At higher doses, it is possible that ion channels other than sodium channels may be altered by DM. In dogs, cumulative doses of 30 mg/kg produced substantial increases in QTc, likely to reflect interaction with a potassium channel(s).

Since the ability of DM and/or its major metabolite (s) inhibit sodium channels and possibly also inhibit other ion channels at the dose proposed for the use of this drug in CCNV, and since drugs that inhibit sodium[8] of potassium [9]predispose to proarrhythmic cardiac events, the pivotal issue is what data is adequate to assure the safety of this drug at the proposed CCNV dose.

Over 700 subjects/patients were exposed to single doses of 200 mg or greater (Table 1) for either PONV or CCNV (Table 1). Dr. Pratt's review concluded that no conduction or proarrhythmic events could be attributed to DM[2].

Both Drs. Fredd's and Botstein's memos express discomfort with the adequacy of the safety data base for the use of the proposed dose of DM for CCNV. There are several ways of augmenting the safety data base. One strategy would be to markedly increase the size of the data base. Absent any events in the larger data base, the drug would be considered safe. If any event occurs,however, the interpretation of this event becomes pivotal but subjective. With the occurrence of single events, the attribution would as likely be ascribed to concurrent therapies or the play of chance (by the sponsor) , as to the index drug (by the

[8]Sodium channel blockers, particularly those that are classified as Ic, such as encainide and flecainide, were associated with an excess of death in patients who were status post myocardial infarction who had concurrent ventricular rhythm alterations (mostly PVCs).

[9] Many drugs that inhibit inward potassium rectifier currents predispose to a form of malignant arrhythmia termed torsades de pointes.

FDA). A placebo or control group would be helpful in defining a set of events as drug related, but would not be helpful in the interpretation of single or infrequent events. Only when there are several related events, which are rare in the general population, would their be general agreement that the event was drug related.

A second strategy would be to explore and expand the edges of the safety margin of this drug. The nature and type of such study or studies would depend on the specifics of the drug. The underlying assumption is that there are populations that handle the drug differently (kinetically) and those that respond to the drug differently (dynamically). Furthermore, this population would be infrequently represented in the normal population. The safety margin study would either enrich for these subjects or generate drug concentrations anticipated for these subjects. In general, these safety margin studies would not be looking for adverse clinical outcomes, but for some surrogate measurement for these adverse outcomes. For DM the surrogate would be ECG changes i.e. PR, QRS, QTc and JTc intervals.

DM does not have a chiral center. Upon either intravenous or oral administration, DM is rapidly reduced to the corresponding alcohol (MDL 74,156) with a $t_{1/2}$ of approximately 10 minutes. The bioavailability of DM is low. The bioavailability of DM, as DMA, however, is approximately 74%. The enzyme responsible for the metabolism of DM to DMA is carbonyl reductase. The reduction to DMA generates a chiral center with the R (+) isomer accounting for between 70-90% of the AUC, C_{max} and urinary excretion of the isomers. DMA is subsequently metabolized by one of several cytochrome P450 enzymes or it is renally excreted. Based on the *in vitro* or *ex vivo* studies, the R- (+)-and the L-(-)-isomers have different cardiovascular activity. The dV/dt of the action potential of guinea pig papillary muscle was inhibited at 1 uM concentrations by 18 and 50% by the R-(+)- and L-(-)-isomer, respectively.

Carbonyl reductase is cytosolic enzyme that belongs to the family aldo-ketoreductases. In a small number of subjects. the inter-individual variability of liver activity was slightly greater than 3[10]. The enzyme activity may be modified by a decrease in the necessary cofactor or by the presence of concurrent competing xenobiotics.

With respect to cofactors, NADPH is a necessary cofactor for carbonyl reductase activity. NADPH is produced solely through the hexose monophosphate shunt. Subjects who are G-6PD deficient[11] and, therefore, deficient in producing NADPH, may be functionally limited in carbonyl reductase activity. Patients who are G-6PD deficient may, consequently, be less able to clear DM, resulting in higher concentrations of the parent drug. Some data for another substrate has been published. Carbonyl reductase is responsible for the reduction of daunorubicin to its corresponding alcohol, daunorubicinol. Decreased metabolism of G-6PD subjects can be demonstrated in vitro. Erythrocytes harvested from G-6PD subjects demonstrated decrease ability to transform daunorubicin to its corresponding alcohol daunorubicinol[12]. Although no clinical correlates have yet been described between individuals who are G-6PD deficient and the metabolism of any drug, there is equally sparse data suggesting such an interaction is not clinically relevant.

DMA is subsequently cleared either by renal mechanisms (approximately 60% of the dose can be accounted for in the urine) and to a lesser extent by one of several cytochrome P 450s. Since the intended use

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[10] Iwata, N.; Inazu, N.; Hara, S.; Takeshi, Y.; Kano, S.; Endo, T.; Kuriwa, F.; Sato, Y.; Satoh, T.; " Inter-individual Variability of Carbonyl Reductase Levels in Human Livers". Biochem. Pharmacol. 45 1711-1714, 1993

[11] G-6PD is a single gene. There are, however, many different mutations and variants of G-6PD deficiency. Some variants have nearly normal activity of this gene. Others express < 10% activity.

[12] Amitai, Y.; Bhooma, T.; Frischer, H.; "Glucose-6-Phosphate Dehydrogenase Deficiency Severely Restricts the Biotransformation of Daunorubicin in Human Erythrocytes" J. Lab. Clin. Med. 127: 588-98, 1996. Figure 5 of this paper suggests that erythrocytes from subjects who are G-6PD deficient, of the mediterranean type, form daunorubicinol at a rate of approximately 10% of those of normals.

of this drug is as a single dose, clearance of DMA is less crucial in defining the safety of DM . Peak concentrations of DM and its active metabolite DMA would define the safety of administering DM. Drug clearance would reflect the duration of vulnerability. Should the dosing regimen change to multiple doses, with interdosing intervals approximating the terminal half-life (6-8 hours), then interactions with other competing drugs should be explored.

DMA is approximately 70% bound to plasma proteins so it is, therefore, unlikely that competing drugs would substantially alter its concentration. It is unclear if the proportion of R-(+)-and the L-(-)-isomers of DMA are produced in the same percentage in all subjects. It is also unclear if each isomer is equivalently active in modifying cardiac conduction in humans or differentially bound to plasma proteins.

In summary, there are several populations that might handle DM differently, namely, those who have lower inherent activities of carbonyl reductase , those who are G-6PD deficient and limited in their ability to generate NADPH, and those with competing concurrent drugs on board. There are probably those whose peak concentrations of DMA may be on the high side (or the more cardiac active optical isomer of DMA might be increased), due to the randomness of those factors that determine the volume of distribution of this metabolite, or those whose fraction of the individual optical isomers differ.

On the other hand, there might be substantial heterogeneity in the handling of DMA. Subjects such as those with renal insufficiency , those with deficiencies in the CYP 450 necessary for DMA's further transformation and those who are taking medications that interact with these CYP 450 would be expected to clear DMA at slower rates than normals . Unless multiple dose regimens, at relatively frequent intervals are also to be studied, any metabolic or excretory pathways that remove DMA are not likely to alter the safety margin of DM.

There are therefore two studies that would further increase our comfort that kinetic aberrations do not modify the safety of this drug. One study would define the kinetics nd corresponding dynamics in subjects who are G-6PD deficient[9].

The second study would be a higher dose single administration study . The purpose of this study would be several-fold. First, this study would explore the extremes of the concentration of DM and DMA. It would determine whether there are non-linear relationships of serum concentrations of DM or DMA to dose and also define the relationships to PR, QRS, QT, QTc, JT and JTc intervals at the extremes in concentrations. Higher serum concentrations of DMA would unequivocally answer the question as to whether depolarization i.e. JTc is prolonged with DMA.

The data base for DM includes some experience with doses higher than 200 mg (or approximately 3 mg/kg). In study # 73147-1-C-006 , there is some experience with single doses of 4, 4.5 and 5 mg/kg (n=4/dose). In study 73147-1-C-028 subjects apparently received doses as high as 200 mg BID[13] . If these doses were well tolerated, higher single doses of DM can and should be explored with respect to kinetics and dynamics.

Aside from those who may have kinetics different from the general population, there are those whose electrocardiographic response to the usual concentrations of DM and DMA may be excessive. Subjects with underlying cardiovascular disease, those with aberrations of electrolytes, those treated with concurrent drugs that modify cardiac conduction may have excessive ECG responses to DM. Unfortunately, all clinical studies excluded subjects with underlying cardiovascular disease, rhythm disturbances that required antiarrhythmic therapy, or those with abnormal ECG intervals at baseline. It is, therefore, unlikely that the already accumulated safety data base will adequately address whether there is a sub-population that is more sensitive to electrocardiographic alterations. I am presuming that

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13 See Table p. 11 of Dr. Pratt's review.

warnings or precautions, about the use of this drug in such a population would of course appear prominently in labeling.

It might, however, be of interest to analyze the ECGs of those who had previous exposure of high cumulative doses of doxorubicin (> 450-550 mg/M^2) or daunorubicin (> 1000 mg/M^2). These subjects would likely be treated with this drug for the prevention of CCNV. These subjects would also likely be most compromised in their cardiac status due to the cardiotoxicity of these anthracyclines.

In the absence of such data, it might be worthwhile to perform a small study in such subjects.

Glucose-6-phosphate dehydrogenase deficiency severely restricts the biotransformation of daunorubicin in human erythrocytes

YONA AMITAI,* THANIKACHALAM BHOOMA, and HENRI FRISCHER

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Recognition and analysis of distinct mechanisms by which primaquine and other hemolytic drugs activate the hexose monophosphate shunt (HMS) have suggested a hitherto unsuspected pharmacogenetic interaction between daunorubicin metabolism and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Because this deficiency is very common, and because anthracyclines are indispensable antitumor antibiotics that are biotransformed mainly by carbonyl reductase, we have compared the reductase-mediated conversion of daunorubicin to daunorubicinol and the conversion of doxorubicin to doxorubicinol in G6PD-deficient and nondeficient erythrocytes. We found that even without G6PD deficiency, the HMS dehydrogenases selectively limited daunorubicin metabolism, as contrasted with that of doxorubicin. The milder GdA$^-$ variety of G6PD deficiency restricted the biotransformation of daunorubicin at therapeutic levels, in hemolysates and intact erythrocytes, within 15 minutes, for at least 24 hours. The bioconversion defect was even more severe in Gd Mediterranean G6PD deficiency. Primaquine aldehyde competed with daunorubicin as a substrate for carbonyl reductase. These studies show that HMS dehydrogenase activity controls carbonyl reductase–dependent biotransformation. New issues arise concerning possible effects of G6PD deficiency on the oncolytic and toxic properties of anthracyclines that are effective substrates for carbonyl reductase and also on nonxenobiotic reactions catalyzed by this enzyme. (J Lab Clin Med 1996;127:588-98)

Abbreviations: BCNU = 1,3-*bis*-(2-chloroethyl)-1-nitrosourea (carmustine); Fe(II) = ferrous iron; Fe(III) = ferric iron; G6PD = glucose-6-phosphate dehydrogenase; HMS = hexose monophosphate shunt; GSH = glutathione reduced form; GSSG = glutathione oxidized form (glutathione disulfide); GSSG-R = glutathione reductase; HPLC = high-performance liquid chromatography; NADP = nicotine adenine dinucleotide phosphate; NADPH = reduced nicotinamide adenine dinucleotide phosphate; O_2^- = superoxide; RBC = red blood cell

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Many agents, including antitumor anthracyclines, activate the HMS in normal erythrocytes and to a much lesser extent in G6PD-deficient erythrocytes. To better understand how drugs interact with the HMS and to understand their propensity to destroy RBCs, we have previously carried out studies to characterize and distin-

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guish the effects of primaquine and several other drugs that are particularly hemolytic in G6PD deficiency.[1] We were able to dissect the activation of the HMS in intact human RBCs by using BCNU to inhibit GSSG-R almost completely without disturbing G6PD and 6-phosphogluconic dehydrogenase.[2-4] Agents such as ascorbate, which mobilize the dehydrogenases of the HMS by oxidizing NADPH indirectly in a GSH-dependent manner, are almost totally blocked by BCNU. In contrast, compounds such as methylene blue or pyrroline carboxylate, which activate the HMS by oxidizing NADPH directly without first involving GSH, are markedly insensitive to the nitrosourea. Chemicals such as primaquine trigger the HMS by independently oxidizing both NADPH and GSH.

