Tolerance Study of Tiludronate in the dromedary camel

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Abstract

To evaluate the systemic tolerance and the possible adverse effects of the tiludronate at the therapeutic dosage in camels, a study was carried out on 10 healthy race male one-humped camels (*Camelus dromedarius*), 3 to 6 years old. They were located in a camel farm near the camel race course of Marrakech (Morocco). Camels were divided in two groups: control group (4 animals) and treated group (6 animals). A commercial formulation of tiludronate (TILDREN®, CEVA) was administrated to the treated group by slow intravenous injection at a dose of 0.17 mg/.kg/day (1 vial containing 50 mg of active mixed in 10 ml of solvent) per animal during 6 days, every day at the morning (06 am) when the control group received an intravenous injection of 10 ml of sodium chloride (0.9%). Camels were examined daily for general conditions, rectal temperature, heart rate, respiratory rate, mucous status, plumb, rumination salivation, appetite, urination, defecation and water intake. Blood samples (10 ml) were collected from each animal into heparinized vacutainer tubes at 0 (pre-treatment), 1, 7, and 13 days after the first IV administration. The plasma samples were analyzed for sodium, potassium, chloride, bicarbonate, urea, creatinine, total bilirubine and AST, ALT, AP, GGT and CK activities.

This study demonstrated that the intravenous administration of tiludronate at the therapeutic dose of 1mg/kg in toto in the camel had no significant side effects on the clinical or biochemical parameters. Therefore, tiludronate is well tolerated by the racing camel and could be used safely at the therapeutic dose of 50 mg of active per day for 6 days.

Key words: Camel, tiludronate, tolerance, clinical parameters, biochemical parameters.

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1. Introduction

The genus Camelus has two species: the dromedary or Arabian camel or one humped camel (*Camelus dromedarius*) and the Bactrian camel or two humped camel (*Camelus bactrianus*). The camel is specie well-known for its ability to survive in harsh conditions, especially in arid countries. By its adaptation to drought and low quality foodstuff, the camel allows maintenance of rural activity in marginal zones from dry areas of the ancient world. After a decrease in camel numbers in 60s and 70s linked to political events, technical effects and climatic factors in most of the countries, the camel's world population has been on the increase for the last twenty years. This new interest in the camel is also linked to a new feature of camel herding with a higher interest for milk and meat production and also in camel races in the Gulf countries. Racing is an important activity in the Arabian Peninsula. Regular race meetings are organized in many countries. Three race breeds are used: "Hajan Omania", "Hajan hurra" and "Hajan sudania".

Specific problems were observed for the race camels in term of diseases, nutrition and management. Bone and articular problems with associated lameness are frequent especially in young camels. The intensive training for young camels can induce stress fracture, areas of osteolysis and osteoporosis.

Generally, the dromedary camel is well known for its sensitivity to bone diseases. The Craft disease characterized by an increase of osteoporotic fractures was described in camel in many countries. It could be related to repetitive dehydration and rehydration cycles inducing a renal osteodystrophy (Bengoumi *et al.*, 1996). Tiludronate is an active substance of therapeutic classification of bisphosphonate characterized by inhibition of bone resorption. Licensed as an intravenous formulation for use in horses, it's also used in racing camels but no information has been published about its tolerance in camels. This approach may lead to irrational tiludronate use in dromedary. Indeed, the physiological and biochemical peculiarities which differentiate the dromedary from others species, may influence drug disposition and metabolism (Ali & Oukessou, 1996).

This study aims to evaluate the systemic tolerance and the possible adverse effects of the Tiludronate containing dip made of the product TILDREN at the therapeutic concentration in camels by using clinical and plasma biochemical parameters.

2. Material and methods

2.1. Animals and experimental design:

The study was carried out on 10 healthy race male one-humped camels (*Camelus dromedarius*), 3 to 6 years old and weighting from 265 to 512 kilograms (Table 1).

