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Bioavailability of silicon and aluminum from Zeolite A in dogs

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Abstract

A study in beagle dogs was carried out to estimate the bioavailability of silicon and aluminum from Zeolite A administered as a capsule, oral suspension, and oral solution relative to an intravenous bolus infusion (i.v.) administered over a 1–1.5 min interval. Twelve dogs received single doses of Zeolite A after a 1 week control period in a randomized five-way crossover design. Plasma samples were drawn at time 0 and for 36 h after dosing. The concentrations of silicon and aluminum were determined by graphite furnace atomic absorption at the University of North Carolina School of Medicine (Bioanalytical Laboratory, UNC). The plasma silicon and aluminum data from i.v. infusion were best described by two-compartment and three-compartment open models, respectively. The mean elimination half-life and clearance of silicon from the i.v. dose were 17.5 h and 0.221 $\pm$ 0.0192 ml/min per kg. The mean extent of absorption of silicon from the oral capsule, oral solution and oral suspension was 2.33%, 3.44% and 2.73%, respectively, relative to the intravenous bolus. The mean elimination half-life and clearance of aluminum were 91.2 h and 0.0497 $\pm$ 0.0082 ml/min per kg. The extent of absorption of aluminum from the oral dosage forms was less than 0.1%, relative to the intravenous infusion. The plasma aluminum AUC values from the oral capsule and suspension showed no statistical difference from those during the control period, but the aluminum AUC of the oral solution was statistically greater than the AUC of the corresponding control period.

Keywords: Zeolite A; Bioavailability; Dogs; Osteoporosis; Silicon; Aluminum; Pharmacokinetics

1. Introduction

Zeolite A is a synthetic zeolite which may have therapeutic utility in osteoporotic individuals because of its ability to stimulate bone formation. In vivo studies have suggested a physiological role for silicon in the bone calcification process (Carlisle, 1970; Carlisle, 1980; Carlisle, 1981). The results from previous studies indicate that silicon may be responsible for the pharmacologic activity of Zeolite A (Keeting et al., 1992). Recently, Zeolite A has been shown to induce osteoblast proliferation in vitro (Keeting et al., 1992). Zeolite A has also demonstrated improved calcium utilization in chickens fed low calcium diets (Leach et al., 1990; Ballard and Edwards, 1988). A 2 month study of dietary administration of Zeolite A in female monkeys indicated that both aluminum and silicon were absorbed; however, the blood sampling schedule was too short for pharmacokinetic evaluation (Cefali).

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Toxicology studies are based on the assumption that the administered compounds are in some part absorbed via the administration route; therefore, it is important to identify the best absorbed formulations prior to initiating toxicology studies. This study was designed to investigate the bioavailability of silicon and aluminum from Zeolite A as a capsule, solution, and suspension, relative to an intravenous bolus.

2. Materials and methods

Twelve female beagle dogs, 6–8 months of age at initiation of dosing, weighing from 7.3 to 8.7 kg were used in the study. The dogs were identified by ear tatoo. Zeolite A (N-0974) raw material (Ethyl, lot no. 6), Zeolite A suspension 10 mg/ml (MF no. 2705, Whitby, lot no. 908-11), empty clear gelatin capsules (Jorgensen Laboratories, lot no. 614), glacial acetic acid, ACS reagent grade (Fisher, lot no. FL030589), sodium chloride (USP, lot no. 127 F-03021), and sterile water for injection (USP, lot no. J1A001C) were used. The encapsulated test article was prepared at the study site. All doses were body weight adjusted. Solutions containing 0.4% by weight Zeolite A were prepared in a 0.1 M acetic acid/sterile water solution with the proper amount of sodium chloride present to maintain osmolality.

