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Table 6.4. Treatment-related lesions and clinical signs incidence

Group	Dose (mg/kg/d)	Clinical signs (lameness, painful limbs)		Macroscopic lesions		Microscopic lesions	
		3 weeks	13 weeks	3 weeks	13 weeks	3 weeks	13 weeks
1	0	0/4	0/6	0/4	0/6	0/4	0/6
2	15	0/4	0/6	0/4	1/6	0/4	0/6
3	30	0/4	0/6	0/4	0/6	0/4	0/6
4	60	0/4	0/6	0/4	0/6	1/4	0/6
5	150	0/4	0/6	1/4	1/6	2/4	2/6

out of 10 dogs (2M–2F) were necropsied after 3 weeks of treatment and six out of 10 were necropsied after 13 weeks of treatment. Clinical observations, morbidity, mortality and locomotor disturbances were checked once or twice daily. Blood sampling for test article blood concentrations and alkaline phosphatase analysis were performed once pre-test, on Day 1 and during weeks 3, 8 and 13. The necropsy procedures for all animals included the examination of the external surface, the carcass, all orifices and all the articulation surfaces. Macrophotographs of joints were performed at the time of necropsy (elbow, shoulder, knee and hip, right and left). All these joints and all gross abnormalities were sampled for all animals. Histopathological examination was performed for right shoulder and hip joints of each animal and for all the gross abnormalities observed.

Results

No mortality occurred during the study. No locomotor disturbance and no treatment-related changes in alkaline phosphatase values were noted during the study. The observed lesions, confined to high-dose groups, were described as erosion, cavities and fibrillation of the matrix. These lesions were minimal to slight, appeared after 3 weeks of treatment and persisted at the same severity during 13 weeks of treatment (Table 6.4).

Discussion and conclusion

The intensity of the cartilage damage caused by quinolones usually depends on the molecule, the dose and the treatment duration [3]. In the present study where a wide range of doses was tested ranging from the therapeutic dose to 10-fold the therapeutic dose in the dogs, the arthropathy observed was of similar nature to that which occurs after treatment with quinolones [2]. For flumequine the treatment duration does not seem to have an influence on the incidence and severity of the lesions, which remain minimal to slight over the study. No toxic signs on juvenile articular cartilages of young dogs (the most sensitive species) were observed at the therapeutic and two-fold the therapeutic doses in dogs after 3 months of treatment.

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Functional characterization of elegantin isoforms, rgd-polypeptides isolated from the venom of trimeresurus elegans

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Introduction

Disintegrins represent a novel family of low-molecular weight proteins, isolated from the venom of various vipers [1]. They contain a tripeptide Arg-Gly-Asp (RGD) sequence, a common cell attachment recognition site [2]. Disintegrins bind with high affinity to the integrin receptors on platelet and cell surface [1,2]. Their structural and functional characterization is of great interest for a better understanding of integrin-ligand interactions as well as for providing the basis for the design of potent integrin inhibitors which could find practical application in a variety of human diseases. Two main isoforms of the disintegrin elegantin were isolated from the venom of the viper *Trimeresurus elegans*: elegantin 1a and 2a. The former corresponds to the only disintegrin previously isolated [1]. In this study we evaluated the ability of the two elegantin isoforms to interfere on platelet aggregation process and on cell adhesion.

Materials and method

The elegantin isoforms were purified from crude *Trimeresurus elegans* venom, using a simple two-step procedure consisting of membrane filtration and reversed-phase HPLC. The inhibition of ADP-induced platelet aggregation by disintegrins was assessed either in human platelet rich plasma (PRP) or in canine PRP. Cell adhesion assay was performed as previously described [3] using mouse melanoma B16-BL6 cells.

Results

Both elegantin isoforms 1a and 2a resulted to be strong inhibitors of ADP-induced platelet aggregation either in human or in canine PRP. However, elegantin 1a was more active than elegantin 2a towards human platelets, whereas elegantin 2a showed greater inhibitory activity on the aggregation of canine platelets. Both isoforms inhibited melanoma cell adhesion to fibronectin- and laminin- coated dishes. The effect was dose-dependent, non-

cytotoxic and reversible. Elegantin 1a was more effective than isoform 2a in inhibiting cell adhesion to both substrates.