While studying anthracyclines in the above manner, we found that doxorubicin and daunorubicin enhanced the HMS by different mechanisms. Although both agents were potent stimulators of the HMS, doxorubicin behaved like ascorbate, while daunorubicin behaved like primaquine. Thus in normal RBCs, doxorubicin activated the HMS almost exclusively through NADP generated by GSSG-R in response to rising glutathione disulfide. On the other hand, daunorubicin—but not doxorubicin—could not only oxidize GSH but also stimulate the HMS by utilizing NADPH directly (the reason why doxorubicin and daunorubicin activate the HMS in such a different manner is addressed in the Discussion).

These data, based on the production of CO_2 derived from glucose, complement remarkably well pharmacologic studies of the biotransformation of anthracyclines. Daunorubicin is indeed converted to its main metabolite daunorubicinol by an NADPH-requiring carbonyl reductase, EC 1.1.1.184,[5-13] while the capacity of this system to convert doxorubicin into doxorubicinol is very low in human erythrocytes without or with G6PD deficiency.[14] Persons with G6PD deficiency are particularly susceptible to the hemolytic toxicity of primaquine.[5-18] This vulnerability is evident not only when G6PD-deficient and nondeficient persons are compared but also when the effect of primaquine is contrasted with that of another hemolytic agent such as diaminodiphenylsulfone.[19] The special sensitivity to primaquine-induced hemolysis has been linked to the drug's capacity to oxidize NADPH both directly and indirectly[1]: moreover, the dual manner of activating the HMS is shared by primaquine and daunorubicin.[1] These considerations suggested that there might exist a hitherto unrecognized pharmacogenetic interaction between the metabolism of certain anthracyclines like daunorubicin and G6PD deficiency. Such an interaction could potentially affect antitumor selectivity and drug toxicity. Furthermore, anthracyclines are indispensable antitumor antibiotics. G6PD deficiency is extremely common, and the influence of this enzymopathy on the bioconversion of daunorubicin has not yet been addressed in previous studies. For these reasons we have compared the rate and extent of biotransformation of daunorubicin to daunorubicinol and of doxorubicin to doxorubicinol in the blood of persons with and without G6PD deficiency of the GdA^- and Mediterranean types. To obtain additional information regarding the control of cellular anthracycline metabolism, we have also investigated whether carbonyl reductase–mediated drug bioconversion was limited by metabolic NADPH or by the amount of constitutive enzyme. We have also examined whether daunorubicin and primaquine aldehyde can compete for carbonyl reductase.

METHODS

Materials. The following substances were obtained from Sigma Chemical Co., St. Louis, Mo.: daunorubicin, doxorubicin, NADP monosodium salt, NADPH tetrasodium salt, and glucose-6-phosphate monosodium salt crystalline. Idarubicin was donated by Farmitalia, Carlo Erba, Milan, Italy. Primaquine aldehyde was prepared from primaquine as previously described.[20] BCNU was obtained from Bristol-Myers, Evansville, Ind.

Blood samples and red cell enzyme assays. Informed consent was obtained from apparently healthy adult volunteers with or without G6PD deficiency. Venous blood was withdrawn into ethylenediaminetetraacetic acid–treated vacuum tubes (Vacutainer: Beckton Dickinson Vacutainer Systems, Rutherford, N.J.). We also used blood samples without and with G6PD deficiency identified as follows. The remainder of sequential samples used for routine blood counts were selected for G6PD deficiency screening if all the values of the complete blood counts were within normal limits. Enzyme activities and the Gd phenotypes were verified, and neighboring samples without or with G6PD deficiency were used for the metabolic studies with anthracyclines. Complete blood counts were obtained by standard automated cell counter analysis (Coulter Counter; Coulter Electronics, Hialeah, Fla.). G6PD, 6PGD, and GSSG-R were screened, assayed, and phenotyped electrophoretically as previously described.[21,22]

Drug incubations in whole blood and in hemolysates. The biotransformation of daunorubicin or doxorubicin was studied at 37° C in whole blood as well as in ghost-free hemolysate. For the whole blood studies, the freshly obtained sample was incubated with a known amount of anthracycline without adding G6P, NADP, or NADPH. For the experiments in hemolysates, the drug was incubated in parallel either with 1 mmol/L NADP and G6P or with 1 mmol/L NADPH alone. The effects of various

potential competitive substrates or inhibitors of carbonyl reductase were studied in hemolysate systems containing daunorubicin and NADPH. The timed samples of whole blood were analyzed for anthracyclines and their metabolites separately in plasma and red cells. The blood was centrifuged (3000 g for 10 minutes at room temperature, model HN-S IEC; International Equipment Co., Needham Heights, Mass.), to obtain buffy coat–free plasma and red cells. Plasma was analyzed as for hemolysates (see below). The packed red cells were washed three times in twice their volume of normal saline solution, hemolyzed with Tris buffer solution (0.05 mol/L, pH 7.4) containing 20 mg/dl saponin, and the ghosts were removed by centrifugation (17,000 g at 4° C). Incubations of anthracyclines in ghost-free hemolysate were carried out in samples adjusted to a hemoglobin concentration of 2.5 gm/dl with 0.01 mol/L potassium dihydrogen phosphate buffer adjusted to pH 7.4 with NaOH. Hemoglobin was measured by the standard Drabkin method.

Sample preparation and extraction for drug analysis. Idarubicin was used as an internal standard. Samples of 0.2 ml of plasma or RBCs hemolyzed as described earlier were supplemented with idarubicin and extracted for drug analysis as follows. In a glass tube containing the plasma or hemolysate sample, 3 ml of acetonitrile was added. Ten minutes later, 100 mg NaCl was added, and after 5 more minutes, the tubes were centrifuged for 7 minutes at 1500 g. A 2 ml sample of the supernatant was transferred into another glass tube and evaporated under nitrogen at 60° C. The residue was reconstituted into 200 μl of mobile phase (see below), and 40 μl was injected into the column.

HPLC analysis of anthracyclines. Daunorubicin, daunorubicinol, doxorubicin, and doxorubicinol levels were determined with HPLC by using a modification of a recently described method.[23] The HPLC instrumentation included a Gilson 620 Data Master with model 302 pumps, a fluorescence detector, and a Waters (Milford, Mass.) intelligent sample processor. A Spherisorb phenyl 4.6 mm × 25 cm column with a Spherisorb phenyl guard column (Phenomenex, Torrance, Calif.) were used for the assay. The fluorescence detection (model 121; Gilson, Middleton, Mass.) was set at 480 nm for excitation and 590 nm for emission. The mobile phase consisted of acetonitrile (30%) and citrate buffer (pH 4.0), 0.03 mol/L (70%). The flow rate for the mobile phase was 1.5 ml/min. Doxorubicinol and doxorubicin eluted at 5.8 and 6.5 minutes, respectively; daunorubicinol, daunorubicin, and idarubicin eluted at 7.4, 9.9, and 11.3 minutes, respectively. Recovery of idarubicin from red cells or plasma was 71% ± 10%. Individual standard curves for daunorubicin in plasma and RBCs were linear over the concentration range of 50 to 200 ng/ml in plasma and RBCs ($r = 0.99$). Plasma and red cell drug levels were determined from a four point standard curve. In experiments with whole blood, the concentrations of the parent compounds and metabolites were determined separately in plasma and RBCs. Values were expressed in nmol/dl whole blood, taking into account the measured donor blood hematocrit. The rate of disappearance of daunorubicin (or doxorubicin), or the rate of appearance of daunorubicinol (or doxorubicinol), by carbonyl reductase activity was expressed as nanomoles per hour per gram of hemoglobin.

Statistical analysis. The mean conversion rate of daunorubicin to daunorubicinol in subjects without and with G6PD deficiency was compared by the two-tailed unpaired Student *t* test.

RESULTS

Fig. 1 illustrates the chromatographic separations in blood of doxorubicinol (peak 1), doxorubicin (peak 2), daunorubicinol (peak 3), daunorubicin (peak 4), and idarubicin, which was used as internal standard (peak 5). In samples processed 40 minutes after the addition of daunorubicin and doxorubicin to whole blood, the red cells (Fig. 1, *A*)—and to a somewhat lesser extent the plasma (Fig. 1, *B*)—contained daunorubicinol as well as a trace amount of doxorubicinol. No metabolite was found in the t_0 samples processed immediately after addition of the parent compounds to whole blood, and incubations with either daunorubicin or doxorubicin alone confirmed the assignment of peak 1 to doxorubicinol and peak 3 to daunorubicinol (data not shown).

Fig. 2 shows the biotransformation of daunorubicin (Fig. 2, *A*) or doxorubicin (Fig. 2, *B*) in non-G6PD-deficient hemolysates supplemented either with buffer alone (control), with NADP and G6P (HMS dehydrogenase-coupled carbonyl reductase), or with NADPH without G6P (HMS dehydrogenase-independent direct carbonyl reductase). In the control systems, no metabolite appeared within 24 hours. Fig. 2, *A*, shows that within this time period most of the daunorubicin became daunorubicinol in hemolysates that had been supplied directly with NADPH. Conversion to daunorubicinol was slower in hemolysates supported with G6P and NADP.

Fig. 2, *B*, shows that the amount of doxorubicin converted into doxorubicinol within 24 hours was very small, whether NADPH was provided directly or indirectly. Hence most subsequent work focused on the metabolism of daunorubicin.

Fig. 3 illustrates the stoichiometric conversion of daunorubicin into daunorubicinol in hemolysates supplied with 100 ng/ml daunorubicin, a concentration within therapeutic range. Biotransformation depended on the amount of hemolysate, and the rate was linear at lower hemoglobin concentrations.

Tables I and II compare the biotransformation of daunorubicin at 15, 30, and 60 minutes in hemolysates from persons without or with the GdA^- type of G6PD deficiency (G6PD activities, respectively >6 or <2 IU/gm hemoblobin). The cell lysates sup-

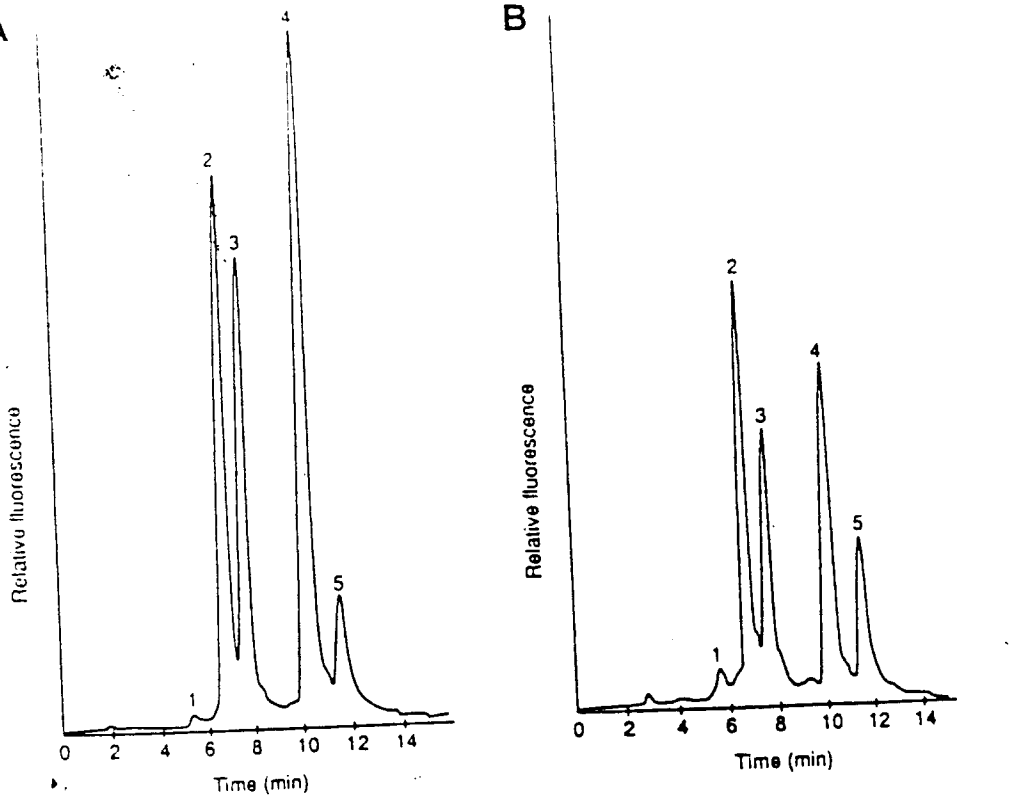

Fig. 1. Distribution of anthracyclines and their metabolites in erythrocytes and plasma. Whole blood was incubated with doxorubicin (100 ng/ml) and daunorubicin (100 ng/ml) at 37° C for 40 minutes. At the end of incubation, erythrocytes and plasma were separated, idarubicin (25 ng/ml, internal standard) was added, and the drugs were extracted. **A**, Distribution of peaks in erythrocytes. **B**, Distribution of peaks in plasma. The retention times of the anthracyclines and metabolites are as follows: doxorubicinol (peak 1): 5.8 minutes; doxorubicin (peak 2): 6.5 minutes; daunorubicinol (peak 3): 7.4 minutes; daunorubicin (peak 4): 9.9 minutes; idarubicin (peak 5): 11.3 minutes.

