"National Toxicology Program."

Al-Awwadi, N., F. Bichon-Laurent, et al. (2004). "Differential effects of sodium tungstate and vanadyl sulfate on vascular responsiveness to vasoactive agents and insulin sensitivity in fructose-fed rats." Can J Physiol Pharmacol **82**(10): 911-8.

High fructose feeding induces insulin resistance, impaired glucose tolerance, and hypertension in rats and mimics most of the features of the metabolic syndrome X. The effects of a 6-week treatment with the transition metals administered in drinking water, vanadium (VOSO4.5H2O, 0.75 mg/mL) or tungsten (Na2O4W, 2 g/mL), were investigated on the reactivity to norepinephrine (NEPI) or acetylcholine (ACh) of thoracic aorta rings isolated from fructose (60%) or standard chow fed rats. Maximal effect (Emax) and pD2 (-log EC50) values were determined in each case in the presence or absence of endothelium, while the degree of insulin resistance was determined using the euglycemic hyper insulinemic glucose clamp technique. Aortic segments isolated from 6-week fructose-fed animals were characterized by NEPI hyperresponsiveness (increase in Emax) and endothelium-dependent NEPI supersensitivity (increase in pD2) without any change in the reactivity to ACh. Vanadium or tungsten administered in fructose-fed animals prevented both hypertension and NEPI hyperresponsiveness, while vanadium, but not tungsten, reduced NEPI supersensitivity. Vanadium, but not tungsten, increased the relaxing activity of ACh, both in control and fructose-fed animals. Insulin resistance associated with high fructose feeding was reversed by vanadium but not by tungsten treatment. The differential effects of the two transition metals on vascular responsiveness to NEPI or ACh may be explained by their differential effects on insulin sensitivity.

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tungsten treatment. The differential effects of the two transition metals on vascular responsiveness to NEPI or ACh may be explained by their differential effects on insulin sensitivity.

Ballester, J., J. Dominguez, et al. (2005). "Tungstate treatment improves Leydig cell function in streptozotocin-diabetic rats." J Androl **26**(6): 706-15.

Oral administration of sodium tungstate to adult male streptozotocin-diabetic rats for 3 months normalized serum levels of glucose, insulin, luteinizing hormone, and follicle-stimulating hormone. These effects were accompanied by an increase in reproductive performance, which was related to a strong improvement in Leydig cell function markers, such as the recovery of the number of Leydig cells and serum testosterone levels. Moreover, this in vivo recovery was related to a concomitant increase in the cell expression of insulin receptors. Tungstate treatment did not modify Leydig cell function in healthy rats. Furthermore, the addition of tungstate or insulin to the mTLC-1 cell line from Leydig cell origin increased the phosphorylation states of MAP-kinase and glycogen synthase kinase-3. Our results indicate that tungstate treatment in diabetic rats leads to a recovery of reproductive performance by increasing the number of Leydig cells. This increase contributes to the recovery of their functionality, thereby improving the overall function of these cells. We propose that this improvement is caused by the combined effect of the tungstate-induced normalization of insulin glucose and luteinizing hormone serum levels and a direct action of the effector on Leydig cells through modulation of at least MAP-kinase and glycogen synthase kinase-3 activities.

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Ballester, J., M. C. Munoz, et al. (2007). "Tungstate administration improves the sexual and reproductive function in female rats with streptozotocin-induced diabetes." Hum Reprod **22**(8): 2128-35.