This study was designed at Whitby Research in Richmond Virginia and conducted at International Research and Development Corporation (IRDC) in Mattawan Michigan. The dogs were individually housed in stainless-steel cages and maintained in an environmentally controlled room (72 ± 1.4°F, humidity 42 ± 7.9%) with a 12-h light/dark cycle. Water was available ad libitum. Diet was available for 2–3 h per day, and was provided at approximately 4 h after dosing for 3 h on the days of dosing. A 7 day control phase was initiated prior to the start of dosing. Each animal was then dosed with an oral capsule 30 mg/kg, oral solution 30 mg/kg (4 mg/ml), oral suspension 30 mg/kg (10 mg/ml) and i.v. solution 20 mg/kg (4 mg/ml) administered over 1–1.5 min The oral solution and suspension were delivered via gavage, followed by oral gavage with deionized water to ensure that all of the test article was delivered. The i.v. dose was administered into a vein in either leg. The duration of each injection was generally 1–1.5 min. Blood was obtained via the jugular vein at 0 h (prior to dosing) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h after dosing. Plasma samples were stored frozen at −15°C until analysis.

2.1. Assay procedures

The plasma samples were assayed for silicon and aluminum at the University of North Carolina School of Medicine Bioanalytical Laboratory by graphite furnace atomic absorption spectrometry (Gitelman and Alderman, 1989; Gitelman and Alderman, 1990). The assay uses standards of silicon or aluminum to estimate plasma concentrations. All sample storage and preparation was carried out using polypropylene equipment to avoid silicon contamination from glass.

2.2. Pharmacokinetic modeling

Pharmacokinetic parameter estimates for silicon were obtained using baseline corrected data. Baseline correction was carried out by subtracting control data from treatment data for each dog. All negative values obtained by baseline subtraction were treated as zero. Non-baseline corrected AUC silicon values were compared with the silicon AUC data obtained during the control period in order to establish that statistically significant absorption of silicon from Zeolite A had occurred. Pharmacokinetic parameter estimates for aluminum were determined using non-baseline corrected data, due to the low aluminum concentrations achieved in the treatments and the irregular nature of the plasma aluminum data during the control period. Initial estimates of the coefficients and exponents for the pharmacokinetic models of silicon and aluminum were obtained using JANA (Dunne, 1987). The intravenous data were fitted assuming a bolus input. The final pharmacokinetic parameter estimates for silicon were obtained by fitting the data to a standard bi-exponential equation using PCNONLIN (Metzler and Weiner, Version 3.0). Estimates for alu-

Table 1
Percent silicon and aluminum content of Zeolite A solution, suspension and capsule

Zeolite ADosage form	% Silicon content	% Aluminum content
Solution 4 mg/ml[a]	14.0	15.3
Suspension 10 mg/ml	13.2	14.6
Capsule	15.4	14.8

[a]Solution used for both i.v. and oral dosing.

minum were obtained by fitting the data to a standard tri-exponential equation also using PCNONLIN. Area under the curve (AUC) was calculated using the linear trapezoidal rule. The dose of silicon or aluminum given (X_o) was calculated by multiplying the percentage of silicon or aluminum measured in each dosage form (Table 1) by the corresponding Zeolite A dose. AUC was extrapolated to infinity (AUC_∞). Volume of distribution (Vd_{area}), volume of the central compartment (V_c), clearance (Cl) and extent of absorption were calculated from the intravenous data by standard pharmacokinetic equations. The mean β half-life for both silicon and aluminum were estimated by dividing 0.693 by the mean elimination rate constant.

3. Results and discussion

3.1. Pharmacokinetics: silicon

The mean concentration versus time plot of non-baseline corrected plasma silicon is shown in Fig. 1. Plasma silicon levels in the control period increased substantially after 4 h. This corresponds to the start of feeding, suggesting that increased plasma silicon levels during the control period were due to silicon uptake from the diet. Therefore the plasma profiles after Zeolite A dosing were baseline corrected to account for the contribution by diet.

The mean uncorrected i.v. curve demonstrated a slight decrease in plasma levels between 1.5 and 4 h, returning to higher concentrations at 6 h and continuing with a log-linear decline (Fig. 1). Review of the individual data revealed that the observed initial drop in the mean curve was due to decreased values observed in only 4 of 12 dogs. A reason for the different profiles could not be ascertained.

