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Influence of food and diabetes on pharmacokinetics of sodium tungstate in rat

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Abstract

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 μg/ml) and AUC (70.7 versus 105 mg h/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.

Keywords: Sodium tungstate; Healthy rats; Streptozotocin-induced diabetic rats; Food; Population approach

1. Introduction

Tungsten is a transition metal naturally occurring as a mineral (group VIb of the periodic system). Sodium tungstate was found to possess antidiabetic activity, correcting hyperglycaemia in insulin- and noninsulin-dependent models of diabetes (Barbera et al., 1994, 1997). Indeed, this compound increases the effects of insulin both in vivo and in isolated cells and tissues (Barbera et al., 1994; Li et al., 1995). During long-term treatment, sodium tungstate has the capacity to restore glucose homeostasis and to prevent some diabetes-related complications (Barbera et al.,

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2001). The reported reduction of glycemia caused by tungstate administration could be due to its direct insulinotropic activity (Rodríguez-Gallardo et al., 2000). Furthermore, chronic tungstate treatment might prime the B-cell, leading to over-response to a glucose-stimulus (Rodríguez-Gallardo et al., 2000). Sodium tungstate represents therefore a potential treatment of diabetes and should be tested clinically. Nowadays, this compound is under investigation and some preclinical studies have been carried out. However, studies on the behavior of sodium tungstate in animals are scarce (Kaye, 1968; Aamodt, 1973; Mullen et al., 1976; Young et al., 1982; Mason et al., 1989; Leggett, 1997).

We recently reported detailed pharmacokinetics in rat and dog after single and repeated doses (Le Lamer et al., 2000, 2001, 2002). Preclinical pharmacokinetic studies using single doses (intravenous, iv and oral) are generally performed in fasted animals, while toxicokinetic studies are performed in the fed state. It is well known that the food intake can alter the pharmacokinetic parameters of many compounds and consequently influence significantly the in vivo determination of activity and toxicity. Moreover, the pathological state could influence the pharmacokinetic behavior of the drug.

The present study was therefore conducted to determine the influence of food and diabetes on the pharmacokinetic parameters of sodium tungstate in rat. Individual pharmacokinetic parameters were estimated using a population methodology. Such an approach is increasingly used and advocated in drug development, not only in clinical trials with sparse data but also in the early stages of the development (Aarons, 1993; Burtin et al., 1996; Bouzom et al., 2000).

2. Materials and methods

This research adhered to the 'Principles of Laboratory Animal Care' (NIH publication #85-23, revised 1985).

2.1. Animals

This study was conducted in male Sprague–Dawley rats. Two hundred and seventy six rats (IFFA CREDO, L'Arbresle Cedex, France) at age 10 weeks were used (six animals per time-point). They were gang-housed in stainless steel cages with suspended wire-mesh floors (maximum of three rats per cage). All animals underwent a period of two weeks of observation and acclimatization before treatment. The rats had free access to a pelleted rat diet (UAR sterile food, Usine d'Alimentation Rationnelle, Villemoisson, Epinay s/Orge, France) and the water was offered to the animals ad libitum, in bottles. The housing rooms had controlled environmental conditions with temperature and relative humidity of approximately 18–21 °C and 40–70%, respectively and artificial lighting, alternating 12 h light/dark cycle.

Diabetes was induced by a single iv injection of streptozotocin (STZ, 60 mg/kg body weight) in 0.9% sodium chloride. Diabetes was confirmed by determination of glycemia. Treatment started 8 days after STZ administration.

2.2. Drug administration

Single iv and oral doses of sodium tungstate were administered to each rat. For which, two different solutions of sodium tungstate were prepared on the day of administration; one solution in 0.9% sterile isotonic saline (4.5 mg/ml) for iv administration and one solution in distilled water (3.6 mg/ml) for oral administration. These solutions were used to treat animals, under the administered volume of 2 ml/kg (iv) and 10 ml/kg (oral).

These studies are summarized as follows: (i) 66 healthy rats received a single dose of sodium tungstate (9 mg/kg) by iv route; (ii) 66 healthy rats received a single oral dose of sodium tungstate (36 mg/kg) after an overnight fast (12 h); (iii) 66 healthy fed rats received a single oral dose of sodium tungstate (36 mg/kg) and (iv) 60 STZ-induced diabetic fed rats received a single oral dose of sodium tungstate (36 mg/kg).

