

SUMMARY

Detection of Apoptosis in Experimentally Induced Fibrosarcomas Using DNA Fragmentation and Immunohistochemical Methods

Fibrosarcoma is deriving from mesenchymal tissue. It is one of the malignant tumors and usually seen in cats and dogs, however, it can be encountered in all animals. It is growing quite fast and invades other tissue rapidly. It is one of the mesenchymal tumors in rodents varying between 1-3 %, and in rats 1-6 %. Apoptosis is used for eliminating cells which are not functioning properly. Early diagnosis of tumors and follow-up prognosis are of great importance for the extension of life of cancer patients. Especially, fibrosarcomas in cats occur in vacation areas due to chronic inflammation. Recent studies on tumors showed that there is a connection between cancers and apoptosis. In this study, apoptosis in fibrosarcomas induced by 3-methylcholanthrene was investigated with two different methods. Thus, the relationship between apoptosis and fibrosarcomas is investigated. On the other hand, programmed cell death was investigated using immunohistochemical and ELISA methods and differences in results are compared.

Sixteen male Sprague Dawley rats were used in this study. They were 8 weeks old and their body weights were between 150-200 grams. Rats were allocated into two groups, each containing 8 rats. During the experiment, which took between 150-210 days depending on the appearance of tumor tissue, they were allowed free access to water and feed. In order to induce fibrosarcoma in rats, at the beginning of the experiment, animals were injected subcutaneously on the neck with 0,2 mg 3-methylcholanthrene dissolved in 0,25 ml sesame oil. To find out whether any tumoral tissue occurred, animals were palpated daily. At the end of the experiment, animals were killed under ether anesthesia and necropsy was performed. DNA fragments of tumor tissue cells were analyzed using ELISA (Roche), and localization of Bcl-2 and Bax was determined by immunohistochemical method.

Tissues obtained from the control and the experimental animals were homogenized, centrifuged, and the supernatant was used for ELISA procedure. In the experimental group, absorbance of the DNA fragmentation was 0,262 U, but in the control group it was 0,069 U. In

other words absorbance of DNA fragmentation of experimental animals was 4 times higher than that of controls.

Immunohistochemically there was a lot of Bcl-2 and Bax positive cells. Contrary to this in control animals there was hardly Bcl-2 and bax positive cells. This data indicates that in the tumor tissue it is observed some cells programmed to death by apoptosis but the others not.

In the light of this findings we got in the present study apoptosis is encountered frequently in fibrosarcoma and fragmentation of the DNA indicates apoptosis. Researchers who are engaged in curing of cancer patients should take into consideration of levels of Bcl-2 and Bax proteins. Furthermore, similar to the results of previous studies the rate of apoptosis seems to be very low at the beginning of the cancer however in the course of the time concomitantly the increase in proliferation of the tumor cells the rate of apoptosis increased dramatically.

Key words; Fibrosarcoma, apoptosis, Bcl-2, Bax, DNA fragmentation, immunohistochemistry, 3-methylcholanthrene, rat.