Table 1. Characteristics of the camels

N°	Group	Age (year)	Body weight (kg)	Injected volume	Observations
1	Treated	4	319	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days	NR
2	Treated	5	322	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days	NR
3	Control	4	265	10 ml of NaCL every day for 6 days	NR
4	Treated	4	299	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days	NR
5	Control	5	360	10 ml of NaCL every day for 6 days	NR
6	Treated	5	304	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days)	NR
7	Treated	4	314	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days	NR
8	Treated	4	307	1 vial of powder containing 50 mg of tiludronate mixed in 10 ml of solvent every day for 6 days)	NR
9	Control	6	512	10 ml of NaCL every day for 6 days	NR
10	Control	5	347	10 ml of NaCL every day for 6 days	

NR: Nothing to report

They were located in a camel farm near the camel race course of Marrakech (Morocco) and kept in a stable under normal day length and temperature and they were receiving 4 kg per day each of barley and 6 kg of alfalfa hay. Water was given twice a day at noon and in the evening. During the experiment, water consumption was measured daily. None had received any drug for at least 60 days prior to the study. Camels were divided in two groups: control group (4 animals) and treated group (6 animals). The body weight varied from 265 to 512 kg in the control group and from 299 to 322 kg for the treated group.

2.2. Drug administration:

A commercial formulation of tiludronate (TILDREN®, CEVA) was administrated to the treated group by slow intravenous injection at a dose of 50 mg/camel (1 vial of powder mixed in 10 ml of solvent)every day at the morning (06 am) during 6 days. The total dose administered was 300 mg for a 300 kg camel, corresponding to posology of 1mg/kg in toto, like in horses. The control group received an intravenous injection of 10 ml of sodium chloride (0.9%) in the same conditions than for the treated group (Table 2).

Table 2. Calendar of the tolerance study of tiludronate in the race camel.

Day	Stage	Date	Injection	Blood samples	Clinical examination
Sunday	d-1	11/09/2005	No	No	Yes
Monday	d0	12/09/2005	No	Yes	Yes
Tuesday	d1	13/09/2005	Yes	No	Yes
Wednesday	d2	14/09/2005	Yes	Yes	Yes
Thursday	d3	15/09/2005	Yes	No	Yes
Friday	d4	16/09/2005	Yes	No	Yes
Saturday	d5	17/09/2005	Yes	No	Yes
Sunday	d6	18/09/2005	Yes	No	Yes
Monday	d7	19/09/2005	No	Yes	Yes
Tuesday	d8	20/09/2005	No	No	Yes
Wednesday	d9	21/09/2005	No	No	Yes
Thursday	d10	22/09/2005	No	No	Yes
Friday	d11	23/09/2005	No	No	Yes
Saturday	d12	24/09/2005	No	No	Yes
Sunday	d13	25/09/2005	No	Yes	Yes

2.3. Clinical examination and blood sampling:

Camels were examined daily for general conditions, rectal temperature, heart rate, respiratory rate, mucous status, plumb, rumination, salivation, appetite, urination, and defecation. Blood samples (10 ml) were collected from each animal into heparinized vacutainer tubes at 0 (pre-treatment), 1, 7, and 13 days after the first administration. Within 30 minutes after collection, blood samples were centrifuged at 3000 g for 15 minutes. The plasma was separated in two aliquots and stored at –20°C until analysis.

2.4. Biochemical analysis:

The plasma samples were analyzed for sodium, potassium, chloride, bicarbonate, urea, creatinine, total bilirubine and AST, ALT, AP, GGT and CK activities. The plasma sodium and potassium were measured by using flame spectrophotometer (PHF 104, Hycel diagnostics). The other parameters were determined by an automate (Kone specific supra, Thermo clinical systems). Plasma urea concentration was measured according to the urease method. Creatinine concentration was determined according to Jaffe's method. Total bilirubine was measured according to the diazoreaction method. AST, ALT, AP, □ GGT and CK activities were determined by using their specific catalyzed reactions (Bengoumi *et al.*, 1997).

2.5. Statistical analysis

Results were expressed as the mean ± SD (standard deviation). The effect of the tiludronate administration and stage was evaluated by the nested multivariate analysis of variance using the Systat software.

3. Results and discussion

Results concerning the clinical and biochemical parameters are reported in table 3 and 4 respectively. The administration of tiludronate to camels did not induce any significant change on these clinical parameters. The general conditions, appetite, salivation, rumination, defecation, urination and plumb stayed normal in the two groups during all the experimental period. However, softening faeces were observed among 2 treated animals between d3 and d9. The mean body temperature varied from 37.4 to 37.6 with no significant difference between the treated and control groups. The mean heart and respiratory rates varied respectively from 37 to 38 pulses per minute and from 7 to 8 movements per minute with no significant difference between the treated and control groups. The daily mean water intake which varied between 19 and 24 litres was not influenced by the administration of tiludronate.