For iv administrations, the dose was administered over 1 min into the tail vein. For oral

administrations, the drug was given by gavage through stomach tubing using a polypropylene catheter.

2.3. Blood sampling

After iv and oral administrations, blood samples (one sample per rat) were collected (six animals per time-point) before administration (to evaluate basal tungsten levels), then 5, 10, 15, 30 min, and 1, 2, 4, 8, 12, 16 (except for STZ-induced diabetic fed rats) and 24 h postdose.

Blood samples were collected in heparinized polypropylene tubes then immediately centrifuged at $2000 \times g$ for 20 min. Plasma was removed and transferred to other tubes, frozen and stored at -20 °C, until assayed.

2.4. Assay method

Tungsten was quantified using an inductively coupled plasma emission spectrometric method (Poucheret et al., 2000). Samples were directly nebulized; each determination was performed in replicate ($n = 5$). Precision ranged from 0.4 to 17%, and accuracy was between 89 and 105%. Dilution has no influence on the performance of the method, which could then be used to quantify plasma samples containing up to 90 μg/ml. The limit of quantification was 100 ng/ml (precision, 17%).

2.5. Population pharmacokinetic analysis

Individual pharmacokinetic parameters were estimated using a Bayesian methodology and the baseline-corrected plasma concentrations. The population analysis was performed using the P-PHARM computer program (P-PHARM User's guide, 1993; Gomeni et al., 1994). The population estimation algorithm used in P-PHARM is an EM-type procedure (Dempster et al., 1977).

2.5.1. Pharmacostatistical model

As previously published (Le Lamer et al., 2000), a two-compartment structural pharmacokinetic model with first-order absorption and elimination was used to fit tungsten plasma concentration–time data. Thus, the six-dimensional vectors, θ, of kinetic parameters considered in the population analysis consist of clearance ($\theta_1 = \mathrm{CL}$), initial volume of distribution ($\theta_2 = V$), transfer rate constants ($\theta_3 = k_{12}$ and $\theta_4 = k_{21}$), absorption rate constant ($\theta_5 = k_a$), and bioavailability ($\theta_6 = F$). The error variance was described by an homoscedastic model. On the basis of the examination of the objective function and of the inspection of weighted residuals and individual predicted-versus-observed concentration plots, results indicate that, for V, the probability distribution of the random effect parameter was better described by log normal rather than normal distribution; for the other parameters a normal distribution was used. Estimation of population parameters was performed using a three-step approach.

Several secondary pharmacokinetic parameters were calculated from the individual (Bayesian estimates) primary pharmacokinetic parameters: (i) the model-predicted maximum concentration (C_{max}); (ii) the time of peak (t_{max}); (iii) the area under the plasma concentration–time curve (AUC) computed as F dose/CL; (iv) the elimination half-life ($t_{1/2\ \mathrm{elim}}$) computed as

$$t_{1/2\ \mathrm{elim}} = \frac{0.693 \times 2}{(k_{12} + k_{21} + k_{el}) - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4k_{21}k_{el}}} \quad (1)$$

with $k_{el} = \mathrm{CL}/V$; and (v) the volume of distribution ($V_{\text{d-beta}}$) computed as $V_{\text{d-beta}} = (\mathrm{CL}\, t_{1/2\ \mathrm{elim}})/0.693$. Moreover, the plasma concentration of tungsten at any time can be easily predicted using the empirical Bayes estimate of the pharmacokinetic parameters.

