EMEA/MRL/024/95

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

POLYSULPHATED GLYCOSAMINOGLYCAN

SUMMARY REPORT

1. Polysulphated glycosaminoglycan (PSGAG) is a highly sulfated polysaccharide consisting of an alternating chain of approximately equal numbers of hexuronic acid and hexosamine. In regard to its chemical structure and biological behaviour it is to a great extent similar to natural heparin. PSGAG is proposed for the treatment of posttraumatic and degenerative joint diseases in horses. The recommended dose for intra-muscular (i.m.) injection, 500 mg/horse every four days for a total of seven injections.

2. The chondroprotective and antiarthrotic activities of PSGAG were investigated in a battery of in vitro and in vivo test models, In summary, PSGAG causes inhibition of cartilage degrading enzymes, reduces the loss of proteoglycans in cartilages and possesses stimulatory effects on the synovial cell metabolism. In horses, there is a significant improvement of lameness both after artificially induced carpitis and spontaneous lameness.

   Due to the heparinoid nature of the drug, the main undesirable effects are caused by its anticoagulant properties.

3.1 Rapid absorption was observed following parenteral administration of the radiolabelled substance (^{3}H-PSGAG, ^{35}S-PSGAG) to rabbits (i.a., i.m., i.v.), horses (i.m.) and humans (i.a., i.m.). The synovial membrane is not a barrier for rapid distribution since serum levels of radioactivity after 12 h and 24 h were similar both after i.a. and i.m. administration in humans. In horses, highest plasma levels (approx. 2 µg/ml) were measured 2 hours after i.m. administration (500 mg ^{3}H-PSGAG/horse) followed by a rapid decline over the following 22 h and nearly constant levels (1 µg/ml) until 96 h post dose.

3.2 The absorption of PSGAG from the digestive tract is low. 24 h after a single oral administration of 2.5 mg/kg bw ^{3}H-PSGAG to rats, maximum radioactivity levels in serum corresponding to about 0.1 µg/ml were measured. The cumulative amounts of radioactivity excreted in urine and in faeces were 2% and 68%, respectively, after six days.

3.3 From the results (blood clotting time) of two separate studies in rabbits treated either with a single oral (1000 mg/kg bw) or subcutaneous dose of PSGAG (10 mg/kg bw) the applicant postulated that the oral absorption rate was only 1/100 of that from s.c. injection site because an almost identical prolongation of the activated partial thromboplastin time (aPPT) was observed in both experiments. The uptake of PSGAG following oral administration was not studied in more detail, however, the existing data indicate a very low gastrointestinal absorption rate.

3.4 The tissue distribution of radiolabelled PSGAG was investigated in rabbits following a single i.m. (7.5 mg ^{3}H-PSGAG/kg bw) or i.a. (1 mg ^{35}S-PSGAG/kg) injection. After either application mode radioactivity levels were found highest in the kidneys followed by liver and the injection site. Up to 10 days after an i.a. administration, the radioactivity levels in the kidney were more than ten times higher than those in liver, spleen and bone marrow, The decrease in radioactivity was slow in all tissues examined. Approximately 25% to 35% of the initial maximum levels were still present in the kidney and liver, respectively, 10 days after treatment.

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3.5 In rabbits, urinary excretion of i.m. administered ^{3}H-PSGAG amounted to approximately 45% within 24 h. A further 10% were excreted within the following 24h. Combined analysis by gel electrophoresis, electrophoresis and radiometry showed increasing degradation of PSGAG during the course of elimination to weaker anionic and to less high molecular products. The elimination from the blood did not follow a first order kinetics. It appeared to be rather a complex process with threshold limitation due to tissue deposits beginning at 6 h post dose.

3.6 10 days after a single i.a. administration of ^{35}S-PSGAG to rabbits, approximately 32% of the radioactivity was found in urine and less than 1% in faeces. Analysis of the urine revealed that the parent compound is degraded to desulfated smaller molecules and inorganic sulfate.

3.7 Similarly, it was found in human patients treated i.m. or i.a. with ^{3}H-PSGAG that the chain length and degree of sulphatisation of the eliminated urinary metabolites were reduced.

3.8 There are no studies on pharmacokinetics following repeated treatment with PSGAG. The distribution of PSGAG in horses was not investigated. No metabolism study in horses was submitted.

4. The acute oral toxicity of PSGAG is low. In rats and mice, the LD_{50} was found to be higher than 20 g/kg bw. Reduced spontaneous activity was the only sign of intoxication noted at 20 g/kg bw.