plied with anthracycline were incubated without further addition(s) (control), with 1 mmol/L G6P and 1 mmol L NADP (coupled NADPH), or with 1 mmol L NADPH (direct uncoupled biotransformation). The data based on the disappearance of daunorubicin (Table I) and on the appearance of daunorubicinol (Table II) gave concordant information. Significantly less daunorubicin was converted into daunorubicinol in hemolysates supplied with G6P and NADP than in those provided with NADPH. The difference in biotransformation rates in the systems with coupled versus direct NADPH was seen without or with G6PD deficiency. Thus even in normal hemolysates the HMS dehydrogenases limit the rate of biotransformation of daunorubicin. Within the GdA^- deficient samples, the quantitative difference in daunorubicin metabolism between the G6P plus NADP system and the NADPH system was magnified ($p < 0.001$). When NADPH was generated through G6P and NADP, the biotransformation of daunorubicin to daunorubicinol was markedly decreased in the G6PD-deficient hemolysates as compared with the nondeficient controls ($p < 0.001$ at all time points tested). However, when NADPH was supplied directly, there was no difference between normal and G6PD GdA^- hemolysates (all $p > 0.1$). Non-G6PD-deficient hemolysates produced so little doxorubicinol from doxorubicin (even after 24 hours) that further comparisons with G6PD-deficient samples became pointless (data not shown).

Fig. 4 shows studies in which daunorubicin was added without doxorubicin, G6P, or NADP to whole blood rather than to hemolysate. The data show that the difference in the bioconversion of daunorubicin found in GdA^- and non-G6PD-deficient hemolysates supplied with G6P and NADP (Fig. 2 and Table I) was also demonstrable in intact erythrocytes supported solely by plasma glucose as the source of energy. The biotransformation of daunorubicin was lower in GdA^--deficient whole blood

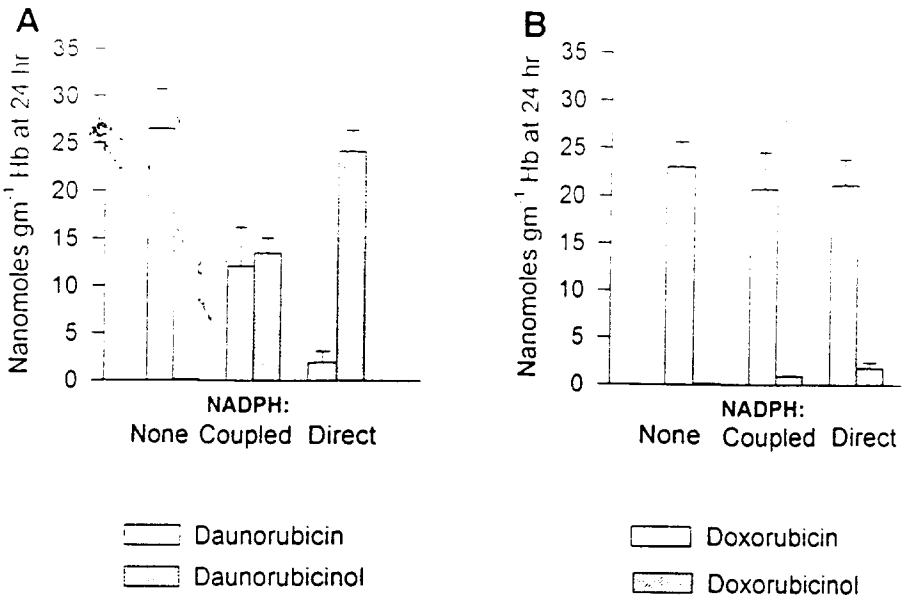

Fig. 2. Biotransformation of daunorubicin and of doxorubicin in destromatized hemolysates from non-G6PD-deficient persons. A. Biotransformation of daunorubicin. B. Biotransformation of doxorubicin. Daunorubicin (200 ng/ml) and doxorubicin (200 ng/ml) were each incubated either with buffer alone or with 1 mmol/L each of G6P and NADP, or with 1 mmol/L NADPH (direct) for 24 hours at 37° C. *Bars* represent mean ± SD (n = 4). Values are expressed as nanomoles per gram of hemoglobin at the end of 24 hours of incubation.

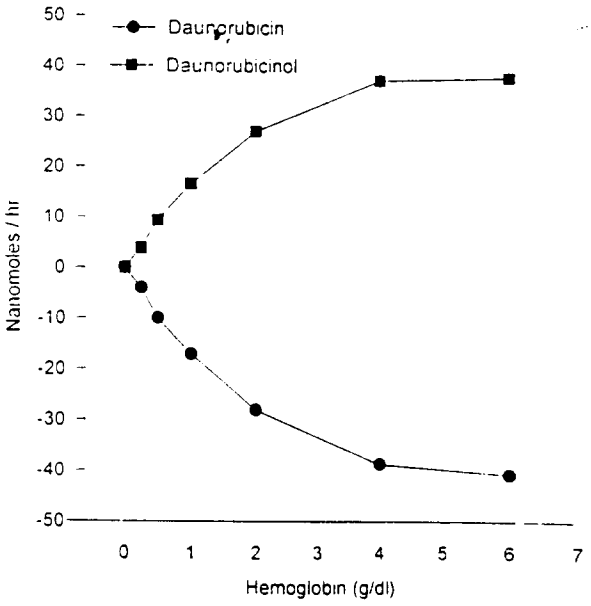

Fig. 3. Daunorubicin metabolism at varying hemoglobin concentrations. The *upper part* of the figure shows the daunorubicinol formed, and the *lower part* shows the amount of daunorubicin decreased when incubated with 1 mmol/L NADPH.

than in nondeficient blood at both time points monitored (20 and 60 minutes).

Fig. 5 extends the previous results by carrying out the incubations with whole blood for 24 hours and by comparing the biotransformation of daunorubicin in blood samples with either GdA⁻ or Gd Mediterranean G6PD deficiency. The difference between the G6PD GdA⁻-deficient and control blood samples persisted for at least 24 hours. GdA⁻ G6PD-deficient erythrocytes supported at body temperature by physiologic plasma glucose needed about 24 hours to match quantitatively the biotransformation of daunorubicin to daunorubicinol achieved within 2 hours by erythrocytes without G6PD deficiency. The biotransformation of daunorubicin was even more depressed in the Mediterranean type of G6PD deficiency so that Gd Mediterranean blood generated only half the amount of daunorubicinol found in GdA⁻ blood after 24 hours.

Table III shows that known inhibitors of carbonyl reductase—including phenanthrene quinone, rutin, menadione, and Cibacron blue A—blocked, as expected, the biotransformation of daunorubicin to daunorubicinol. Primaquine aldehyde interfered with the carbonyl reductase–mediated conversion of daunorubicin to daunorubicinol, while BCNU, the compound used in earlier studies to dissect drug-induced activation of the HMS, did not.

DISCUSSION

The data presented reveal that G6PD deficiency interacts with the main carbonyl reductase–dependent pathway for anthracycline metabolism in hu-

Table I. Restricted daunorubicin removal in G6PD deficiency (GdA⁻)

Group	Coupled (G6P+NADP) 15 minutes	30 minutes	60 minutes	Direct (NADPH) 15 minutes	30 minutes	60 minutes
Control	9.3 ± 1.3 (9)	9.5 ± 2.5 (9)	15.4 ± 3.6 (9)	7.2 ± 1.4 (8)	12.3 ± 3.6 (7)	19.7 ± 2.9 (7)
G6PD	1.0 ± 0.8	2.5 ± 2.1	6.0 ± 3.0	6.2 ± 3.4	8.7 ± 5.1	15.3 ± 7.6
GdA⁻	(8)	(9)	(9)	(5)	(9)	(5)
p	<0.001	<0.001	<0.001	>0.1	>0.1	>0.1

Removal of daunorubicin (200 ng/ml) in destromatized hemolysates from G6PD-deficient or nondeficient persons incubated with either 1 mmol/L G6P and 1 mmol/L NADP or with 1 mmol/L NADPH. Results are in nmoles daunorubicin removed per gram of hemoglobin at the indicated times ± SD (number of different individuals). The last row provides the statistical comparisons between G6PD-deficient and nondeficient persons. Drug biotransformation was also compared at equivalent times in coupled versus direct systems (not shown in the table). For these comparisons, the p values in the non-G6PD-deficient controls were <0.01, <0.05, and <0.001 at 15, 30, and 60 minutes, respectively. In G6PD deficiency the corresponding p values were all <0.001.

*The disappearance of daunorubicin as well as the production of daunorubicinol were similar to those in controls in a hemolysate prepared from a blood sample with reticulocytosis (310,000 reticulocytes/mm³) obtained from a patient with idiopathic congenital nonspherocytic hemolytic anemia.

Table II. Restricted daunorubicinol formation in G6PD deficiency (GdA⁻)

Group	Coupled (G6P plus NADP) 15 minutes	30 minutes	60 minutes	Direct (NADPH) 15 minutes	30 minutes	60 minutes
Control	4.8 ± 1.3 (9)	9.8 ± 2.4 (9)	14.9 ± 2.5 (9)	7.6 ± 1.9 (8)	11.9 ± 3.5 (7)	19.1 ± 2.6 (7)
G6PD	1.0 ± 0.9	2.5 ± 2.2	5.0 ± 4.3	6.0 ± 3.3	8.9 ± 4.4	14.4 ± 6.4
GdA⁻	(8)	(9)	(9)	(5)	(9)	(5)
p	<0.001	<0.001	<0.001	>0.1	>0.1	>0.1

Appearance of daunorubicinol in destromatized hemolysates from G6PD-deficient or nondeficient persons. Experimental conditions are as described for Table I and in Methods; results are expressed in nanomoles per gram of hemoglobin ± 1 SD (number of different individuals). The *last row* refers to the comparisons between G6PD-deficient and nondeficient persons. For the non-G6PD-deficient controls, the p values (not shown in the table) for the comparisons at equivalent times in coupled versus direct systems are <0.01, <0.05, and <0.001 at 15, 30, and 60 minutes, respectively. In G6PD deficiency the corresponding p values are all <0.001.

man erythrocytes.[5-13,24,25] Moreover, we found that this pathway is limited by NADPH generated through the HMS dehydrogenases, even without G6PD deficiency. In G6PD-deficient red cells, the conversion of daunorubicin is markedly restricted as compared with non-G6PD-deficient cells. Persistence of unmetabolized daunorubicin in G6PD-deficient erythrocytes is strictly stoichiometric with defective formation of daunorubicinol. The effect of the enzymopathy is seen at therapeutic levels of daunorubicin, in hemolysates as well as in intact erythrocytes. The depressed biotransformation of daunorubicin in G6PD deficiency is clearly evident within 15 minutes and persists for at least 24 hours. Quantitatively, the removal of daunorubicin with generation of daunorubicinol is very inadequate, even in the milder GdA⁻ variety of G6PD deficiency. In the Gd Mediterranean variety of G6PD deficiency, the bioconversion defect is even more severe. Daunorubicin is confirmed to be a far better substrate for carbonyl reductase than doxorubicin. Correspondingly, G6PD deficiency impacts the metabolism of daunorubicin in the red cell much more than that of doxorubicin.