2.5.2. Model acceptance

The adequacy of the model to the data was judged by using graphics and descriptive statistics. Individual predicted concentrations (IPRED) were plotted versus observed concentrations (DV) and results were compared to the reference line of slope = 1 and intercept = 0. Moreover, residuals (DV–IPRED) were plotted versus time and versus predicted concentrations. A standardized concentration prediction error (SCPE) was calculated as follows: SCPE = [DV − IPRED]/S.D.(IPRED),

Table 1
Population pharmacokinetic parameters of sodium tungstate in rat

Parameters	Distribution	Population parameters without covariates		Population parameters with covariates	
		Mean value	CV (%)	Mean value	CV (%)
CL (l/h/kg)	Normal	0.187	49.6	0.188	37.4
V (l/kg)	Log-normal	0.191	65.3	0.178	63.0
k_{12} (h^{-1})	Normal	1.19	53.2	1.15	53.4
k_{21} (h^{-1})	Normal	1.32	46.8	1.34	45.6
k_a (h^{-1})	Normal	0.27	53.2	0.26	50.9
F	Normal	0.72	26.2	0.70	23.1
Sigma[a]		0.215		0.146	
Objective function[b]		967.2		947.9	

CL, total plasma clearance; V, initial volume of distribution; k_{12} and k_{21}, two transfer rate constants; k_a, absorption rate; F: bioavailability.

[a] The sigma value is the residual variability.

[b] Computed from the log-likelihood value.

where S.D.(IPRED) represents the estimated standard deviation on the predicted values computed using all sources of random variability including

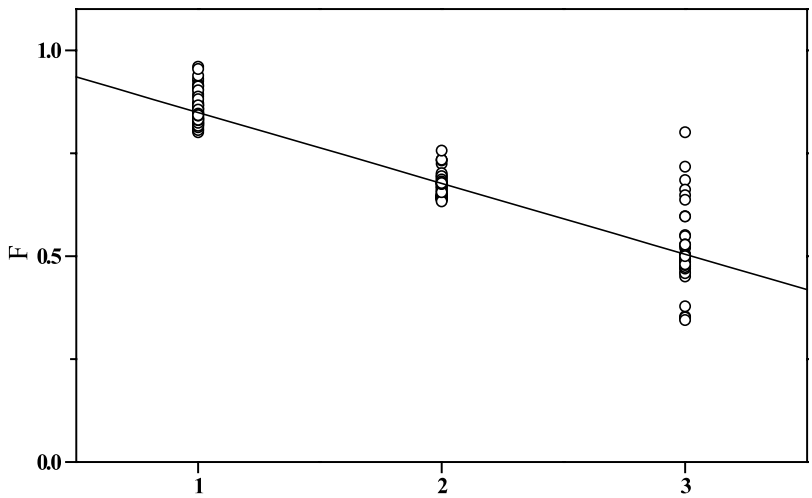

Fig. 1. Relationship between the bioavailability (F) and the status of the animals (1) healthy fasted rats; (2) healthy fed rats; (3) diabetic fed rats.

the residual error. Then, the student t-test was used to compare the mean residual value to zero; the Kolmogorov–Smirnov test was used to compare the sampled distribution to the expected one ($N(0,1)$) (Sokal and Rohlf, 1969).

The model was accepted when (i) plots showed no systematic pattern and, (ii) descriptive statistics did not show any systematic deviation from the initial hypothesis.

3. Results

The mean endogenous tungsten concentration was 0.14 μg/ml. Pharmacokinetic analyses were performed using the baseline-corrected plasma concentrations. 12–16 h after iv administration (16 rats) and 16–24 h after oral administration (21 rats), concentrations returned to the baseline value.

3.1. Population pharmacokinetic analysis

A total of 221 concentrations were used to compute population parameters. In step 1 of the analysis, population pharmacokinetic parameters with their inter-individual variabilities were computed assuming that no dependency exists between pharmacokinetic parameters and the status of the animals. Results are given in Table 1. In step 2, the relationship between the posterior individual estimates and the status of the animals (fasted rats, fed rats or STZ-induced diabetic fed rats) was investigated. The stepwise inclusion (Draper and