5. Three subchronic parenteral toxicity studies were carried out in rats (2, 10 or 25 mg/kg bw/day, i.m.) and dogs (5, 15 or 50 mg/kg twice weekly, i.m., and 2, 5 or 10 mg/kg bw, 3 times a week, i.a.). In rats, an increased weight of liver and kidneys, and in dogs compound related histopathological alterations in the liver (i.m.) and kidneys (i.m., i.a.) were found in the mid and high dose groups. In rats a NOEL of 2 mg/kg bw and in dogs a NOEL of 5 mg/kg bw following i.m. and 2 mg/kg following i.a. administrations was established. The slight reticuloendothelial cell vacuolization of the spleen and a minimal to moderate medullary histiocytosis of the lymph nodes after i.a. administration of 2 mg/kg bw in dogs can be explained as a result of the deposit of PSGAG and/or PSGAG-metabolites and are, apparently, of low toxicological relevance.

   No subchronic oral toxicity study using PSGAG was submitted. Repeated dose studies with oral administration are not considered to be necessary because PSGAG is only very poorly absorbed from the gastrointestinal tract.

6. Two fertility studies were conducted in male (2, 10, 25 mg/kg bw) and female rats (2, 8, 32 mg/kg bw) treated with i.m. injections of PSGAG prior to and throughout the mating phase. The only notable effect was observed in the female fertility study and included a significantly reduced body weight of the offspring in the highest dose group (32 mg/kg) on day 21 post partum. A NOEL of 2 mg/kg bw was established.

   Two embryotoxicity studies with PSGAG were carried out in rats (40, 80, 160 mg/kg bw, s.c.) and rabbits (2, 8, 32 mg/kg bw, i.m.). In rats mean food consumption and body weight gain of the parent animals were reduced in all dose groups. In the highest dose group 9 out of 25 dams died. A significant dose related increase in the number of foetuses showing unilateral and bilateral cervical ribs, interpreted to be caused by maternotoxicity, was noted. In rabbits, a significant increase in the resorption rate was found in the low (2 mg/kg bw) and high dose group (32 mg/kg bw). A NOEL can not be established from these studies. A two generation study was not provided.

7. The mutagenic potency of PSGAG was investigated in the Ames test with and without metabolic activation and in a chromosome abberation test in rats. No mutagenic effects were observed. No gene mutation test using mammalian cell cultures was carried out.

8. Longterm carcinogenicity studies were not performed. PSGAG does not belong to any of the substance groups for which carcinogenic properties have been assumed or proven.

9. Various blood coagulation parameters (e.g. aPPT, factor Xa) were investigated in a published doubled blind study in humans following single s.c. doses ranging between 10 to 70 mg PSGAG/human. 10 mg/human was proposed by the applicant as NOEL on the basis of aPPT. However, in one test a transient anti-factor Xa effect and a significant release of serum lipoproteinlipase seemed to occur at the lowest dose tested (10 mg/human). The toxicological relevance of these finding remains unclear and a NOEL can not be established at present. After repeated PSGAG administration (day 1 : 3 x 40 mg/human, then 1 x 40 mg/human/day for 11 days) no accumulation or tachyphylaxis regarding anticoagulant effects was measured.

10. After parenteral application of high doses of PSGAG to humans (250 mg/person) antibody reactions to PSGAG/platelet complexes and thrombocytopenia were observed in rare cases. No study on the immunotoxic risk after oral application was submitted.

11. A clear NOEL which could serve as basis for the establishment of an ADI was not established. With regard i) to the overall chemical and biological properties of PSGAG, ii) its similarity to endogenous substances, iii) its low intestinal absorption rate as concluded from studies using rats (3.2) and rabbits (3.3), and iv) its intended clinical use in horses the substance is considered a candidate for Annex II.

    The Committee considers that there is no need to establish an MRL for this substance and recommends the inclusion into Annex II of Council Regulation (EEC) No 2377/90 of Polysulphated glycosaminoglycan for Horses in accordance with the following table.

Polysaccharides

Pharmacologically active substance	Animal species	Other provisions
Polysulphated glycosaminoglycan	Horses	

The original source of PSGAG is bovine trachea. The national authorities will assume responsibility for checking the measures taken by the manufacturer against microbial contamination of the material and against the transmission of the infective agent of bovine spongiform encephalopathy.