The observation that G6PD deficiency severely restricts the carbonyl reductase–dependent metabolism of daunorubicin in a relatively selective manner as compared with its effect on doxorubicin rekindles long-standing interest in how biotransformation affects the pharmacology of these anthracyclines. The pioneering studies of Bachur and Huffman[7] led them to state as early as 1971 that "... since blood cells are the site of action of the drug in leukaemias, the concentration of daunorubicin reductase in these cells, as well as in other tissues, may bear a relationship to the pharmacodynamics of daunorubicin therapy." This insight has been supported by much subsequent work. For example, in a recent study of patients with acute leukemia induced with daunorubicin, therapeutic efficacy did not correlate with plasma levels or with multiple drug resistance status, while the levels of cellular daunorubicin and daunorubicinol discriminated significantly between responders and failures.[26] The clinical relevance of our observation depends in part on whether G6PD deficiency modifies the biotransformation of daunorubicin in tissues other than

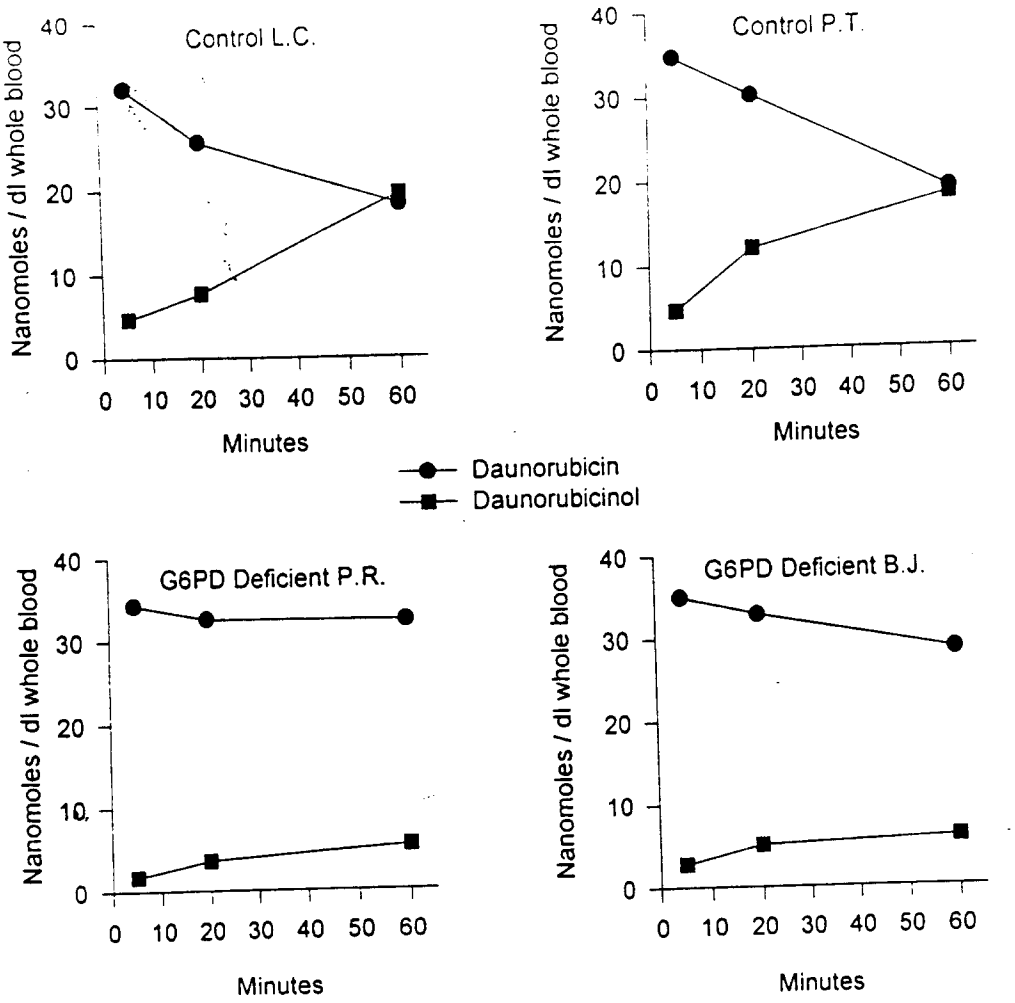

Fig. 4. Biotransformation of daunorubicin from 0 to 1 hour in whole blood from persons without or with G6PD GdA⁻ deficiency. Whole blood was incubated with daunorubicin (200 ng/ml) for 5, 20, and 60 minutes. Daunorubicin and daunorubicinol were determined separately in plasma and erythrocytes. Values were calculated, taking into account the measured whole blood hematocrits.

erythrocytes. Currently this information is not available. It is known, however, that G6PD activity is diminished in leukocytes,[27] platelets,[28] liver,[29] skin,[30] muscle,[31] and saliva[32] in the Mediterranean deficiency variant (Gd Mediterranean). Likewise, in Chinese G6PD deficiency variants, enzyme activity is markedly reduced in liver, adrenals and kidneys.[33] Thus, although in GdA⁻ G6PD deficiency the lowered enzyme activity is limited to erythrocytes (with the possible exception of liver cells),[34] this is not the case for other common types of G6PD deficiency.

Restricted biotransformation of daunorubicin in G6PD deficiency may affect antitumor activity and toxicity. Both doxorubicin and daunorubicin inhibit DNA and RNA syntheses and cell proliferation more effectively than doxorubicinol and daunorubicinol even though the metabolites retain some antitumor activity.[35] Thus metabolism diminishes the antitumor efficacy of anthracyclines. Hence it has been tempting to view active drug metabolism as contributing to an impaired therapeutic range and loss of therapeutic activity. Indeed, a number of years ago Loveless et al.,[8] in considering the implications of anthracycline metabolism, raised the possibility that "... simultaneous administration of a pharmacologically acceptable reductase inhibitor could increase the therapeutic efficacy of these drugs." We have now shown that the function of carbonyl reductase is severely restricted in the red cells of the many persons throughout the world who are G6PD deficient. Does this mean that anthracy-

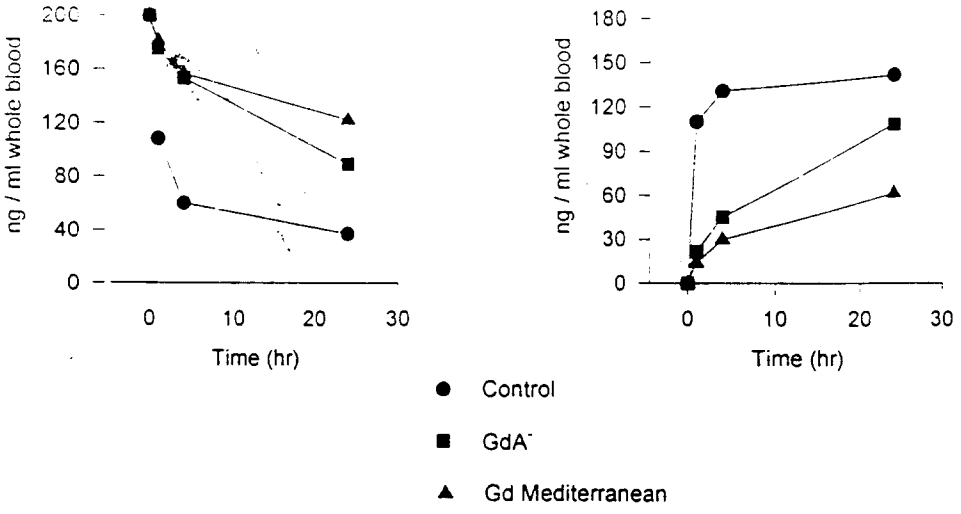

Fig. 5. Biotransformation of daunorubicin from 0 to 24 hours in whole blood from persons without or with G6PD deficiency of the GdA⁻ or Gd Mediterranean types. A. Disappearance of daunorubicin (200 ng/ml). B. Formation of daunorubicinol.

Table III. Inhibitors of daunorubicin metabolism

Substrate	Conc (mmol/L)	Daunorubicin removed		Daunorubicinol formed	
		nmol/hr/gm Hb	Percent inhibition	nmol/hr/gm Hb	Percent inhibition
None		9.4	0	9.5	0
Phenanthrene quinone	0.0025	0.4	95.7	0.3	96.8
	0.01	0.4	95.7	0.3	96.8
Rutin	0.01	2.7	71.0	2.7	71.5
	0.025	1.8	80.9	1.7	82.1
Menadione	0.25	1.1	88.3	1.0	89.5
	0.62	0.2	97.8	1.6	83.2
Cibacron blue A	0.5	5.4	42.5	5.0	47.4
	1.25	2.0	78.7	1.6	83.2
Primaquine aldehyde	1.4	5.5	41.4	5.2	45.3
BCNU	0.05	10.2	0	10.5	0

Daunorubicin 100 ng/ml was incubated for 1 hour at 37° C in destromatized hemolysate with 1 mmol/L NADPH and with or without the indicated compounds. Conc. Concentration.

clines that are effectively metabolized by carbonyl reductase—such as daunorubicin or its 4-demethoxy derivative idarubicin[35-37]—become better oncolytic agents in a patient with a severe type of G6PD deficiency? Although this is possible and there is evidence that daunorubicin-resistant murine cells have an elevated HMS activity,[38] we believe that such an interpretation would be premature and may be misleading. The difficulty stems from uncertainties concerning the key differences between nonmalignant and malignant cells that are responsible for the relative antitumor selectivity of anthracyclines.

In leukemic tissue there is evidence that blasts have lower carbonyl reductase activity than nonleukemic cells.[26,39-41] Consequently, the tumor cells might be exposed for a longer period, to higher levels of therapeutically more-active parent drug when compared with their nonleukemic neighbors in the bone marrow. A relative deficiency of carbonyl reductase in malignant cells may therefore contribute to or even be responsible for the antitumor selectivity of the drug. Taking this into consideration, it is possible that restriction of daunorubicin metabolism in G6PD-deficient hematopoietic tissue would decrease rather than increase the therapeutic index of the drug by impairing anthracycline metabolism preferentially in the nonmalignant cells. If so, the enzymopathy would narrow a useful or perhaps critical metabolic difference between the tumor target and the surrounding cells. Currently it is not

possible to decide whether antitumor chemotherapy with an anthracycline that is an effective substrate for carbonyl reductase is or is not deleterious in G6PD-deficient persons, particularly those with the more severe common Mediterranean or Oriental subtypes. Resolution of this important issue will require future studies to compare the antitumor efficacy and safety of anthracyclines in persons with and without various types of G6PD deficiency and to quantify the impact of the enzymopathy on drug metabolism in tissues other than red cells, particularly in leukemic and nonleukemic cells.

Additional considerations that arise from the present findings concern the possible effect of G6PD deficiency on the toxicity of anthracyclines. Much effort has focused on developing less cardiotoxic anthracyclines, on minimizing abrupt blood level peaks of available agents through continuous infusion protocols or drug encapsulation, and on increasing our understanding of the mechanisms responsible for anthracycline cardiomyopathy.[14,25,35,42-45] There is mounting evidence for the pathogenic role of reduced oxygen moieties created by semiquinones or by a drug-induced elevation of cellular Fe(II). The unequal biologic effects of anthracyclines depend on their different availability as a substrate for carbonyl reductase and also on their ability to generate semiquinones through flavoenzymes and to mobilize, chelate, or reduce Fe(III) to Fe(II).[36,42-44,46] For instance, three drug molecules chelate one atom of Fe(III) that remains oxidized with daunorubicin (ketone side chain: R-$C_{13}O$-$C_{14}H_3$) but not with doxorubicin (ketol: R-$C_{13}O$-C_{14} H_2OH).[42,46,47] Excess production of O_2^- and more reactive oxygen radicals generated by Fe(II) but not by Fe(III) may influence doxorubicin's therapeutic range while increasing cardiotoxicity and the risk of necrotizing enterocolitis.[36,43,44] In the present context it is particularly relevant to note that antitumor and antimyocardial mechanisms can be dissociated,[25,42,45] that the cardiotoxicity of parent anthracyclines and their alcohol metabolites differ in some experimental systems[25,45] as well as in the human myocardium,[45] and that anthracycline-induced iron mobilization in human myocardial biopsy samples depends critically on carbonyl reductase activity.[45] Thus the restricted carbonyl reductase in G6PD deficiency may not only impact the drug's therapeutic value but also its tendency to injure the heart.