PCNONLIN estimates indicated that a two-compartment model produced a better fit of the silicon plasma data. The mean β, $\beta t_{1/2}$, α, Cl, and V_c estimates for silicon from the i.v. data were 0.0487 ± 0.00758/h, 17.5 h, 1.12 ± 0.401/h,

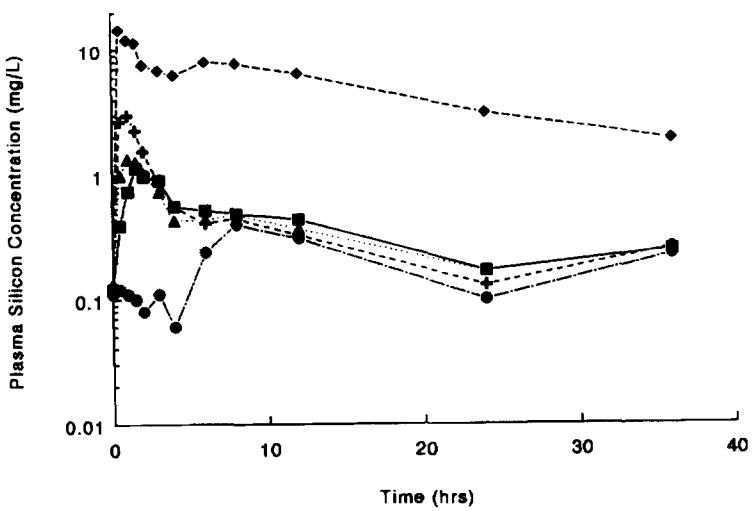

Fig. 1. Mean uncorrected plasma silicon from 12 dogs after receiving the following doses of Zeolite A: ●, control; ■, capsule 30 mg/kg Zeolite A; +, solution 30 mg/kg Zeolite A; ◆, intravenous 20 mg/kg Zeolite A; ▲, suspension 30 mg/kg Zeolite A.

Table 2
Pharmacokinetic estimates for plasma silicon and aluminum from 20 mg/kg i.v. Zeolite A (12 dogs)

	Silicon[a]		Aluminum	
	Mean	S.D.	Mean	S.D.
Dose (mg/kg)	2.799	–	3.067	–
AUC_{∞} (mg/h per l)	213	19.5	1050	193
β (h^{-1})	0.0487	0.00758	0.0076	0.0054
$\beta t_{1/2}$ (h)	17.5	[b]	91.2	[b]
V_c	0.156	0.0235	0.0553	0.0045
Cl (ml/min per kg)	0.221	0.0192	0.0497	0.0082
C_{max} (mg/l)	18.3	3.03	55.71	4.50
$AUC_{(trap)}$ (mg/h per l)	180	14.08	1046	191
α (h^{-1})	1.12	0.401	1.406	0.438

[a]Plasma concentrations were baseline corrected.
[b]Based on mean rate constant.

0.221 ± 0.0192 ml/min per kg, and 0.156 ± 0.0235 l/kg, respectively (Table 2), suggesting slow clearance of silicon from plasma and a small volume of distribution. The silicon AUC values for each individual dog were calculated using both baseline corrected and uncorrected data. The mean uncorrected silicon AUC values were higher for the dogs receiving the Zeolite A solution than for those receiving any other oral dosage form (14.72 μg/h per l). The mean AUC value for the dogs during the control period was the smallest (7.14 μg/h per l). The AUC values of the uncorrected data were compared using a one-way ANOVA. The ANOVA indicated that there was a significant difference between the groups ($P < 0.001$). A comparison using Bonferroni simultaneous confidence intervals further demonstrated that the AUC values for the oral dosage forms were all significantly different from the AUC of the control, indicating that there was statistically significant absorption of silicon from the three oral dosage forms of Zeolite A. No statistical difference between the three oral dosage forms was detected.

