ABSTRACTS

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**EXPRESSION OF ABC-TRANSPORT PROTEINS IN CANINE MAMMARY CANCER: CONSEQUENCES FOR CHEMOTHERAPY**

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**Introduction** - The emergence of canine mammary tumours is a widespread problem in veterinary medicine. Mammary tumours account for approximately 50% of all neoplasms in female dogs [1]. About 71% of them are histological malignant. In the majority of cases, treatment by surgery is unsatisfactory because scattered tumour cells and metastases can not be eliminated. Thus, adjuvant application of chemotherapeutics appears reasonable. Unfortunately, no standard treatment protocols are available for canine mammary cancer.

**Objectives** - In order to facilitate the choice of adequate chemotherapeutics for treatment of mammary cancer, we investigated the ability of tumour cells to express the phenomenon of chemoresistance.

Chemotherapeutics enter the cells by passive diffusion or by active transport, dependent of their lipophility. The tumour cells die, if an intracellular effective dose of the drug is reached. Carrier proteins belonging to three families of ABC-transporters (ATP-binding cassette) mediate an ATP-dependent efflux of chemotherapeutics out of the cell, so that the effective dose can not achieved. MDR1 (P-glycoprotein) is the best-known ABC-transporter involved in chemoresistance of canine tumours. Several studies demonstrated MDR1 expression in canine mammary cancer [2, 3]. Because additional ABC-transporters mediate chemoresistancies in human, we investigated the mRNA-expression of 9 ABC-transport proteins by semiquantitative RT-PCR in 119 canine mammary tumours.

We could show an individual ABC-transporter expression pattern of eight carriers for every tumour, which is independent from breed, age, histopathological diagnosis and malignancy. Hence, the development of a standard protocol for canine mammary cancer is hampered by the individuality of the transporter expression.

However, there were also some similarities between the individual tumours. The transporter MRP2 could not detected in canine mammary tumours. A weak expression of MRP5 (multidrug resistance associated protein) and MRP6 was observed in comparison to liver cells. A moderate expression was noticed for the well known transporter MDR1 (multidrug resistance protein) and for MRP3 as well as MRP7, whereas MRP3 and MRP7 showed a high degree of interindividual differences. Very high expression levels were identified for BCRP (breast cancer resistance protein), MRP1 and MRP9.

Substrates for canine MDR1 and MRP1 are already known, so we investigated possible substrates for BCRP. We cloned the full-length cDNA from canine placenta by RACE-PCR technique. The canine BCRP cDNA (Genbank accession no. DQ222459) consists of 2161 base pairs. The open reading frame (base 111-2078) encodes for a protein of 655 amino acids. Canine BCRP shows a homology of 84.7% to human BCRP, 85.2% to monkey, 78.6% to mouse and 77.9% to rat BCRP. MDCK-II cells were stably transfected with canine BCRP in order to enable a functional characterization. The BCRP induced chemoresistance against cytostatic drugs was tested by cell viability assays. MDCK-II cells transfected with canine BCRP were 100 fold more resistant against doxorubicin than untransfected parental cell line. Canine BCRP mediates no chemoresistance against pacliataxel, carboplatin, cyclophosphamide and 5-fluoro-uracil. So, we conclude that doxorubicin is inappropriate for the treatment of canine mammary cancer.
A comparison of normal breast tissue with the malignant breast tissue from the same bitches showed an increased mRNA expression of MDR1, MRP1, MRP9 and BCRP, indicating that normal mammary cells were more sensible to chemotherapeutics than the tumour cells. The high BCRP mRNA expression in canine mammary tumour cells and the intracellular localization of the protein was verified by immunohistochemical detection. Canine BCRP was localized with the primary antibody 407 (a friendly gift from Rob Robey and Susan E. Bates; National cancer Institute, Bethesda, Maryland, USA) in formalin fixed mammary cancer sections. The canine BCRP protein was highly expressed in epithelial cells of the glandular tissue. But the intracellular localization was disturbed in contrast to normal breast tissue. Besides the normal membrane staining a strong cytoplasmatic staining was observed.

**Conclusions** - canine mammary tumours express an individual pattern of eight ABC-transport proteins. A high mRNA expression level was found for BCRP, MRP1 and MRP9. The BCRP protein is expressed in the epithelial cells of mammary gland, but in malignant cells the intracellular localization is disturbed. Functional studies showed that doxorubicin is not appropriate for the treatment of canine mammary cancer. A check of the individual chemoresistance of tumours could be recommended before application of cytostatic drugs.

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