The clinical parameter observed in this study were comparable to those reported in the camel by other authors (Shwartz & Dioli, 1984; Faye & Bengoumi, 1999; Chaudhary & Akbar, 2000; Wernery *et al.*, 2002). The tiludronate administration did not influence significantly the clinical parameters. However, in the camel it is difficult to evaluate any effect through clinical symptoms. In fact, the camel shows anatomical and physiological peculiarities participating to diseases expression and pharmacological behaviour, notably the metabolism of molecules used for the diseases control (Ali & Oukessou, 1996). For example, hyperthermia is not a useful sign of fever for camel as its body temperature can vary for 8°C in the same day to manage heat change (Bengoumi & Faye, 2002). The immunological peculiarities are also well known. Important differences with other mammals are observed (Atarouch *et al.*, 1997) and the use of vaccines or

diagnosis kit has to be applied with care if they have been tested on other species. The sick camel has often a coarse symptomatology and the diagnosis could be difficult. Some observatories summarised the clinical expression to a simple sentence: the sick animal is lying and died easily with no specific signs.

The lack of specific symptoms in the camel justifies the use of biochemical parameters especially for cell integrity and functional soundness. The mean plasma concentration of sodium, potassium, chloride, bicarbonate, urea, creatinine, bilirubine and the mean plasma AST, ALT, AP, GT and CK observed in this study were comparable to those reported by other authors (Snow *et al.*, 1988; Faye & Mulato, 1991 ; Bengoumi *et al.*, 1997).

The administration of tiludronate had no significant effect on these biochemical parameters. The plasma sodium, potassium chloride concentration are influenced by dehydration or oedema, acid base disorders or in renal failure. The plasma bicarbonate concentration is a specific marker of salt balance; it increases during alkalemia and decreases during acidosis. Blood urea nitrogen which is synthesised in the liver and excreted by the kidney is a biochemical marker of liver and kidney functions; it increases during renal failure and decreases in case of liver damage. The creatinine is synthesised in the muscle, carried in the blood and filtered and excreted by the kidney. It is also a specific biochemical marker for renal filtration. The plasma creatinine concentration increases according the renal filtration diminution. The plasma total bilirubine is a marker of haemolytic or hepatobiliary jaundice. The absence of significant effect of tiludronate on these biochemical parameters in the racing camel indicates that the administration of this compound at recommended therapeutic dose did not induce salt disorders, nor liver or kidney functional disturbances.

The mean plasma AST activity varied between 83 and 125 U/l. These values are comparable to those reported in the bibliography (37 to 131 U/l) (Bengoumi *et al.*, 1998). There was no significant difference between control and treated groups even if a slight increase of AST activity was observed in the treated group one day after the last administration of tiludronate. AST is a mixed enzyme: cytoplasmic and mitochondrial. Contrary to the other ruminants where it is concentrated in the skeletal and cardiac muscle, and secondarily in the liver (Braun, 1985), among the dromedary it is an enzyme first of all contained in the kidney then the muscle, the liver and finally the myocardium (Bengoumi *et al.*, 1997). However, the increase of its plasmatic activity would reveal more a muscular or hepatic damage whereas the increase of its urinary activity would indicate a renal failure.

The mean plasma ALT activity varied between 12 and 18 U/l. These values are comparable to those reported in the bibliography (6 - 25 U/l) (Bengoumi *et al.*, 1998). There was no significant difference between control and treated groups even if a slight increase of ALT activity was observed in the treated group during the second week (days 7 and 13). ALT is a cytoplasmic enzyme. As for AST, ALT is contained first in the camel in the kidney then the muscle, the liver and finally the myocardium (Bengoumi *et al.*, 1997). However, the increase of its plasmatic activity would indicate more a muscular or hepatic damage whereas the increase of its urinary activity would indicate a renal failure.