The dose-corrected extents of silicon absorption for the oral capsule, oral solution and oral suspension relative to the i.v. dose (Table 3) were 2.33%, 3.44%, 2.73%, respectively. The mean baseline corrected C_{max} values for the oral capsule, solution and suspension were 1.28 ± 1.13, 2.87 ± 0.37, and 1.44 ± 0.990 mg/l, respectively. The mean T_{max} values for the oral capsule, solution and suspension were 3.67 ± 4.00, 0.83 ± 0.25 and 5.88 ± 7.44 h, respectively. Both the C_{max} and T_{max} data from dogs receiving suspension and capsules were highly variable. Five dogs receiving capsules exhibited C_{max} plasma levels which were nearly an order of magnitude lower than the values obtained for the other seven dogs. Two of these five had a T_{max} of 12 h, in contrast to T_{max} values ranging from 1 to 4 h for the other ten dogs. Similarly, four of the 12 dogs receiving suspension manifested low C_{max} values. All four demonstrating low C_{max} values, had T_{max} values greater than or equal to 12 h. All dogs receiving the solution had similar C_{max} values ranging from 2.33 to 3.66 mg/l, and all had T_{max} values ranging from 0.5 to 1.0 h. Although absorption of silicon occurs from all three dosage forms, the data suggest that absorption from the capsule and suspension is more variable than absorption from solution. Also, the extent of absorption from the oral solution appears to be higher and faster than from the capsule and the suspension.

3.2. Pharmacokinetics: aluminum

Plasma aluminum concentrations during the control phase were highly variable and demonstrated no discernable trend (Fig. 2). The random nature of these concentrations suggested that baseline correction would be futile. While the plasma aluminum concentrations after oral doses were not very different than control concentrations, plasma concentrations of aluminum after the i.v. dose were high. Because of the high plasma aluminum concentrations following the i.v. Zeolite A dose, and the apparent long half-life of aluminum, a distinct sequence effect was observed which affected the oral doses of Zeolite A (Table 4). Therefore, the initial estimates for i.v. aluminum were obtained using the data from Dosing Group 3. The terminal elimination phase of the aluminum plasma data was estimated by inclusion of the zero time-point data of subsequent oral-dose treatment weeks. This is acceptable since the oral doses resulted in little

Table 3
Silicon bioavailability estimates for Zeolite A from oral capsules, oral solution and oral suspension using baseline corrected plasma data

Dosage form	Oral capsule		Oral solution		Oral suspension		Control	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Dose (mg/kg)	4.62	–	4.198	–	3.96	–	0	–
[a]AUC∞ (mg•h per l)	8.18**	3.90	11.0**	3.31	8.21**	3.30	8.89	1.64
C_{max} (mg/l)	1.28**	1.13	2.87**	0.37	1.44**	0.990	0.44	0.12
T_{max} (h)	3.67**	4.00	0.83**	0.25	5.88	7.44	11.33	7.97
% Extent of absorption	2.33		3.44		2.73			

[a]Beta from i.v. data used in oral AUC∞ estimation.
**Significantly different from control.

absorption of aluminum compared with the i.v. doses. Fig. 3 displays the continued log-linearity of these additional points in the elimination phase. Pharmacokinetic estimates from PCNONLIN indicated that i.v. profiles for aluminum were best fit by a three-compartment model. The three-compartment model for aluminum is an oversimplification of a complex process involving equilibria between blood and tissues, binding to plasma transferrin, binding to citrate and phosphate, competition with Ca^{2+}, and Mg^{2+} for other binding sites, and formation of high molecular weight aggregates.

The mean γ, $\gamma t_{1/2}$, Cl and V_c estimates from the i.v. data were 0.0076 ± 0.0054/h, 91.2 h, 0.0497 ± 0.0082 ml/min per kg, and 0.0553 ± 0.0045 l/kg, respectively (Table 5). Accurate estimation of the elimination half-life was difficult because of the small number of useable points in the terminal elimination phase, and the 1000-fold higher plasma levels during the early time-points. The volume of distribution when extrapolated to humans represents little more than plasma volume. This is consistent with observations in the literature which indicate that aluminum is 95% bound in the plasma (Trapp, 1983). Of this bound aluminum, a model has been presented in which 81% is bound to transferrin and the remaining 19% exists primarily as $Al(PO)_4(OH^-)$ with minor amounts of citrate or hydroxide species (Harris, 1992). The long half-life and slow clearance suggest slow equilibration and/or elimination from tissues. Formation of high molecular weight aggregates may occur at high aluminum concentrations reducing filtration and the rate of clearance (Xu et al., 1991). This may also explain the small V_c and the slow elimination of aluminum after the i.v. dose, since the plasma aluminum levels after i.v. administration were over a thousand times greater than baseline plasma levels. Xu et al. observed decreasing clearance and increasing elimination half-life corresponding to increasing i.v. doses in rats (Xu et al., 1991). The elimination of aluminum following i.v. administration in this study may therefore be substantially slower than the elimination at lower concentrations, such as those obtained during oral dosing.

