Instructions for use

Measurement of keratan sulfate in sera and synovial fluid from horses by a monoclonal antibody 1/14H9 reacting with equine keratan sulfate

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product, as an indicator of myocardial oxygen consumption, decreased during and after the administration of carperitide in all dogs. The PAWP was remarkably reduced and the cardiac output increased during and after the administration of carperitide in the three dogs with overt CHF (CHF+) among them in comparison with the other three dogs without CHF (CHF−). But the urine volume and the excretion of sodium increased during and after the infusion of carperitide in the CHF− dogs, compared with the CHF+ dogs. On the relationship between PAWP and cardiac index, all plots of the CHF+ dogs shifted upward and to left, meaning an improvement in cardiac function, during and after the injection of carperitide. In contrast, all plots of the CHF− dogs showed little change.

In conclusion, it was suggested that carperitide is effective in the treatment for dogs with CHF caused by chronic mitral regurgitation.

Measurement of keratan sulfate in sera and synovial fluid from horses by a monoclonal antibody 1/14/16H9 reacting with equine keratan sulfate

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The inhibition-ELISA for the measurement of equine keratan sulfate (KS) concentration was established by using an equine KS monoclonal antibody 1/14/16H9, and the usefulness of 1/14/16H9 monoclonal antibody was evaluated for this purpose.

An inhibition-ELISA using 1/14/16H9 antibody for determination of KS was optimized. The sensitivity of the assay could be substantially improved by increasing the dilution of primary antibody (1/14/16H9, 1:25,000), and the binding was detected by a peroxidase-conjugated antibody (second antibody, 1:2,500). Precision data were obtained over the range 39–319 ng/ml of KS concentration in this assay.

KS concentration were measured in sera from foals (from 1 week after birth to the age of 5 months), and sera and synovial fluids from horses which were experimentally induced arthritis by the injection of chymopapain (30 mg/joint) into the carpal joint. The KS concentration in these samples were also evaluated by using 1/20/5D4 antibody.

In this assay system, serum and synovial fluid samples were diluted as follows; sera of foals: 20–40 times, sera of arthritic horses: 1–2 times, and synovial fluids: 100–800 times.

In the sera, values of 1/20/5D4 monoclonal antibody were significantly higher than that of 1/14/16H9 monoclonal antibody (p<0.01). In synovial fluids, no statistical difference was found between the values by using both monoclonal antibodies. The values of samples in the arthritic horses measured by both antibodies showed similar fluctuations.

In sera, there was a close correlation between the values measured by both antibodies in 3–14 days after injection of chymopapain, while no obvious correlation was found in 0–3 and 14–49 days. In synovial fluids, the correlation index was low in 3–17 days, while a close correlation was detected in 0–3 days and 17–31 days after injection of chymopapain.

These results show that 1/14/16H9 antibody
recognizes a different epitope and has a different reactivity to KS from 1/20/5D4 antibody, the correlation between the values measured by two antibodies suggest the measurement and comparison of these KS concentrations would be valuable in monitoring the catabolism of articular cartilage and the degree of of synovial function injury.

Study on lung injury related to neutrophils induced by subcutaneous administration of large doses of chitosan in dogs

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Subcutaneous administration of large doses of chitosan causes fatal acute hemorrhagic pneumonia in dogs. This chitosan-inducing hemorrhagic pneumonia activity in dogs is reported to be pathophysiologically similar to acute respiratory distress syndrome (ARDS) in humans, but the mechanism of this response remains to be clarified. The purpose of this study was to investigate the role of neutrophils sequestered in the lung from this response in dogs, in which the changes in levels of several components of the bronchoalveolar lavage fluid (BALF) were examined. It was also evaluated whether the level of interleukin (IL)-8, the cytokine playing an important role in the pathogenesis of ARDS, was involved in this response using rat model as a substitute for dogs.

Before administration of chitosan, BALF was collected from each dog under general anesthesia. After 6 days, each dog was administered with 200mg/kg chitosan by subcutaneous injection. Physical and blood examinations were performed over 24 hours. After that, BALF was collected from each dog in the same way. Concentrations of lipid peroxide (LPO) and total protein (TP), activities of myeloperoxidase (MPO) and neutrophil elastase, and the production of nitric oxide (NO) in the BALF were measured and compared with those in the pre-administration BALF. After BAL, pathological examination was performed and concentrations of LPO in the lungs, livers, and kidneys were measured in comparison with normal dogs. Subcutaneous administration of chitosan on rats was also done and then the IL-8 levels in serum were assayed.

After chitosan administration, the concentrations of LPO and TP, and the activities of MPO and neutrophil elastase in the BALF increased in all dogs. Increase of neutrophil and eosinophil numbers in the BALF was observed. The change of NO production in the BALF was not found. IL-8 level in the serum of rats increased after chitosan administration, the highest was 9 hours after administration. However a response similar to that in dogs was not observed.

The pathogenesis of the increase in the concentrations of LPO and TP, and the activities of MPO and neutrophil elastase in the BALF after chitosan administration in dogs may be due to the activation of neutrophils in lungs similar to ARDS. Subcutaneous administration of chitosan in rats did not cause lung injury as in dogs, but high levels of IL-8 were observed. Similar change in the level of IL-8 may also be caused after chitosan injection in dogs.