Discussion

Elegantin isoforms demonstrate distinct biological activity, thus supporting the evidence that the particular conformation of the RGD sequence within the molecule, as well as the aminoacid sequences flanking the RGD locus determine the specificity and/or affinity of a disintegrin for integrin receptors on cell surface. Further, elegantin isoforms showed a different specificity towards the platelet integrin GP IIb/IIIa from different mammalian species. The molecular heterogeneity for the disintegrins might represent a selective advantage for the viper that feeds different species of animals and needs to defend against different predators.

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Effects of hypertonic saline solutions on blood oxygen transport in healthy calves

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Introduction

Gustin *et al.* [1] showed that *in vitro*, in standard conditions (pH=7.4, pCO_2=40 mm Hg and T=37 °C chloride (Cl) modulated the binding of oxygen to bovine red blood cells, shifting the oxygen dissociation curve to the right. These experiments suggest that, *in vivo*, chloride could be used as a pharmacological agent to improve oxygenation of the tissues in calves.

Materials and method

Healthy male Friesian calves between 7 and 44 days old were infused, via the jugular vein, with a hypertonic saline solution (NaCl 7.5%; 5 mL/kg; n=6 or 7.5 mL/kg; n=6). The control solution was isotonic saline (NaCl 0.9%; 5 mL/kg; n=4). The rate of infusion was 1 mL/kg/min and the beginning of the perfusion was considered to be time zero. At time 0, 15, 30, 60, 120 and 300 min, venous blood samples were collected from the jugular vein. At each time, the following measurements were made: oxyhaemoglobin dissociation curve in standard conditions [2], haemoglobin content and chloride concentration. Moreover, the venous and arterial gases and blood pH were taken into account. The arterial samples were collected from the brachial artery. The oxygen dissociation curves of oxyhaemoglobin were obtained in *in vivo* conditions, for the venous and the arterial blood samples, taking into account the standard ODC corrected for the blood pH, pCO_2 and temperature, using a statistical model. The *in vivo* oxygen exchange fraction (OEF *in vivo*) was calculated as the difference in saturation between PO2 values measured in arterial and venous blood. The amount of oxygen released, *in vivo*, by 100 mL of bovine blood was calculated by:

$$OEF\ (Vol\%) = Hb \times POX \times [OEF\%/100] \times a\ (PaO_2 - PvO_2)$$

where POX is the hemoglobin oxygen capacity (1.39 mL O_2 /g Hb); Hb is the level of haemoglobin and a is the oxygen solubility coefficient for blood at the experimental temperature (0.003 mL 100 mL^{-1} mm Hg^{-1}).

Results

Following infusion of 7.5% NaCl, the increase in the plasma chloride concentration shifted the ODC to the right. For example, due to the 13% increase observed in the 7.5 mL/kg group, the P50, measured in standard conditions, significantly increased from 24.23 ± 0.65 to 26.29 ± 0.94 mm Hg. The right shift was more sustained in the 7.5 mL/kg than in the 5 mL/kg group (60 vs. 30 min). The acidosis and the mild hypercapnia induced by infusion reinforced the shift of the ODC in the venous and in the arterial compartments. While the right shift of the arterial and venous ODC was induced by hyperchloremia, an increase in the venous PO_2 and a fall in the haemoglobin concentration explained why the OEF Vol % was not significantly modified.

Discussion

Our data confirm our previous *in vitro* observations [1]. Thus *in vivo*, chloride can induce a right shift of the ODC in the venous and the arterial compartments. These effects could favour enhanced release of oxygen at the tissue level in hypoxic animals. However, as there is no reason, in healthy animals, for a change in oxygen consumption, the right shift of the ODC is counterbalanced by an increase in venous PO_2, limiting the desaturation of haemoglobin.

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Poster Communications

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Tetracycline-induced eosinophilia in the dog

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