Due to the sequence effect incurred by the administration of i.v. Zeolite A, mean aluminum AUC values were determined for the oral dosage forms using data uncomplicated by a previous i.v. administration of Zeolite A (Table 5). Fig. 3 represents the mean of all non-confounded oral dose data. Plasma aluminum concentrations following oral Zeolite A were slightly higher than those during the control week. Comparisons in Table 2 of the AUC data for oral dosage forms and the AUC data of the control suggest slightly larger group mean AUC values for the solution (312 μg/h per l) followed by the suspension (262 μg/h per l), capsule (222 μg/h per l) and controls (191 μg/h per l). Because of the observed sequence effect, the group mean AUC values for the non-confounded oral dosages were compared with the group control means using a paired t-test. The paired t-test indicated that there was a statistically

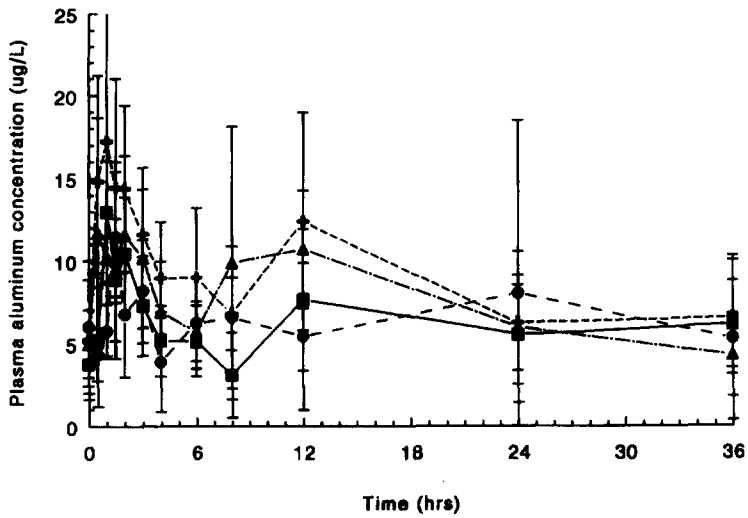

Fig. 2. Mean uncorrected plasma aluminum from the 3 dogs in Dosing Group 3 that received 20 mg/kg Zeolite A i.v. in the first period of the study. Zero time points prior to subsequent oral doses correspond to measurements at 168, 336 and 504 h.

significant difference between the control and oral solution ($P = 0.0154$); however, no significant difference was observed between control and the oral capsule or suspension ($P = 0.6328$ and $P = 0.1331$, respectively). The mean AUC for each of the oral dosage forms was then divided by the mean i.v. AUC for Group 3, and dose corrected, to determine what fraction of the i.v. dose was represented by the observed plasma aluminum levels. The mean extent of absorption of the aluminum from the oral capsule, solution and suspension were 0.023%, 0.032% and 0.028% respectively. The aluminum AUC resulting from the oral solution, however, is 64% greater than that in the control periods. Combined, these data suggest that the extent of absorption of aluminum from oral Zeolite A is less than 0.1%.