Table 3. Effect of tiludronate on clinical parameters in the race camel

Parameter	Group	D0	D1	D7	D13
General	Treated	Good	Good	Good	Good
conditions	Control	Good	Good	Good	Good
Temperature	Treated	37.6 ± 0.1	37.6 ± 0.1	37.6 ± 0.1	37.6 ± 0.1
(°C)	Control	37.4 + 0.1	37.6 + 0.1	37.6 + 0.1	37.6 + 0.1
Heart	Treated	38 ± 1	37 ± 1	37 ± 0	37 ± 2
rate	Control	37 ± 3	37 ± 1	37 ± 1	37 ± 3
Respiratory	Treated	7 ± 1	7 ± 1	7 ± 1	7 ± 1
rate	Control	7 ± 1	7 ± 1	7 ± 2	8 ± 1
Mucous	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR
Plumb	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR
Rumination	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR
Salivation	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR
Urination	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR
Defecation	Treated	NR	NR	SF	NR
	Control	NR	NR	NR	NR
Water intake	Treated	21 ± 2	21 ± 1	21 ± 3	21 ± 1
	Control	24 ± 5	22 ± 5	19 ± 3	20 ± 4
Appetite	Treated	NR	NR	NR	NR
	Control	NR	NR	NR	NR

NR / Nothing to report SF : Softening faeces in two animals

The mean AP activity varied from 216 and 269 U/l. These values are higher than those reported in the bibliography (32 - 110 U/l in adult animals and 172 - 386 U/l in young camels) (Bengoumi *et al.*, 1997). There was no significant difference between control and treated groups even if a slight increase of AP activity was observed in the treated group during the second week (days 7 and 13). AP constitutes a group of isoenzymes present in several cells. In general, their activity is associated to the cellular microvilli of absorption and secretion, as the bile duct epithelium, intestinal tracts, renal tubules and the placenta. AP is concentrated first in the osteoblast cells responsible of ossification (Whitby, 1984). Its low concentration in the

muscle does not allow any influence of physical exercise on the plasmatic activity of AP (Snow and al., 1988; Bengoumi *et al.*, 1998). The major source of the plasmatic AP is represented by the bone (Kaneko, 1989). Otherwise, this activity increases also in case of cholestasis (Kaneko, 1989).

Table 4. Effect of tiludronate on blood biochemical parameters in the camel

Parameter	**Group**	**D0**	**D1**	**D7**	**D13**
Urea	Treated	5,7 ± 0.9	6,6 ± 0.7	6,0 ± 0.9	6,4 ± 0.9
(mmol/l)	Control	5,9 ± 1.0	6,0 ± 1.0	5,8 ± 1.0	5,6 ± 0.8
Creatinin	Treated	185 ± 12	182 ± 12	166 ± 18	165 ± 9
(µmol/l)	Control	189 ± 10	185 ± 4	170 ± 22	167 ± 16
Sodium	Treated	165 ± 5	168 ± 4	160 ± 3	166 ± 5
(mmol/l)	Control	166 ± 3	167 ± 4	163 ± 3	164 ± 5
Potassium	Treated	4.35 ± 0.37	5.48 ± 0.58	4.65 ± 0.57	5.02 ± 0.58
(mmol/l)	Control	4.50 ± 0.32	5.10 ± 0.36	4.90 ± 0.29	5.10 ± 0.29
Chloride	Treated	107 ± 2	107 ± 2	107 ± 3	108 ± 3
(mmol/l)	Control	105 ± 3	105 ± 3	108 ± 5	106 ± 5
Bicarbonate	Treated	24.3 ± 2.3	24.7 ± 2.0	23.5 ± 2.4	24.3 ± 2.9
(mmol/l)	Control	24.5 ± 1.9	25.5 ± 2.6	24.5 ± 2.6	25.5 ± 2.6
Bilirubin	Treated	2.8 ± 0.5	2.4 ± 0.4	3.1 ± 0.4	2.9 ± 0.2
(mmol/l)	Control	3.2 ± 0.5	2.9 ± 0.5	3.0 ± 0.3	3.1 ± 0.5
AST	Treated	87 ± 18	89 ± 16	125 ± 46	87 ± 18
(U/l)	Control	83 ± 10	83 ± 8	97 ± 18	83 ± 10
ALT	Treated	12 ± 3	11 ± 2	15 ± 3	18 ± 7
(U/l)	Control	14 ± 3	12 ± 3	13 ± 3	16 ± 5
AP	Treated	216 ± 39	240 ± 53	269 ± 28	265 ± 23
(U/l)	Control	232 ± 34	246 ± 31	236 ± 28	247 ± 24
GT	Treated	20 ± 5	20 ± 5	24 ± 6	25 ± 6 *
(U/l)	Control	18 ± 4	19 ± 3	19 ± 5	19 ± 4
CK	Treated	75 ± 19	90 ± 24	88 ± 17	82 ± 18
(U/l)	Control	80 ± 17	85 ± 14	79 ± 15	83 ± 13

* Significant difference for $p<0.05$ ** Significant difference for $p<0.01$

The mean GT activity varied from 19 to 25 U/l. These values are close to those reported in the bibliography (8 - 28 U/l) (Bengoumi *et al.*, 1997). There was no significant difference between control and treated groups even if a slight increase of GT activity was observed in the treated group during the second week (days 7 and 13). The GT is a membrane related enzyme located essentially in the

kidney with low concentration in the liver (Bengoumi *et al.*, 1997). However, the increase of its plasmatic activity would indicate more hepatobiliary damage whereas the increase of its urinary activity would indicate a renal failure (Bengoumi *et al.*, 1998).