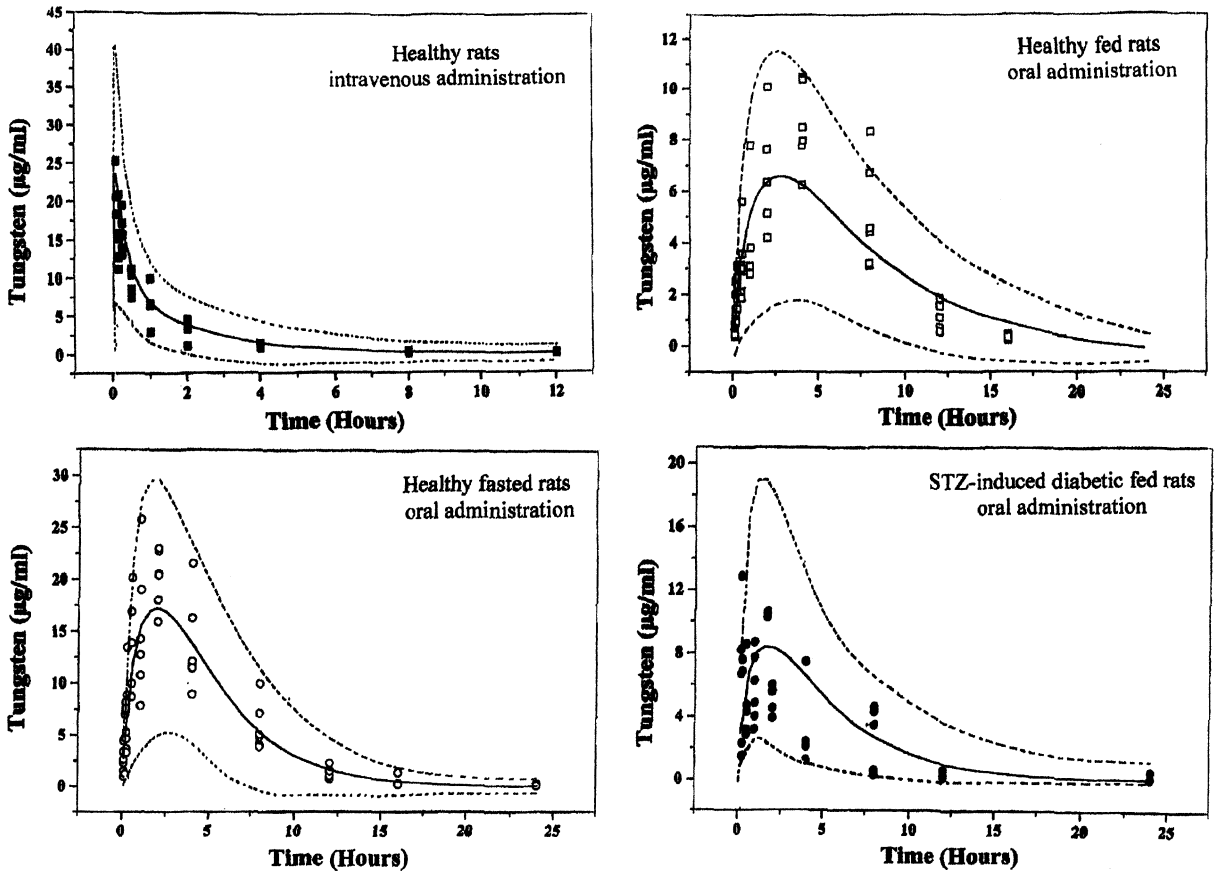

Fig. 2. Population fitting of data. The solid lines represent the average plasma concentrations and the dotted lines represent the 95% confidence interval.

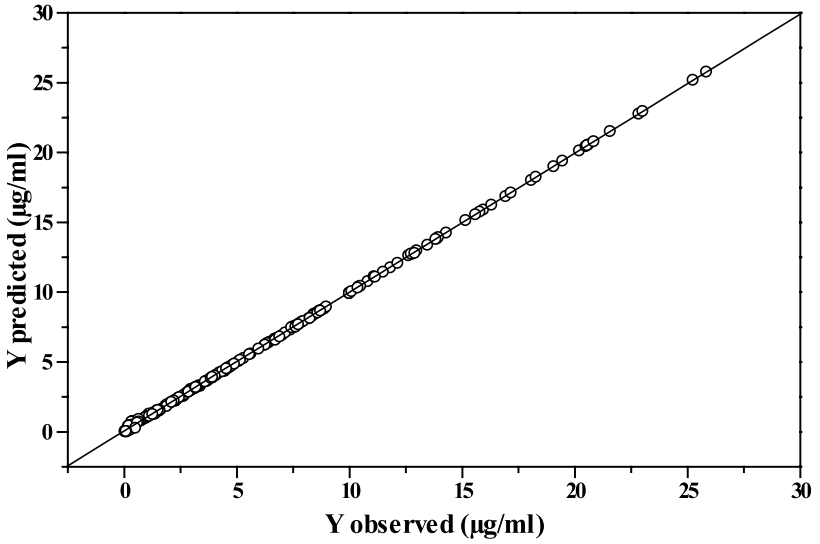

Fig. 3. Scatter plot of predicted concentrations (Bayesian estimates) vs. observed concentrations.