BACKGROUND Diabetes induces great alterations in female reproductive function. We analyzed the effects of tungstate, an anti-diabetic agent, on the reproductive function of healthy and diabetic female rats. METHODS Healthy and streptozotocin-induced diabetic rats were treated with sodium tungstate (2 mg/ml in their drinking water) for 12 weeks. Markers of reproductive function and diabetes were measured in serum, and in uterus and ovaries by Western blot or RT-PCR. Reproductive function was also assessed by mating. RESULTS Diabetic rats showed great impairment of libido, which was accompanied by a total loss of fertility ($P < 0.05$) and a decrease in the serum levels of FSH ($P < 0.05$) and LH ($P < 0.05$) compared with healthy rats. Tungstate treatment of diabetic rats partially recovered libido while fertility rate increased to 66.6%. This improvement was accompanied by a recovery of serum FSH (to a level higher than healthy rats) and LH. Moreover, tungstate treatment normalized ovarian expression of GLUT 3 hexose transporter, and estrogen, progesterone and FSH receptors, whereas only GLUT 3 and FSH receptors were normalized in the uterus. CONCLUSIONS Our results indicate that the alterations in female reproduction in diabetes were partially reversed after tungstate treatment by a mechanism(s) involving the normalization of serum FSH and LH levels, and ovarian and uterine expression of FSH receptors and GLUT3.

Caujolle, F. and C. Pham Huu (1967). "[Comparative toxicity of sodium chromate, molybdate, tungstate and metavandadate. IV. Tests on dogs]." Agressologie **8**(3): 265-73.

Gomez-Ramos, A., J. Dominguez, et al. (2006). "Sodium tungstate decreases the phosphorylation of tau through GSK3 inactivation." J Neurosci Res **83**(2): 264-73.

Tungstate treatment increases the phosphorylation of glycogen synthase kinase-3beta (GSK3beta) at serine 9, which triggers its inactivation both in cultured neural cells and in vivo. GSK3 phosphorylation is dependent on the activation of extracellular signal-regulated kinases 1/2 (ERK1/2) induced by tungstate. As a consequence of GSK3 inactivation, the phosphorylation of several GSK3-dependent sites of the microtubule-associated protein tau decreases. Tungstate reduces tau phosphorylation only in primed sequences, namely, those prephosphorylated by other kinases before GSK3beta modification, which are serines 198, 199, or 202 and threonine 231. The phosphorylation at these sites is involved in reduction of the interaction of tau with microtubules that occurs in Alzheimer's disease.

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Le Lamer, S., G. Cros, et al. (2002). "An application of population kinetics analysis to estimate pharmacokinetic parameters of sodium tungstate after multiple-dose during preclinical studies in rats." Pharmacol Toxicol **90**(2): 100-5.

The purpose of this study was to use a population approach in the preclinical development program of sodium tungstate in the rat in order i) to compute individual pharmacokinetic parameters of this compound after repeated oral administrations, until the 4-week toxicology study, using an empirical Bayes methodology; and ii) to study the influence of the administered dose, of the gender and of the duration of treatment on the pharmacokinetic parameters. Four studies were used representing a mixture of single intravenous administration and multiple oral administrations. The treatment duration ranged from 7 to 28 days. Intravenous dose was 9 mg/kg; three different oral doses were tested, 50, 100 and 200 mg/kg/day. Plasma concentration profiles versus time were compatible with a two-compartment model. A significant gender effect was found on bioavailability. The duration of treatment and the administered dose did not significantly explain part of the interindividual variability of pharmacokinetic parameters. The absorption of tungsten was rapid (1-3 hr). Total plasma clearance and elimination half-life averaged 2.8 ml/min/kg and 3.04 hr in males, and 3 ml/min/kg and 2.74 hr in females. The bioavailability was on an average 70%; being significantly higher in females than in males (0.78 versus 0.61). This compartmental approach should be considered as complementary to the usual non-compartmental approach used for analysis of preclinical data and should be a valuable tool to characterise the pharacokinetic/pharmacodynamic behaviour of a drug.

Le Lamer, S., G. Cros, et al. (2001). "Estimation of pharmacokinetic parameters of sodium tungstate after multiple-dose during preclinical studies in beagle dogs." Eur J Pharm Sci **14**(4): 323-9.