Daunorubicin and doxorubicin have dissimilar toxic effects on human erythrocytes in which they have been encapsulated, so that entrapped daunorubicin perturbs the loaded cells[48] much more than does doxorubicin.[14] Moreover, daunorubicin differs from doxorubicin in boosting the demand for erythrocyte NADPH by detoxifying peroxides through GSH-peroxidase and GSSG-R, as well as by serving as a substrate for carbonyl reductase (see Results) in a BCNU-insensitive manner (reference 1, Table III). To mitigate oxygen toxicity, hydrogen peroxide formed from O_2^- by superoxide dismutase is rapidly removed through defensive recycling of glutathione by GSH-peroxidase supported by increased GSSG-R activity.[3,21,22] The disulfide reductase depends in turn on G6PD, which it stimulates by removing inhibitory NADPH while providing NADP. Thus doxorubicin that produces more semiquinone, Fe(II), and peroxide activates the HMS powerfully in an indirect BCNU-sensitive manner. Daunorubicin, on the other hand, being an effective substrate for carbonyl reductase, stimulates the HMS strongly with a direct BCNU-insensitive component (see Introduction). Thus daunorubicin resembles primaquine rather than doxorubicin in its dual mode of consuming NADPH as well as in its propensity to damage erythrocytes. Consequently G6PD deficiency, which is responsible for primaquine-sensitivity in erythrocytes, may also influence differentially how these cells interact with daunorubicin, idarubicin, and other quinone substrates for carbonyl reductase.

These studies and the preceding discussion have dealt primarily with the effect of G6PD deficiency on anthracycline metabolism by carbonyl reductase. Nonetheless, we found that drug metabolism was limited by the HMS dehydrogenases even without G6PD deficiency. Further, carbonyl reductase metabolizes substances other than xenobiotics,[10-13] including 9-keto prostaglandins,[49] C_{21} steroids of the progesterone family,[50] cellular alkylglyoxals,[50] and naturally occurring quinones with the potential of generating reactive semiquinone radicals as well as detoxified hydroquinones.[11-13] Our observations suggest that these seemingly unrelated non-xenobiotic reactions of uncertain physiologic significance may be modulated by an enhanced flux of glucose through the HMS and be altered in persons with G6PD deficiency.

The present studies have shown that HMS dehydrogenase activity controls carbonyl reductase–dependent biotransformation. New issues arise concerning the possible effects of G6PD deficiency on the oncolytic and toxic properties of anthracyclines that are effective

substrates for carbonyl reductase and on non-xenobiotic reactions catalyzed by this enzyme.

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SHORT COMMUNICATIONS

Interindividual variability of carbonyl reductase levels in human livers

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Abstract—Interindividual variability of carbonyl reductase levels in human livers (N = 11) was examined by measuring reductase activity toward various substrates and by western blot analysis using anti-rat ovarian carbonyl reductase CR2 antibody. The carbonyl reductase activity toward *p*-nitrobenzaldehyde (PNBA) (58.1 ± 5.4 nmol/mg protein/min, mean ± SE) was highest among the substrates examined, followed by 4-benzoylpyridine (4BP) (14.4 ± 2.0 nmol/mg protein/min) and *p*-nitroacetophenone (PNAP) (2.00 ± 0.37 nmol/mg protein/min). The reductase activity (6.33 ± 0.56 pmol/mg protein/min) toward 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (15KD-$PGF_{2\alpha}$), which is a diagnostic substrate for rat ovarian carbonyl reductases, was relatively high compared to that in other species. Western blot analysis revealed that each human liver contained several immunoreactive proteins to anti-CR2 antibody. The activities toward 15KD-$PGF_{2\alpha}$ ($r = 0.85$, $P < 0.01$) and 4BP ($r = 0.84$, $P < 0.01$), but not PNBA ($r = 0.53$, not significant) or PNAP ($r = 0.52$, not significant), were closely correlated with the relative amounts of the high molecular weight immunoreactive proteins determined with a densitometer. Thus, the major carbonyl reductases in human liver are similar to those of rat ovarian enzymes.

Carbonyl reductase (EC 1.1.1.184) is a cytosolic, monomeric oxidoreductase that catalyses the NADPH-dependent reduction of a large number of biologically and pharmacologically important endogenous and xenobiotic carbonyl compounds, such as prostaglandins (PGs*), steroids, quinones and anthracycline antibiotics [1–3]. Based on its broad substrate specificity, the enzyme is distinguished from alcohol dehydrogenase (EC 1.1.1.1), aldehyde reductase (EC 1.1.1.2) and aldose reductase (EC 1.1.1.21). Multiple forms of the enzyme differing in size and charge have been isolated from various sources and even from the same tissue [1–3].

Recently, we have purified from rat ovary two isoforms designated as carbonyl reductases CR1 and CR2, which are very similar to each other in terms of substrate specificity and immunological cross-reactivity, and we demonstrated that the isoforms were specific for PGs rather than steroids, i.e. they were able to catalyse the reduction of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (15KD-$PGF_{2\alpha}$) to 13,14-dihydro-$PGF_{2\alpha}$, as well as the interconversion of PGE_2 to $PGF_{2\alpha}$ [4]. Western blot analysis using anti-CR2 antibody revealed that immunoreactive proteins, which migrated to the same position as CR1 and CR2, were present in various tissues [4, 5]. The immunoreactive proteins were found only in tissues which exhibited detectable 15KD-$PGF_{2\alpha}$ reductase activity [4, 5], suggesting that 15KD-$PGF_{2\alpha}$ may be a diagnostic substrate for isoforms of carbonyl reductase from rat ovary.

Human liver possesses considerable carbonyl reductase activity toward 15KD-$PGF_{2\alpha}$, as well as toward *p*-nitroacetophenone (PNAP) and *p*-nitrobenzaldehyde (PNBA), which are non-specific substrates for carbonyl reductases [5]. In the present study, we investigated interindividual variability of carbonyl reductase levels in human livers by measurement of 15KD-$PGF_{2\alpha}$ reductase activity and by western blot analysis using anti-CR2 antibody, and examined the correlation between the activities and the immunoreactive proteins.

* Abbreviations: PG, prostaglandin; 15KD-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$; PNAP, *p*-nitroacetophenone; PNBA, *p*-nitrobenzaldehyde; 4BP, 4-benzoylpyridine; HMW, high molecular weight components; LMW, low molecular weight components.

Materials and Methods

Materials. Portions of human livers were obtained from 11 cadavers during medico-legal autopsy and stored at −70° until use. Each liver was homogenized with 3 vol. (v/w) of ice-cold 1.15% KCl. Cytosolic fractions were prepared, as described previously for laboratory animals [4, 5]. 15KD-$PGF_{2\alpha}$ was obtained from Upjohn Pharmaceuticals Ltd (Kalamazoo, MI, U.S.A.) and [5,6,8,9,11,12,14-^{3}H]15KD-$PGF_{2\alpha}$ (sp. act. 80 Ci/mmol) from Amersham International (Amersham, U.K.). 13,14-Dihydro-$PGF_{2\alpha}$ was kindly supplied by the Ono Pharmaceutical Co. (Osaka, Japan). Other chemicals of reagent grade were products of either Wako Pure Chemical Industries (Osaka, Japan) or the Japan Bio-Rad Lab. Co. (Tokyo, Japan).

Enzyme assay and protein determination. Two different techniques were employed to measure reductase activities. One involved radiochemical measurement of [^{3}H]13,14-dihydro-$PGF_{2\alpha}$ formed by the enzymatic reduction of [^{3}H]-15KD-$PGF_{2\alpha}$ as described by Inazu *et al.* [6]. The other involved spectrophotometric measurement of the oxidation rate of NADPH at 340 nm and 37° [4, 5]. The standard assay mixture consisted of 100 mM phosphate buffer (pH 6.5), 0.1 mM NADPH, enzyme and either PNAP, PNBA or 4-benzoylpyridine (4BP) at 1.0 mM in a total volume of 1.0 mL. The reaction was initiated by addition of cofactor to the assay mixture. Blanks without substrate or enzyme were routinely included. Water-insoluble substrates were dissolved in ethanol and the final concentration of ethanol in the assay mixture did not exceed 2%, a concentration which had no effect on the catalytic activity of the enzymes. One unit was defined as the amount of enzyme that catalysed the oxidation of one micromole of cofactor at 37°. Protein concentration was determined by the method of Lowry *et al.* [7].

Western blot analysis. Western blot of human liver cytosolic proteins (10 μg) was performed by a modification of the method of Towbin *et al.* [8] using polyclonal antiserum raised against the purified rat ovarian carbonyl reductase CR2 as described previously [4, 5]. The relative intensity of each band was calculated as a percentage of that of sample 1, based on densitometric measurements (λ_r 400 nm, λ_t 650 nm).

Statistical analysis. Results were correlated using linear regression analysis. Student's *t*-test was used and the cor-

Table 1. Carbonyl reductase activities and relative contents in human livers

Specimen (age, sex)	Reductase activity				Content of immunoreactive proteins		
	15KD-$PGF_{2\alpha}$ (pmol/mg protein/15 min)	PNAP	PNBA (nmol/mg protein/min)	4BP	Total	HMW (Relative intensity)	LMW
1 (47, M)	8.36	3.25	68.5	22.9	100	74	26
2 (36, M)	2.97	1.06	37.0	5.9	37	27	10
3 (40, M)	6.64	0.83	51.6	19.4	68	48	20
4 (40, M)	5.22	1.76	51.9	14.2	49	28	21
5 (69, M)	6.01	1.72	32.5	11.8	73	54	19
6 (22, M)	7.80	4.73	64.7	19.2	91	46	44
7 (50, M)	8.01	2.31	76.3	20.6	107	76	31
8 (51, F)	5.88	2.06	77.2	13.7	96	37	59
9 (47, M)	5.02	0.73	45.5	4.9	53	23	30
10 (51, M)	9.14	2.73	87.9	20.8	89	NS	NS
11 (55, M)	4.52	0.68	46.5	5.5	45	19	27
Mean ± SE	6.33 ± 0.56	2.00 ± 0.37	58.1 ± 5.4	14.4 ± 2.0			

NS, not separated.

The detection limit for reductase activity toward 15KD-$PGF_{2\alpha}$ is 0.75 pmol/mg protein/15 min and that toward PNAP, PNBA and 4BP is 0.02 nmol/mg protein/min.

Table 2. Correlation coefficients

	15KD-$PGF_{2\alpha}$	PNAP	PNBA	4BP	CR (HMW)	CR (LMW)
CR (total)	0.86‡	0.70*	0.78†	0.81†	0.83†	0.60
15KD-$PGF_{2\alpha}$		0.70*	0.77†	0.90‡	0.85†	0.38
PNAP			0.57	0.69*	0.53	0.47
PNBA				0.71*	0.52	0.73*
4BP					0.84†	0.23
CR (HMW)						0.06

Statistically significant; * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.
CR, carbonyl reductase.

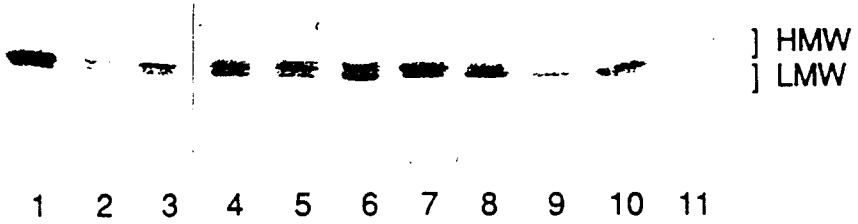

Fig. 1. Interindividual variability of human liver immunoreactive carbonyl reductases by western blot analysis. Human liver cytosolic proteins (10 μg) were electrophoresed on 10% polyacrylamide gel containing SDS, transferred to nitrocellulose membrane and then immunostained with anti-CR2 antiserum as described in Materials and Methods.

relations were considered to be statistically significant at $P < 0.05$.