The mean plasma CK activity ranged from 80 to 90 U/l. These values are comparable to those reported in the bibliography (40 - 120 U/l). The administration of tiludronate had no significant effect on the plasmatic CK activity in the camel. The tissular distribution of the CK is not known in the dromedary camel. In the other domestic species, the CK is especially concentrated in the skeletal muscle and the myocardium with a certain activity in the brain. The plasma CK activity is a specific marker for muscle or myocardium damages.

4. Conclusion

This study demonstrated that the intravenous administration of tiludronate at the therapeutic dose of 1 mg/kg in the race camel had no significant side effect on the clinical or biochemical parameters. Tiludronate did not induce any symptoms, metabolic disorders nor tissue damages. Therefore, tiludronate is well tolerated by the racing camel and could be used safely at the therapeutic dose of 1mg/kg of body weight in toto corresponding to 1 vial of 50mg tiludronate powder per day for 6 days for a camel weighting 300 kg.). The slight but no significant increase of the plasmatic activity of some enzymes could indicate a slight liver reaction, therefore, the strict respect of the dose is recommended.

5. References

Ali, B.H., Oukessou, M. and Bashir, A.K., 1996. Pharmacokineyic consideration in the camel (*Camelus dromedarius*). *Comp. Biochem. Physiol.*, 115, 1-9.

Atarouch T., Bendahman D., Hamers-Casterman C., Hamers R., Muyldermans S., 1997. CDNA sequence coding for the constant region of the dromedary gamma3 heavy chain antibody. *J. Camel Res. Pract.*, **4**, 177-182

Bengoumi M., Robins S.-P., De La Farge F., Davicco M.-J. & Barlet J.-P. (1996) Water restriction and bone metabolism in camels. *Reprod. Nutr. Dev.*, **38**, 545-554.

Bengoumi M., Faye B., El Kasmi K. & De La Farge F. (1997) Clinical enzymology in the dromedary camel. Part I. Effect of some physiological factors on serum AST, ALT, GGT, AP, and LDH activities. *J. Camel. Res. Pract. **4**, 25-29.*

Bengoumi M., Faye B & De La Farge F (1998) Clinical enzymology in the dromedary camel. Part III. Effect of dehydration on serum AST, ALT, GGT, AP, and LDH activities. *J. Camel. Res. Pract. **5**, 119-122.*

Bengoumi M., Faye B & De La Farge F. (1998) Clinical enzymology in the dromedary camel. Part IV. Effect of physical exercise on serum AST, ALT, GGT, AP, and LDH activities. *J. Camel. Res. Pract.* **5**, 123-126.

Bengoumi M. & Faye B. (2002) Adaptation du dromadaire à la déshydratation. *Sécheresse* **13**, 121-129.

Chaudhary Z I., Akbar S.J., 2000. The camel and its diseases. *Ed. Al Bayan Press (Publ.), Dubai, UAE, .*

Faye B. & Mulato C., 1991. Facteurs de variation des parameters protéo-énergétiques, enzymatiques et minéraux dans le plasma chez le dromadaire à Djibouti. *Rev. Elev. Med. Vet. Pays Trop.*,**44**, 325-324.

Faye & Bengoumi, 1999. «Almorchid fi tarbiat al ibil ». Arabic version. *Ed. Sanofi Publ., Libourne, France.*

Shwartz & Dioli, 1992. The One-Humped camel in eastern-Africa. *Ed. Publ. Verlag, Weikersheim, Germany, 282 pp.*

Snow D.H, Billah A. 1& RIDAH A., 988. Effect of maximal exercise on the blood composition of the racing camel. *Vet. Rec.*, **123**, 311-312.

Wernery U. & Kaaden O.R. , 2002. Infectious diseases of camelids. *Verlag Publ., Berlin.*