In this paper, an empirical Bayes methodology was used to determine the pharmacokinetic profile of sodium tungstate in beagle dogs after multiple oral dosing using the P-PHARM computer program. The population estimation algorithm used in P-PHARM is an EM-type procedure. Sodium tungstate was administered orally, three times a day, (i) for 11 days (21 and 42 mg/kg per day) to 18 dogs (nine males and nine females) and (ii) for 13 weeks (15, 30 and 60 mg/kg per day) to 28 dogs (14 males, 14 females). Six other dogs received the compound intravenously (25 and 50 mg/kg). Plasma concentration profiles versus time were compatible with a two-compartment model and first-order kinetics. After oral administration, F (0.61+/-0.086 vs. 0.48+/-0.093), and normalized (to a 7-mg/kg dose of sodium tungstate) AUC (54+/-8.4 vs. 41.2+/-8.5 mg/l x h), C(max) (10.6+/-0.49 vs. 8.5+/-0.57 microg/ml) and C(min) (3.04+/-0.23 vs. 2.04+/-

0.22 microg/ml), were higher in male than in female dogs. However, the introduction of the gender in the final model did not contribute statistically to an improvement of the fit of the population pharmacokinetic model. In males, t(1/2) elimination averaged 3.1+/-0.56 vs. 2.6+/-0.18 h in females. The duration of treatment did not modify statistically the pharmacokinetic parameters. After repeated multiple oral administration of 15-60 mg/kg per day of sodium tungstate, tungsten plasma concentrations increased in proportion to dose. No dose-dependent changes in pharmacokinetic parameters occurred.

Le Lamer, S., P. Poucheret, et al. (2000). "Pharmacokinetics of sodium tungstate in rat and dog: a population approach." J Pharmacol Exp Ther **294**(2): 714-21.

Sodium tungstate has been found to correct hyperglycemia in insulin- and noninsulin-dependent models of diabetes when administered in drinking fluid with a low degree of toxicity; thus, it provides a potential treatment for diabetes. In the present report, pharmacokinetic studies with sodium tungstate were carried out in the Sprague-Dawley rat and beagle dog. This drug was administered either i.v. (8.97 mg/kg in rat; 25 and 50 mg/kg in dog) or orally in the form of solution (35.9 and 107.7 mg/kg in rat; 25 and 50 mg/kg in dog). Tungsten was quantified using an inductively coupled plasma method. Pharmacokinetic parameters were estimated using a population approach. Sodium tungstate followed first order kinetics, and plasma concentration-versus-time data were adequately described by a two-compartment model. In rat, bioavailability was high (92%), whereas it was lower in dog (approximately 65%). The total volume of distribution expressed by unit of body weight was much higher when the animal was smaller (0.46 l/kg in rat versus 0.23 l/kg in dog). The total body clearance normalized by weight, 0.19 l/h/kg in rat versus 0.043 l/h/kg in dog, changed as for the volume of distribution. The elimination half-life was two times higher in dog (approximately 4 h) than in rat (approximately 1.7 h). In the range of 35.9 to 107.7 mg/kg after oral administration in rat and 25 to 50 mg/kg after oral and i.v. administration in dog, tungsten plasma concentrations increased in proportion to dose.

Le Lamer-Dechamps, S., P. Poucheret, et al. (2002). "Influence of food and diabetes on pharmacokinetics of sodium tungstate in rat." Int J Pharm **248**(1-2): 131-9.

In this paper, the influence of food and diabetes on the pharmacokinetics of sodium tungstate in rat was investigated. The compound was administered intravenously (9 mg/kg) and orally in the form of solution (36 mg/kg). An empirical Bayes methodology was used to compute individual pharmacokinetic parameters. Sodium tungstate followed first-order kinetics, and plasma concentration versus time data were described by a two-compartment model. A significant relationship was found between the bioavailability and the status of the animals. Total plasma clearance and elimination half-life averaged 3.1 ml/min/kg and 1.6 h, respectively. Food had some effects on the extent of sodium tungstate absorption. After oral administration, the bioavailability (0.67 versus 0.85), C(max) (6.10 versus 15.2 microg/ml) and AUC (70.7 versus 105 mgh/l) were 20, 60 and 32% lower in fed than in fasted rats, respectively. The presence of cellulose and sulphate anions in rat chow could partially explain the fed state-associated reduction of tungstate bioavailability. In streptozotocin-induced diabetic fed rats, a 25% decrease occurred in

AUC and F, and a 14% increase occurred in the elimination rate constant compared with healthy fed rats. These changes could be explain on the one hand, by the increase of liquid consumption and food intake, and on the other hand, by a gastroparesis in the early diabetic rats.