Results and Discussion

Figure 1 shows the result of western blot analysis using anti-CR2 antibody. The human liver cytosolic proteins contained several immunoreactive proteins having similar electrophoretic mobility to those of the ovarian carbonyl reductases we examined previously [5]. Relative intensity of each band was variable in individual human livers. In samples 1–7, high molecular weight bands were more intense than low molecular weight ones, whereas in samples 8–11, low molecular weight bands were more intense. The results of the cytosolic carbonyl reductase activity determinations and the relative intensity determined by densitometric scanning of western blots in Fig. 1 are summarized in Table 1. As we could not separate several bands in each lane by densitometric scanning, for convenience, we designated higher molecular weight components, low molecular weight components and the sum of them as HMW, LMW and total, respectively. The carbonyl reductase activity toward PNBA (58.1 ± 5.4 nmol/mg protein/min, mean ± SE) was the highest among those toward the substrates examined, followed by 4BP (14.4 ± 2.0 nmol/mg protein/min) and PNAP (2.00 ± 0.37 nmol/mg protein/min). Carbonyl reductase activity toward 15KD-$PGF_{2\alpha}$ was higher (6.33 ± 0.56 pmol/mg protein/min) than in livers of other species such as mouse, hamster, guinea pig, rabbit, cow, dog and monkey [5]. We found that total immunoreactive proteins to anti-CR2 antibody were correlated significantly with activities toward both 15KD-$PGF_{2\alpha}$ and 4BP, but not PNBA or PNAP (Table 2). Further, the correlation coefficients suggested that the former activities may be mostly due to HMW, but not LMW. That is, the immunoreactive HMW recognized by anti-CR2 antibody may contribute predominantly to the carbonyl reductase activities toward 15KD-$PGF_{2\alpha}$ and 4BP. In contrast, no significant correlation of immunoreactive HMW with the enzyme activities toward either PNBA or PNAP was found. Thus, it seems that carbonyl reductases having similar characteristics to those of the rat ovarian enzyme exist in human liver.

In general, the carbonyl reductases have been classified into two groups according to their substrate specificity. One is a group that shows higher affinity for steroids and is reported to be identical with 3α- or 3(17)β-hydroxysteroid dehydrogenases, which have been isolated mostly from hepatic tissues [9–12]. The other is a group having higher affinity for PGs and functions as 15-hydroxyprostaglandin dehydrogenase, PGE_2 9-ketoreductase or PGD_2 11-ketoreductase [4, 13–18]. The carbonyl reductases from rat ovary belong to the latter group [4, 6]. These enzymes are considered to play important roles in the metabolism of both endogenous and xenobiotic carbonyl compounds. When we examined species differences of rat hepatic 3α-hydroxysteroid dehydrogenase in various animals, anti-3α-hydroxysteroid dehydrogenase cross-reacted with proteins in hepatic cytosols of mouse, hamster, guinea pig and rabbit, but not dog, pig, cow, monkey and human, which all possess reductase activities toward PNBA and PNAP [5]. In contrast, the immunoreactive proteins to anti-CR2 antibody were found in livers from all of the above species, all of which exhibit distinct hepatic 15KD-$PGF_{2\alpha}$ reductase activity [5]. Thus, similar enzyme forms to those purified from rat ovary are present in human liver, and appear to be preserved and functionally important in all species examined. The purified carbonyl reductases from both the testis [18] and liver [19] in humans show charge heterogeneity. In this study, there were no great interindividual differences in the carbonyl reductase activities of human livers (Table 1). The heterogeneity of carbonyl reductases in human liver may arise from small structural

modifications, resulting in differences in molecular weight and isoelectric point.

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M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: February 5, 1997 /S/ 2/5/97

FROM: Hugo E. Gallo-Torres, M.D., Ph.D., Medical Officer, Division of Gastrointestinal and Coagulation Drug Products, HFD-180

SUBJECT: Recommendations for Approval of DOLA•Mesyl Tablets, 100 mg

TO: Acting Director, ODE III

THROUGH: Division Director, Division of Gastrointestinal and Coagulation Drug Products, HFD-180 2/5/97 /S/

Hechst Marion Roussel, the sponsor of NDA 20-623 has submitted data in support of approval of DOLA•Mesyl tablets once-a-day for two indications: a) 200 mg given 30 min. before the start of chemotherapy, for the prevention of N&V associated with emetogenic cancer chemotherapy, including initial and repeat doses and b) 50 mg given within two hours prior to surgery, for the prevention of post-operative N&V (PONV). Review of the evidence on efficacy seemed to justify the MO recommendation for approval of DOLA•Mesyl for the prevention of PONV indication (MOR of May 31, 1996). It was recommended to approve a once-a-day dose of 100 mg (not 50 mg, as proposed by the sponsor). Both Dr. Fredd, the Division Director and Dr. Botstein the Acting Director, ODE III, agreed on this recommendation. The MO also recommended approval of DOLA•Mesyl for the prevention of CCNV indication but at a once-a-day dose of 100 mg (not 200 mg as proposed by the sponsor). The MO recommendation was based on results of pivotal trials -043 and -048 (p.335 of MOR of May 31, 1996) which supported efficacy for either 100 or 200 mg. Taking into consideration data from study -087 the Division Director selected a 200 mg dose for the prevention of CCNV indication. But this dose was not recommended for approval because of lack of sufficient safety reassurance. It was thought that, for assurance equal to that available for the proposed dose of the PONV application (100 mg) ca. 2500 more CCNV patients would need to be studied. In addition, Dr. Botstein requested further characterization of DOLA•Mesyl's cardiac effects. A comprehensive submission was made by the sponsor in response to questions about effects on EKG parameters. On December 13, 1996, a consultation request was sent to the Division of Cardio-Renal.

In the present memorandum, Dr. A. Karkowsky's recommendations in consult review of January 16, 1997, regarding the safety profile of DOLA•Mesyl, are considered. In addition to information on DOLA•Mesyl tablets (MOR of May 31, 1996) the MO incorporates brief summary statements from his recently completed review of the data from eleven trials in the DOLA•Mesyl injection NDA (MOR of

February 5, 1997). Notwithstanding cases in individual patients uncovered during the detailed review of the evidence, it can be said that, at the dose of 1.8 mg/Kg (roughly 100 mg one dose fits all), intravenously administered DOLA•Mesyl does not appear to be less safe than when this dose is administered orally. Actually, there is reason to state that at the once-a-day dose of 100 mg, the benefit/risk ratio for the intravenously administered drug may be better. The evidence at hand demonstrates that this dose of i.v. DOLA•Mesyl is effective in the prevention of CCNV induced by high dose cisplatin-based chemotherapeutic regimens and this is considered an important clinical effect. [Evaluations in high-dose cisplatin patients were not carried out with DOLA•Mesyl tablets.]

A. Efficacy

The data on which the MO's recommendation to approve the 100 mg DOLA•Mesyl tablets for both indications are based are summarized in Table 1. This information was taken from the MOR of NDA 20-623, May 31, 1996. (In both instances, ITT data are presented.) For both indications the 100 mg dose is better than the 50 mg and, as stated in Dr. Fredd's memorandum of August 16, 1996, there is no gain in using 200 mg.

TABLE 1

I. Complete Response (CR) in Prevention of CCNV Studies			
Study No.	CR With Comparator (25 mg)	DOLA•Mesyl Dose (mg) Therapeutic Gain (%)/[p-value] 50	100
-043 (n=307)	45%	27% [0.0006]	29% [0.0005]
-048 (n=320)	31%	10% [N.S.]	31% [0.0002]
II. Complete Response (CR) in Prevention of PONV Studies			
	CR With Comparator (PL)	DOLA•Mesyl Dose (mg) Therapeutic Gain (%)/[p-value] 50	100
-095 (n=793)	35%	22% [0.0001]	15% [0.0062]
0292 (n=374)	29%	11% [N.S.]	25% [0.0026]
Source of Data MOR of May 31, 1996, NDA 20-623			

B. Safety

As summarized in Dr. Fredd's memorandum of August 16, 1996, we believe the safety database is sufficient to recommend approval of the prevention of PONV indication at the 100 mg dose. The i.v. database plus the oral database provide a total of ca. 2725 patients for safety assessment [this includes 100 mg and 200 mg safety database, an approach considered appropriate]. Similarly, the MO believes that the safety database is also sufficient to recommend approval of the prevention of CCNV indication at the 100 mg dose. In the three randomized studies with the tablet formulation (-043, -048 and -087) 457 patients received doses of ≥100 mg. In the five randomized studies with the i.v. formulation (-081, -031, -093, -032 and -082), a total of 1293 patients received DOLA•Mesyl at the i.v. dose of ≥1.8 mg/Kg
single dose if patients' body weight ranged from
this dose is considered sufficient to assess the risk of the 100 mg tablets). This represents a total database of 1750 patients. [It is to be noted that Dr. Pratt's review, reproduced on page 3 of Dr. Karkowsky consult review, computes 465 patients as the number of patients receiving a single oral dose in CCNV trials and 1431 as the number of patients receiving a single i.v. dose in CCNV trials for an overall total of 1896 receiving ≥100 mg. The reason for the discrepancy in numbers is that Dr. Pratt is including additional randomized and non-randomized data.] Using Dr. R. O'Neill's table, reproduced on page 6 of Dr. Fredd's memorandum of August 16, 1996, seeing no Torsades in this number of patients would give reassurance that a 0.1% incidence would not be missed with a probability of 0.80 to 0.90.

The above computations, however, although useful for regulatory purposes, are considered rough approximations of what may or may not happen in the clinical setting, where electrolyte disbalances (K, Mg, Ca) or pre-existing arrhythmias or cardiovascular heart conditions or concomitant medications may predispose the patient to serious arrhythmias. It is important to reiterate that the clinical experience with DOLA•Mesyl is limited. Patients with arrhythmias and/or CHF were excluded from pivotal chemotherapy trials -043 and -048. The MO reiterates here that more experience is needed on the potential interaction between DOLA•Mesyl and cardiovascular medications in general and those drugs and conditions that prolong the PR, QRS and - in particular - the QT_c intervals. Also lacking are more data on possible interaction of this drug with clinical conditions involving patients with history of cardiovascular disease. But this additional information can be handled by a) appropriate language in the labeling and b) a close post-marketing monitoring of AEs in association with this drug, especially in patients in whom drugs that accumulate in and induce injury to the heart are being administered long-term, such as anthracyclines and anthracendiones.

C. Recommendations From the Cardio-Renal Consultant

In his review of January 16, 1997, the consultant concludes that the 200 mg dose of DOLA•Mesyl has "modest" (my quotes because there are cases where the changes in individual patients were very marked) effects on the cardiovascular system as judged by its effects on EKG. Listed were composite data for the three CCNV and two prevention of PONV studies with the PR, QRS, QT, QT_c and

JTc intervals from EKG points measured ca. 1 to 2h post drug. The conclusion is reached that both PR- and QRS-intervals are unquestionably increased by the drug and that QT_c but JT_c intervals are also increased. This appraisal means that although the main effects of the drug seem to be on depolarization, there also appear to be (although less frequently and less intensely) prolongations of the ventricular repolarization time [this was particularly evident in i.v. study -093]. This information should be included in the labeling.

The consultant points out that DMA, the main metabolite of DOLA•Mesyl, has a chiral center. The action of the enzyme carbonyl reductase on DOLA•Mesyl generates two isomers R-(+)- and L(-)- which co-exist at an unknown ratio in humans [in *in vitro* or *ex vivo* studies, these two isomers have been shown to possess different cardiovascular activity]. It is further pointed out that **NADPH**, normally produced in the hexose monophosphate (HMP) shunt is required for carbonyl reductase activity and that subjects who are G-6PD (glucose 6-phosphate dehydrogenase) deficient may be functionally limited in carbonyl reductase activity. It is speculated that these G-6PD deficient patients may be less able to clear DOLA•Mesyl, resulting in higher concentrations of the parent drug. The consultant ends up with four recommendations, two of which are as follows: 1. An analysis of the data base for those who are G-6PD deficient or alternatively should be a small study looking at the PK and PD of DM/DMA in subjects who are G-6PD deficient; 2. A study of higher single doses of DM in normals. The dose to be studied should be as high as tolerated and should be performed with adequate monitoring with trained personnel available on site, to treat any adverse events.