Smith, 1966) performed on P-PHARM revealed a status effect on CL and F, explaining 13.0 and 23% of the inter-individual variability of these parameters, respectively. In a further step, the relationships between CL and F, and the status of the animals were individually added to the model and were included on the basis of the objective function value. The difference in the objective function (δ) between the full model and the basic model was only significant when the relationship between F and the status of the animals (Fig. 1) was included in the model, $\delta = 19.3$, $P < 0.001$ (Table 1).

Graphical representations of the population fitting are shown in Fig. 2.

3.2. Model acceptance

Under the assumption of a correct regression model and unbiased parameter estimates, the Kolmogorov–Smirnov test showed that the SCPE distribution was not significantly different from a normal distribution $N(0,1)$. The mean SCPE value (−0.030) was not significantly different from zero (student t-test) and the 95% confidence intervals included the zero value (−0.094, 0.016). The goodness of fit is shown by the analysis of the scatter plot of the posterior predicted values versus the individual observed concentrations (Fig. 3).

3.3. Mean posterior pharmacokinetic parameters

From computed population parameters, a posterior individual estimation of pharmacokinetic parameters was performed. The mean (±S.D.) posterior pharmacokinetic parameters with minimum and maximum values are reported in Table 2. After oral administrations, F, C_{max} and AUC were 20, 60 and 32% lower in fed than in fasted rats, respectively. Compared to healthy fed rats, a 14% decrease in the elimination half-life and a 25% decrease in AUC and F were observed in STZ-induced diabetic fed rats.

4. Discussion

The aim of the present study was to evaluate the influence of food and diabetes on the pharmaco-

Table 2
Mean posterior pharmacokinetic parameters in male rat computed using the population approach

Parameters	Intravenous administration (9 mg/kg)		Oral administration, healthy fasted rats (36 mg/kg)		Oral administration, healthy fed rats (36 mg/kg)		Oral administration diabetic fed rats (36 mg/kg)	
	Mean (±S.D.)	Min–max	Mean (±S.D.)	Min–max	Mean (±S.D.)	Min–max	Mean (±S.D.)	Min–max
C_{max} (μg/ml)	–	–	15.2 (±4.10)	10.3–26.3	6.07 (±2.07)	3.55–11.9	6.92 (±2.84)	3.98–15.2
t_{max} (h)	–	–	2.71 (±0.45)	1.9–4.2	3.34 (±0.62)	2.3–4.6	2.91 (±0.98)	1.6–6.3
CL (l/h/kg)	0.194 (±0.024)	0.12–0.27	0.172 (±0.033)	0.077–0.22	0.191 (±0.016)	0.12–0.22	0.200 (±0.024)	0.15–0.25
V (l/kg)	0.197 (±0.039)	0.122–0.330	–	–	–	–	–	–
$V_{d\text{-beta}}$ (l/kg)	0.483 (±0.157)	0.293–1.23	0.373 (±0.055)	0.23–0.47	0.440 (±0.033)	0.38–0.50	0.400 (±0.06)	0.21–0.48
k_{12} (h^{-1})	1.20 (±0.155)	0.661–1.56	–	–	–	–	–	–
k_{21} (h^{-1})	1.30 (±0.141)	0.457–1.44	–	–	–	–	–	–
AUC (mg h/l)	26.2 (±3.80)	18.5–41.1	104.6 (±29.4)	75.3–216	70.7 (±11.2)	58.3–131	53.0 (±15.6)	27.1–103
$t_{1/2dist}$ (h)	0.22 (±0.0073)	0.21–0.25	–	–	–	–	–	–
$t_{1/2elim}$ (h)	1.71 (±0.426)	0.751–3.45	1.54 (±0.32)	1.09–2.94	1.61 (±0.18)	1.21–2.39	1.39 (±0.25)	0.93–1.74
$t_{1/2}$ ka (h)	–	–	2.37 (±0.62)	1.61–5.69	3.64 (±1.37)	2.06–7.03	3.13 (±2.12)	1.40–11.0
F	–	–	0.85 (±0.04)	0.80–0.96	0.67 (±0.029)	0.63–0.76	0.51 (±0.097)	0.61–0.80