Le Lamer-Dechamps, S., P. Poucheret, et al. (2003). "Validation of an inductively coupled plasma-mass spectrometry method to quantify tungsten in human plasma. Determination of percentage binding to plasma proteins." Clin Chim Acta **327**(1-2): 39-46.

BACKGROUND: The aim of this paper was to validate an inductively coupled plasma-mass spectrometry (ICP-MS) method to quantify tungsten in human plasma and to study its percentage binding to plasma proteins. METHODS: This method was validated with respect to accuracy, precision, selectivity and limits of quantification and of detection according to Good Laboratory Practice Guidelines. Calibration curves were obtained in the range 10-500 ng/ml. The extent of plasma protein binding was determined by ultrafiltration in the range 40-2000 ng/ml. RESULTS: A significant matrix effect was observed. The linearity of this method was statistically proven. Precision ranged from 0.76% to 6.49%, and accuracy from 97% to 102%. The lower limit of quantification (LLOQ) was 10 ng/ml. The mean percentage of unbound fraction was 89%. CONCLUSIONS: The results obtained indicate that the method described fulfills the accuracy and precision requirements necessary to carry out pharmacokinetic studies in man.

Pham Huu, C. (1965). "The comparative toxicity of sodium chromate, molybdate, tungstate and metavanadate. II. Experiments on rabbits." Arch Int Pharmacodyn Ther **157**(1): 109-14.

Pham Huu, C. and S. Chanvattey (1967). "[Comparative study of sodium chromate, molybdate, tungstate and metavandate. V. Experiments on pigeons, chickens and rats]." Agressologie **8**(5): 433-9.

Piquer, S., S. Barcelo-Batllori, et al. (2007). "Phosphorylation events implicating p38 and PI3K mediate tungstate-effects in MIN6 beta cells." Biochem Biophys Res Commun **358**(2): 385-91.

Oral administration of sodium tungstate is an effective treatment for diabetes in animal models. Several lines of evidence indicate the pancreatic beta cell as one of the targets of tungstate action. Here, we examined the molecular mechanism by which this compound exerts its effects on the beta cell line MIN6. Tungstate treatment induced phosphorylation and subsequent activation of p38 and PI3K which in turn are implicated in tungstate PDX-1 nuclear localization and activation. Although no effect was observed in glucose-induced insulin secretion we found that tungstate activates basal insulin release, a process driven, at least in part, by activation of p38. These results show a direct involvement of p38 and PI3K phosphorylation in the mechanism of action of tungstate in the beta cell.

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Rodriguez-Gallardo, J., R. A. Silvestre, et al. (2000). "Effects of sodium tungstate on insulin and glucagon secretion in the perfused rat pancreas." Eur J Pharmacol **402**(1-2): 199-204.

Both the direct effect of sodium tungstate on insulin and glucagon secretion in the perfused rat pancreas, and the insulin response to glucose and arginine in pancreases isolated from tungstate-pretreated rats were studied. Infusion of tungstate stimulated insulin output in a dose-dependent manner. The insulinotropic effect of tungstate was observed at normal (5.5 mM), and moderately high (9 mM) glucose concentrations, but not at a low glucose concentration (3.2 mM). Tungstate-induced insulin output was blocked by diazoxide, somatostatin, and amylin, suggesting several targets for tungstate at the B-cell secretory machinery. Glucagon release was not modified by tungstate. Pancreases from chronically tungstate-treated rats showed an enhanced response to glucose but not to arginine. Our results indicate that the reported reduction of glycemia caused by tungstate administration is, at least in part, due to its direct insulinotropic activity. Furthermore, chronic tungstate treatment may prime the B-cell, leading to over-response to a glucose stimulus.

Takeuchi, I. K. (1981). "Differential staining of nucleoli and chromatin by sodium tungstate." J Electron Microsc (Tokyo) **30**(2): 150-3.