The MO has very carefully considered these two recommendations. These evaluations included meetings with Dr. Ahmad (who has published on the subject of G-6PD deficiency), Drs. Kauss and Pradhan (the Biopharm reviewers who, in addition, elicited an opinion from Dr. J. Collins) and Dr. L. Talarico (an expert on hematology/coagulation). Also obtained was information from up-to-date standard Biochemistry Textbooks and especially from Beck's Hematology (5^{th} Edition). The MO's assessment is succinctly summarized below.

The biochemical reaction leading to the formulation of DMA, the major active metabolite (α- and β-OH), is depicted in Fig. 1. One important piece of information is that although indeed, carbonyl reductase utilizes NADPH, being that the substrate is an aliphatic ketone, reduction could also be accomplished by **NADH-dependent** enzyme systems [K.C. Leibman, Xenobiotica 1:97 (1971); D.L. Felsted and N.R. Bachur, Mammalian Carbonyl Reductases, Drug Met. Rev. 11:1-60 (1980)]. The source of NADH cofactor of several oxidation-reduction reactions is glycolysis. This means that, for the metabolism of DOLA•Mesyl, NADPH deficiency may be clinically irrelevant since disturbances of glycolysis are extremely rare.

Nonetheless, it is recognized that ca. 5 to 20% of utilized glucose is normally metabolized through the HMP shunt and that this pathway is concerned chiefly with the generation of reducing power. In Fig. 1, the oxidative branch reversible reactions, leading to the formation of ribulose-5-phosphate from glucose-6-phosphate are depicted. The HMP shunt is the major source of NADPH in red cells, two molecules of NADPH being produced for each molecule of glucose metabolized. Traffic through the shunt pathway increases when NADPH

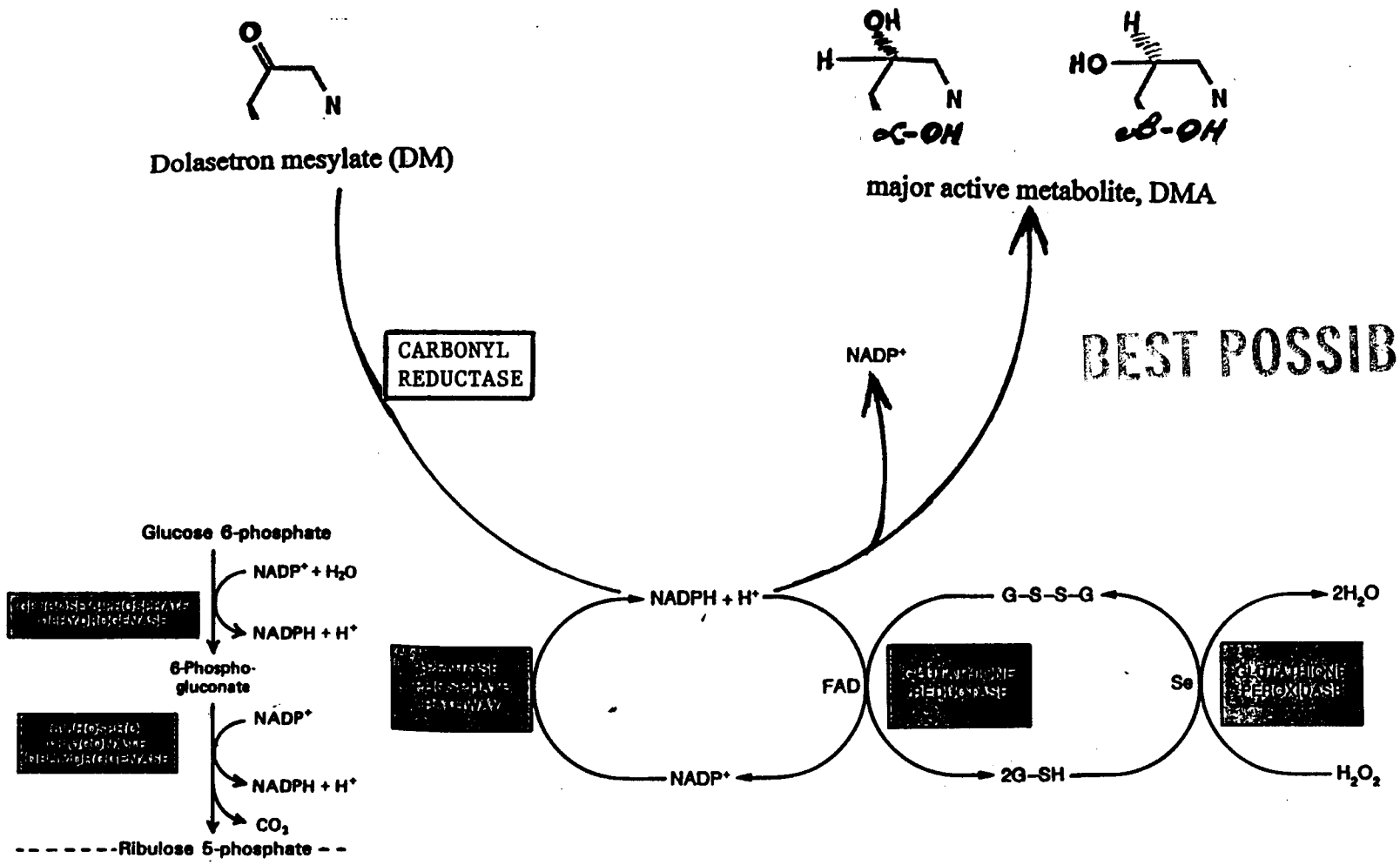

Fig. 1 - DOLA•Mesyl is converted to the major active metabolite by the action of carbonyl reductase which uses NADPH as one of its cofactors. NADPH is generated from the HMP shunt, of which, the irreversible reactions are shown.

Carbonyl reductase may also use NADH as a cofactor (not shown in scheme). NADH originates from glycolysis.

oxidation is accelerated. But, as shown in Fig. 1, the major reactions associated with NADPH oxidation are related to glutathione metabolism.[1] It is true that the majority of shunt defects are associated with diminished G-6-PD activity, which is accompanied by a fall in GSH levels because NADPH synthesis is diminished. Oxidants are thus free to damage cell constituents. Oxidation of Hb produces methemoglobin (in which Fe^{3+} cannot bind oxygen) and denatured Hb (in which globin has been oxidized). The latter precipates as intracellular Heinz bodies. Other aspects of the pathophysiology of the hemolysis associated with G-6-PD deficiency are beyond the scope of the present review.

It is however of interest to mention that more than 350 G-6-PD variants have been described but only a few have been sequenced and in only a few the mutation has been precisely described. It is worth mentioning that, of the known variants, the following are the most important clinically:

- Gd^{B}, the phenotype considered normal, is present in 70% of Caucasians.

- Gd^{A} is a normal variant present in 20% of American blacks. Replacement of asparagine with aspartic acid makes it electrophoretically faster than Gd^{B}.

- Gd^{A-}, the most common variant associated with hemolysis, is found in 11% of American blacks and in higher percentages in many African populations. Its electrophoretic mobility is identical to that of Gd^{A}, but its catalytic activity is impaired. Because it has two nucleotide substitutions, it may be that the A^{-} mutation occurred when A was the predominant genotype.

- Gd^{Med}, the second most common abnormal variant (and the most common among Caucasians), is found in many ethnic groups in the Mediterranean area basin (Italians, Greeks, Sardinians, Sephardic Jews, Arabs, etc.), and in India and southeastern Asia. Its electrophoretic mobility is normal, but its catalytic activity is markedly reduced. It may include several discrete variants.

- Gd^{Canton} is a common variant in Oriental populations that produces a clinical syndrome like that associated with Gd^{A-}.

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[1] Red cells contain a high concentration (2 mM) of reduced glutathione (GSH), a tripeptide (γ-glutamyl-cysteinyl-glycine), that is synthetized de novo by mature red cells and serves as a sulfhydryl buffer, cycling between its reduced form (GSH) and an oxidized form (GSSG), which links two tripeptides by a disulfide bond. GSH acts intracellularly to protect red cells against injury by exogenous and endogenous oxidants, such as superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2), which are produced by macrophages in infection and by red cells in the presence of certain drugs such as primaquine. The ingestion of broad beans (also known as fava beans) **Vicia fava**, can likewise induce a hemolytic anemia in dehydrogenase-deficient people. Accumulation of these agents leads to injury of cell proteins and lipids. This is normally prevented by GSH, which inactivates such oxidants. This detoxification can occur spontaneously, but it is accelerated by **glutathione peroxidase**, a remarkable selenium-containing enzyme. As hydrogen peroxide is reduced in the peroxidase reaction, GSH is oxidized to GSSG and mixed disulfides with protein-thiols (GS-S-protein). (Catalase also degrades peroxides, but under physiologic conditions it is less important.) Regeneration of GSH is catalyzed by glutathione reductase, A flavoprotein in this NADPH-mediated reaction, both GSSG and mixed disulfides are reduced to GSH as NADPH is simultaneously oxidized. This in turn stimulates HMP shunt activity, which regenerates NADPH. The tight coupling of HMP shunt and glutathione metabolism efficiently protects red cells from oxidant injury.

The above-summarized information suggests that Gd^{A-} and Gd^{Med}, the first and second most common abnormal variants associated with hemolysis, are infrequent in the U.S. population.

On the practical side, although it is well established that blood levels of the DOLA•Mesyl metabolite DMA are associated with alterations of the EKG, efficacy of DOLA•Mesyl (the parent drug) does not seem to depend on bioconversion, since unmetabolized DOLA•Mesyl is active (efficacy wise). The consultant recommendations appear to test the hypothesis that the parent drug is the toxic species. But there is no evidence for such a proposal. It appears that the parent drug, which is rapidly and almost quantitatively converted to the metabolite, cannot be more toxic than the metabolite. The hypothesis that the G-6PD deficient patient (actually a variant of the lot) may be more susceptible to EKG alterations following administration of DOLA•Mesyl does not seem tenable. Nevertheless, as proposed by Dr. J. Collins, such a theory may be tested in cardiocytes *in vitro* (see Appendix I, Memorandum of January 31, 1997 from Dr. Pradhan to the MO).

The MO does not believe that the consultant's recommendations Nos. 1 and 2 would be helpful. It is important to mention that studies in small number of subjects have shown that doses as high as 5 mg/Kg (the equivalent of 350 mg single dose) were not accompanied by clinical cardiovascular alterations. Once again, the MO concern is not what would happen in the normal individual given the drug alone and at recommended doses, depending on indication.

On the other hand, the consultant's recommendation No. 3 ["The ECGs of all patients with large cumulative exposures to either daunorubicin or doxorubicin should be analyzed for ECG changes. In the absence of a respectable database a small study should be considered in those who are receiving high cumulative doses"] needs to be carefully considered. Anthracycline (an antileukemic antibiotic; ex. daunorubicin) accumulates in the heart muscle where it induces cardiac toxicity through degeneration and atrophy of cardiac muscle in the area around His's bundle. As pointed out in MOR of May 31, 1996 of NDA 20-623, there is little experience in patients that had been treated with adriamycin long-term and the concomitant administration of DOLA•Mesyl. This situation is compounded by the occurrence of a sudden death reported in NDA 20-624 (DOLA•Mesyl injection). This occurred in a patient six hours after receiving 1.8 mg/Kg intravenous DOLA•Mesyl and concomitant anthracycle. This patient had numerous risk factors including substantial exposure to doxorubicin, prior thoracic irradiation and concomitant cyclophosphamide. But, as pointed out by Dr. C.R. Benedict in his Cardiovascular Export Report for Dolasetron, "there is no way to exclude a causal relationship between dolasetron exposure and this death". The MO recommends that information on this death be succinctly included in the labeling. The MO concludes that there is need for close patient monitoring during DOLA•Mesyl therapy in patients that have received long-term anthracyclines, anthracendiones or other drugs that accumulate in the heart and induce cardiac arrhythmias).

