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Examination of a method using cationic liposomes for transfection of the bovine CD18 gene into peripheral blood leukocytes of cattle affected with leukocyte adhesion deficiency

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In this study, using liposome vesicles, I attempted to transfect the normal bovine CD18 gene into peripheral blood leukocytes of cattle affected with leukocyte adhesion deficiency (BLAD-PBL), and examined the rate of expression of CD18 on BLAD-PBL *in vitro* by the transfection of the gene. pTargeT/CD18 was used as an expression vector for bovine CD18. The pTargeT/CD18 was constructed by transfer of the normal bovine CD18 gene into a mammalian expression plasmid vector. Three commercial liposome vesicles (TransIT-LT1, -LT2, -LT100) were used for the transfection of pTargeT/CD18. The following results were obtained.

A positive correlation between the quantity of liposomes and the number of BLAD-PBL transcripts was recognized. The expression of

CD18 was not recognized in the incubation time of 24 or 96 hours after transfection of pTargeT/CD18. The most effective expression rate of bovine CD18 was obtained when 1.6×10^6 BLAD-PBL were transfected with pTargeT/CD18 using $4 \mu\text{l}$ of TransIT-LT1 and incubated for 48 hours after the transfection.

In the present study, the recover of adhesion activity of BLAD-PBL transfected with pTargeT/CD18 was not recognized. However, some suitable conditions for the gene transfection using liposome vesicles were determined. Although it is necessary to examine conditions for transfection in more detail, BLAD may be useful as a model disease for investigation of the effect of gene therapy in corresponding human diseases.

A study on radiation-induced apoptosis
as a possible predictive assay for radiotherapy on canine tumors

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Predictive assay for the radiocurability of tumors is useful when tumor patients are selected for radiotherapy and individual undergone various radiation schedules. The purpose

of this study was to investigate whether the incidence of radiation-induced apoptotic cells in the tumor tissue irradiated *in vitro* can predict radiocurability of the tumors.

In this study, we used five tumor models namely: Nude mice implanted with canine malignant melanoma (MM), mast cell tumor (MCT), mammary adenocarcinoma (MAC), malignant synovioma (MS), and osteosarcoma (OS). To evaluate the incidence of radiation-induced apoptosis *in vitro*, tumor tissues from each model were cultured and irradiated (0, 4, 16 Gy of X-rays). Apoptotic cells in the irradiated tissues were detected, using the TUNEL method at 2, 4, 6, and 12 hours after irradiation. To evaluate the radiocurability of each tumor *in vivo*, implanted tumors in nude mice were irradiated at 48 Gy administered in 4 Gy fractions over 4 weeks and their growth rates were monitored individually.

The incidence of radiation-induced apoptosis *in vitro* from highest to lowest incidence are: MCT (22% by 4 Gy at 12 hours), MAC (11%), MM and OS (5%), and MS (2%).

The radiocurability of tumors *in vivo* are: MCT showed the highest radiocurability as CR (Complete Response) 4/4, followed by MAC as MR (Minimum Response) 4/4. MM showed lower as CR1, MR1 and PD (Progress Disease) 2/4, OS and MS showed the lowest radiocurability as CR1 and PD4/5, PD5/5 respectively.

These results suggest that the tumors with many apoptotic cells in the irradiated tissue *in vitro* showed higher radiocurability *in vivo*. The efficient detection of radiation-induced apoptotic cells *in vitro* from the various X-ray exposure conditions in this study was at 4 Gy of X-ray exposure and 12 hours apoptosis-developing time post-irradiation.

In conclusion, the detection of radiation-induced apoptosis in this study could be used as a predictive assay for radiotherapy of the five models used, and could be adopted to other canine tumors.

Efficacy of atrial natriuretic peptide administration in dogs with chronic heart failure caused by experimentally induced mitral regurgitation

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Atrial natriuretic peptide (ANP) is a cardiac hormone which is responsible for the regulation of blood pressure and body fluid homeostasis. Recently, the clinical application of recombinant human α -ANP (carperptide: HANP[®]) is possible in human acute heart failure cases. The objective of this study is to evaluate the efficacy of carperptide in the treatment for canine mitral regurgitation which occurs frequently in canine acquired heart diseases.

Chronic mitral regurgitation (MR) was experimentally induced in six dogs by the rupture of

mitral valvular chordae tendineae. Two weeks after that, the time courses of the hemodynamics, diuresis, natriuresis and plasma ANP levels were compared during the continuous administration of different carperptide doses (0.1, 0.5 and 1 μ g/kg/min) for 1 hour and 1 hour after the withdrawal.

The plasma ANP levels and the pulmonary arterial wedge pressure (PAWP) increased after the induction of MR and a good correlation was evident ($r=0.85$). The heart rate, the mean arterial pressure, the PAWP and the double