4. Conclusions

The plasma silicon and aluminum data from an i.v. bolus infusion of Zeolite A were best described by two-compartment and three-compartment open models, respectively. The mean elimination half-life and clearance of silicon from the i.v. dose of Zeolite A were 17.5 h and 0.221 $\pm$ 0.0192 ml/min per kg. The mean extent of absorption of silicon from the oral capsule, oral solution and oral suspension of Zeolite A was

Table 4
Mean uncorrected plasma aluminum area under the curve (AUC) μg/h per l

Dosing group	Week 1	Week 2	Week 3	Week 4	Week 5
1	*Control 227*	*Capsule 280*	*i.v. bolus 529500*	Solution 12487	Suspension 1486
2	*Control 189*	*Solution 376*	*Capsule 164*	*Suspension 218*	*i.v. bolus 639745*
3	*Control 155*	*i.v. bolus 648969*	Suspension 3318	Capsule 1303	Solution 1226
4	*Control 191*	*Suspension 306*	*Solution 249*	*i.v. bolus 622214*	Capsule 6561

Data in italics represent data not confounded by previous i.v. administration.

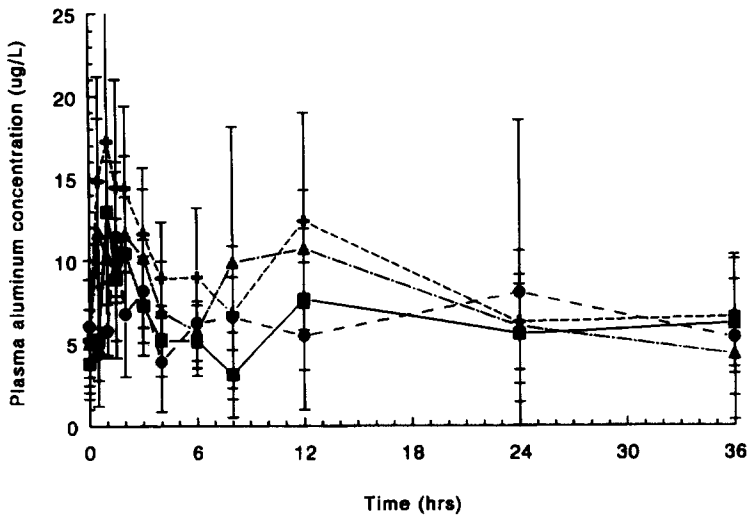

Fig. 3. Mean uncorrected plasma aluminum from all dogs receiving oral doses of Zeolite A which were not confounded by a previous i.v. dose: ●, control; ■, capsule 30 mg/kg Zeolite A; +, solution 30 mg/kg Zeolite A; ◆, intravenous 20 mg/kg Zeolite A; ▲, suspension 30 mg/kg Zeolite A.

2.33%, 3.44% and 2.73%, respectively. The mean peak silicon concentrations for the oral dosage forms ranged from 1.28 to 2.95 mg/l and the mean time to reach maximum concentrations ranged from 0.83 to 5.88 h, with most T_{max} values occurring within 2 h. The mean elimination half-life and clearance of aluminum were 91.2 h and 0.0495 ml/min per kg. The long half-life and slow clearance of aluminum after i.v. dosing was probably due to the high concentration of aluminum in plasma. The absolute extent of aluminum absorption from Zeolite A observed after doses of the oral capsule, oral solution and oral suspension were 0.023%, 0.032% and 0.028% of the i.v. dose, respectively. There was no statistically significant absorption of aluminum from the oral capsule or suspension. Statistically significant absorption of aluminum occurred from the solution over control. Therefore, single-dose oral administration of Zeolite A results in low but appreciable absorption of silicon with little absorption of aluminum. The long elimination half-lives of silicon and aluminum warrant multiple-dose studies to investigate the accumulation of silicon and aluminum after repeated doses of Zeolite A. The small differences in silicon and aluminum bioavailability from these three Zeolite A oral formulations does not preclude the use of any of the three formulations in future in vivo studies.

Table 5
Percent aluminum absorbed based on AUC

Doe	AUC (μg/h per l)	Extent of absorption (relative to i.v.)
i.v. solution* ($n = 3$)	648969	
Oral capsule ($n = 6$)	222	0.023
Oral solution[a] ($n = 6$)	312	0.032
Oral suspension ($n = 6$)	262	0.028
Control ($n = 12$)	191	–

*Statistically different than control.

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