As per consultant's recommendation 3, the sponsor should be asked to analyze the EKGs of all patients with large cumulative exposures to either daunorubicin or doxorubicin for EKG changes. Since a succinct description of

the sudden death in a patient receiving intravenous DOLA•Mesyl and anthracycline and that of complete block in another patient receiving 200 mg of DOLA•Mesyl tablets and verapamil are to be included in the labeling, there seems to be no need to ask the sponsor to consider a "small study in those who are receiving high cumulative doses".

D. Labeling Recommendations

On pages 8-9 of the Consult, the consultant states "I am presuming that warnings or precautions, about the use of this drug in such a population would of course appear prominently in labeling". The consultant is making reference to the following paragraph at the bottom of page 8 of his Consult Review:

> "Aside from those who may have kinetics different from the general population, there are those whose electrocardiographic response to the usual concentrations of DM and DMA may be excessive. Subjects with underlying cardiovascular disease, those with aberrations of electrolytes, those treated with concurrent drugs that modify cardiac conduction may have excessive ECG responses to DM. Unfortunately, all clinical studies excluded subjects with underlying cardiovascular disease, rhythm disturbances that required antiarrhythmic therapy, or those with abnormal ECG intervals at baseline. It is, therefore, unlikely that the already accumulated safety data base will adequately address whether there is a sub-population that is more sensitive to electrocardiographic alterations."

The MO agrees with Consultant's recommendation No. 4. The MO reiterates here his recommendation of including a warning and a precaution section in the labeling for DOLA•Mesyl tablets (as well as for the injection formulation).

/S/ February 5, 1997

Hugo E. Gallo-Torres, M.D., Ph.D.

cc:
NDA 20-623
NDA-20-624
HFD-180
HFD-180/SFredd
HFD-180/HGallo-Torres
r/d 2/5/97 jgw
GEN\20623701.0HG

APPENDIX I

Memorandum

To: Hugo Gallitoris, MD Ph.D.

Through: Lydia Kaus, Ph.D. /S/ 1/31/97 JAN 31 1997

From: Rajendra Pradhan, Ph.D. /S/ 1-31-97

Background: Dolasetron is an antiemetic drug under review by Division of Gastrointestinal and Coagulation Products (HFD-180). The sponsor is requesting an approval for tablet (oral) and injection (IV infusion) forms of dolasetron. Dolasetron is a pro-drug and it is converted to its pharmacodynamically active form (DMA) by an ubiquitous enzyme, carbonyl reductase. Dolasetron and DMA both exhibit cardio-toxicity (Qtc prolongation) to same extent (based on in-vitro studies). The Medical officer (MO) (HFD-180) currently considers 100 mg oral dose to be the safe and effective dose for chemotherapy induced nausea and vomiting and for PONV (for sponsor's proposed doses, refer to proposed labeling). The MO requested the DPE-II, OCPB to compare the systemic exposure at 100 mg dose for the two routes of administration.

In addition, Director of ODE-III had consulted the safety issues on Dolasetron to Division of Cardio-Renal Products (HFD-110). In the response, questions were raised regarding the conversion of pro-drug Dolasetron to DMA. Specifically, concerns were raised about the role glucose 6 phosphate dehydrogenase (G6-PD) plays in carbonyl reductase ability to reduce Dolasetron to DMA. Theoretically, in G6-PD deficiency (due to genetic reasons or administration of other drugs), Dolasetron could stay in systemic circulation for longer duration of time. This however, was not seen in the clinical data base presented by the sponsor. Currently, the MO (HFD-180) is working on this issue and invites any suggestions.

Comments:
In a pharmacokinetic comparison between IV and oral routes at the same doses, for pharmacokinetic parameters such as $AUC_{0-\infty}$ and Cmax for DMA, IV route showed about 30% greater $AUC_{0-\infty}$ and Cmax contribution than oral route. It should also be noted that there is an additional 8% contribution to $AUC_{0-\infty}$ from the prodrug component (DM) when IV is compared to oral. Since, in-vitro DM and DMA are equi-toxic, the 8% contribution from DM could be additive. In other words, **a suitable IV dose to get a similar DMA-oral exposure should be at least 30% lower than the corresponding oral dose**.

Attempts were made to do a similar comparison ($AUC_{0-\infty}$ and Cmax /IV vs. Oral) using a modeling approach. However, the predicted values showed about 8% underestimation bias (predicted Cmax lower than observed Cmax). Therefore, a noncompartmental approach was used to compare the pharmacokinetic parameters between IV and oral route.

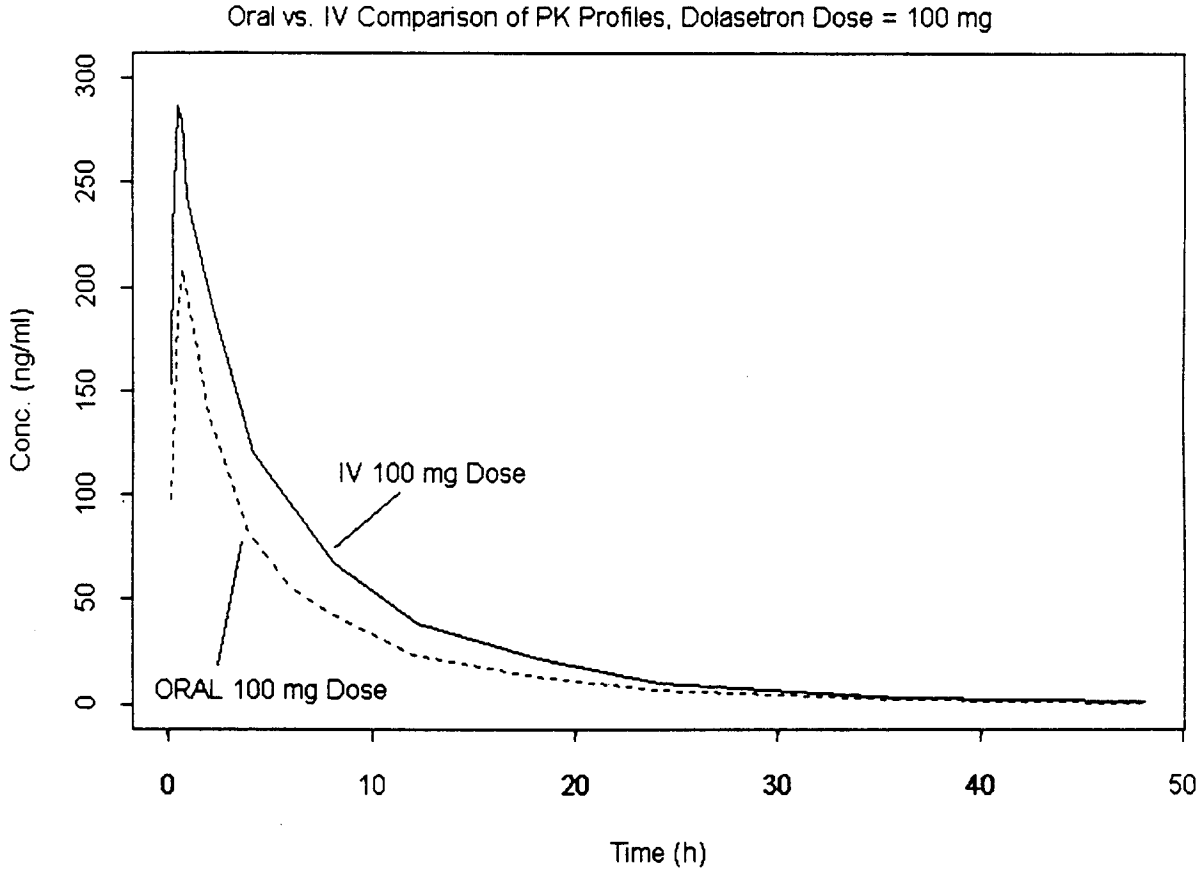
It appears there are few questions unanswered at the current time about the conversion of prodrug to drug. These are as follows:
What is the effect of glucose 6 phosphate dehydrogenase deficiency on the carbonyl reductase that metabolizes DM to DMA?
Is carbonyl reductase, responsible for DM metabolism totally NADPH dependent?

I would advice that we ask these questions to the sponsor.

Lydia Kaus (Team Leader, DPE-II) has discussed this issue with Jerry Collins (Director, DCPR, OTR) and following suggestions were generated.

1. explore the exposure-toxicity in dogs and find out what (DM or DMA) is responsible for the QTc prolongation

2. For QTc prolongation, Dr. Collins think that the simplest test only requires cardiocytes in vitro. Woosley's group at Georgetown used this test system effectively to show that the active metabolite of terfenadine was at least 500-fold less toxic than the parent. [JAMA; 1993; vol 269, p1532]



cc: NDA 20-623 and 20-624, HFD-180, HFD-870 (MChen, Kaus, Pradhan), HFD-850 (Drug, Reviewer), Drug File (Millison)

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: July 24, 1996

FROM: Pharmacology Team Leader
Division of Gastrointestinal and
Coagulation Drug Products, HFD-180

SUBJECT: NDA 20,623 (ANZEMET®/Dolasetron Mesylate) -
Preclinical Portions of the Labeling.

TO: NDA 20,623

The following portions of the attached sponsor's version of labeling (identified) should be replaced or expanded with the accompanying revisions/additions.

1. "PRECAUTIONS"

   a. "Carcinogenesis, Mutagenesis, Impairment of Fertility" - on sponsor's page S4-V1.21-P292 and 293.

   b. "Pregnancy

      Teratogenic Effects. Pregnancy Category B:" - on sponsor's page S4-V1.21-P293.

2. "OVERDOSAGE" - on sponsor's page S4-V1.21-P296.

Revisions

1. PRECAUTIONS

   Carcinogenesis, Mutagenesis, Impairment of Fertility

   In a 24-month carcinogenicity study, mice (CD-1) were treated orally with dolasetron mesylate 75, 150 or 300 mg/kg/day (225, 450 or 900 mg/m^2/day). For a 50 kg person of average height (1.46 m^2 body surface area), these doses represent 1.5, 3 and 6 times the recommended clinical dose (148 mg/m^2) on a body surface area basis. There was a statistically significant (p=0.0001) increase in the incidence of combined hepatocellular adenomas and carcinomas

in males treated with 150 mg/kg/day (450 mg/m^2/day, 3 times the recommended human dose based on body surface area) and above. No increase in liver tumors was observed at a dose of 75 mg/kg/day (225 mg/m^2/day, 1.5 times the recommended human dose based on body surface area) in males and at doses up to 300 mg/kg/day (900 mg/m^2/day, 6 times the recommended clinical dose based on body surface area) in females.

In a 24-month rat (Sprague-Dawley) carcinogenicity study, oral dolasetron mesylate at doses up to 150 mg/kg/day (900 mg/m^2/day, 6 times the recommended human dose based on body surface area) in males and 300 mg/kg/day (1800 mg/m^2/day, 12 times the recommended human dose based on body surface area) in females was not tumorigenic.

Dolasetron mesylate was not genotoxic in the Ames test, the rat lymphocyte chromosomal aberration test, the Chinese hamster ovary (CHO) cell (HGPRT) forward mutation test, the rat hepatocyte unscheduled DNA synthesis (UDS) test or the mouse micronucleus test.

Dolasetron mesylate at oral doses up to 400 mg/kg/day (2400 mg/m^2/day, 16 times the recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

Pregnancy

Teratogenic Effects. Pregnancy Category B. Teratology studies have been performed in pregnant rats at oral doses up to 100 mg/kg/day (4 times the recommended human dose based on body surface area) and pregnant rabbits at oral doses up to 100 mg/kg/day (7.3 times the recommended human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to dolasetron mesylate. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

2. OVERDOSAGE

Single oral doses of dolasetron mesylate at 525 mg/kg in mice (1575 mg/m^2, 10.6 times the recommended human dose based on body surface area) and 400 mg/kg in rats (2400 mg/m^2, 16 times the recommended human dose based on body surface area) were lethal. Symptoms of acute toxicity were tremors, depression and convulsions.

Jasti B. Choudary, Ph.D., B.V.Sc.

cc:
Orig. NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd

JBC/hw/7/25/96
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