C_{max}, maximum plasma concentration; t_{max}, time of C_{max}; CL, total plasma clearance; V, initial volume of distribution; $V_{d\text{-beta}}$, steady-state volume of distribution; k_{12} and k_{21}, two transfer rate constants; AUC, area under plasma concentration versus time curve; $t_{1/2dist}$, distribution half-life; $t_{1/2elim}$, elimination half-life; $t_{1/2}$ k_a, absorption half-life; F, bioavailability.

kinetic profile of sodium tungstate in rat. Since only one sample per rat was taken, an empirical Bayes methodology was used to compute individual pharmacokinetic parameters. This compartmental analysis should be considered as complementary to the usual noncompartmental approach used for analysis of preclinical data (Aarons, 1993; Burtin et al., 1996; Bouzom et al., 2000). In order to estimate the bioavailability after oral administration, part of the animals received iv administrations of the compound. Plasma concentration profiles versus time were compatible with a two-compartment model and first-order kinetics.

Our results indicated that the absorption of tungsten was rapid (t_{max} 1.6–6.3 h). In healthy animals, total plasma clearance and elimination half-life averaged 3.1 ml/min/kg and 1.6 h, respectively. Food had some effect on the extent of sodium tungstate absorption. Indeed, the plasma concentration–time profiles in fasted and fed states were not identical, plasma concentration values were lower in the fed state for about 4 h after administration; afterwards, plasma concentrations were similar (Fig. 2). After oral administration, bioavailability (0.67 versus 0.85), C_{max} (6.10 versus 15.2 μg/ml) and AUC (70.7 versus 105 mg h/l) were 20, 60 and 32% lower in fed than in fasted rats, respectively. These results are similar to those previously published in fasted animals (Le Lamer et al., 2000), and similar to those observed during toxicokinetic studies (Le Lamer et al., 2002).

Cardin and Mason (1976) have found that the maximum rate of transport of tungstate through the small intestine of the rat, studied in vitro using the everted sac technique, occurred in the lower ileum. Molybdate, tungstate and sulphate are readily transported across the lower ileum in the rat by a common transport system subject to competitive inhibition between those anions. Because the dietary level of sulphate is higher than that of tungstate, the extent of tungstat ingested may be lowered by the presence of luminal sulphate levels. It has been also found that gastrointestinal uptake of tungstate was reduced substantially in sheep by diet high in roughage, perhaps due to adsorption of tracer levels of tungstate to feed particles high in cellulose (Bell and Sneed, 1970). The presence of cellulose ($\sim$ 8%) and sulphate anions ($\sim$ 1%) in rat chow can therefore explain, at least partially, the fed state-associated reduction of tungstate bioavailability.

In STZ-induced diabetic fed rats, a 25% decrease occurred in AUC and F compared with healthy fed rats. Moreover, a slight increase ($\sim$ 14%) was detected in the elimination rate constant of sodium tungstate. The observed decreases in bioavailability and elimination half-life 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 (Chang et al., 1997). Actually, acute hyperglycaemia is far from benign and causes rapid endothelial impairment (Bohlen et al., 2002). Hyperglycaemia is one of the mechanisms to delay liquid gastric emptying. Moreover, dysmotility is probably one of factors enabled to impair the body weight gain (Chang et al., 1997). This gastroparesis involves a ferment with diarrhoea. In these diabetic rats, the inter-individual variability in pharmacokinetic parameters was higher than that observed in healthy animals. Sodium tungstate has been administered 8 days after STZ injection. This administration induced pancreas impairments during the following 3 weeks; thus, the day of kinetics, different degrees of diabetes occurred according to the animal.

In conclusion, concomitant food intake decreases significantly the bioavailability of sodium tungstate. In STZ-induced diabetic rats, pharmacokinetic parameters of sodium tungstate are significantly different from those computed in healthy rats.

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