Aim: To study the effects of Paclitaxel (Ptx), γ-irradiation (IR) and their combination on the growth of xenografted tumors derived from undifferentiated thyroid cancer cells. Materials and Methods: Experiments were performed in nude mice with tumors developing from implanted undifferentiated thyroid carcinoma cells (FRO). Animals were treated with Ptx i.p. and exposed locally to a single dose of 5 Gy of IR. Apoptosis in situ was detected using ApopTag Peroxidase Kit. Results: In the in vivo experiments, IR significantly inhibited but did not abrogate tumor growth. Ptx effect was stronger, and the combination therapy with Ptx and IR led to the decrease of tumor volume to 0-0.3% of the control (P < 0.01). The systemic administration of Ptx to the animals with advanced tumors resulted in a profound tumor growth suppression and in apoptosis in tumor tissues in time-dependent manner. Conclusion: The combination of Ptx and IR is a promising strategy for further preclinical and clinical trials aimed at the development of new therapeutic approaches to the treatment of undifferentiated thyroid cancer.

Key Words: Paclitaxel, ionizing radiation, thyroid cancer.
Histological estimation of apoptosis in the tumors. Needle biopsies of tumor tissues were fixed in 10% neutral-buffered formalin, and embedded in paraffin. Apoptotic cells were detected in 5-µm sections with an ApopTag Peroxidase Kit (Intergen Co., Burlington, MA). Positively stained cells were counted in four fields (x100) for each specimen, and the apoptotic index was determined as the ratio of apoptotic cell number to total cell number.

Statistical analysis. All data were expressed as a mean ± SD. Differences between groups were examined for statistical significance using Kruskal—Wallis test (nonparametric ANOVA), Mann—Whitney test and one-way analysis of variance (ANOVA). P < 0.05 was considered indicating statistical significance.

RESULTS AND DISCUSSION

Treatment of anaplastic thyroid cancer cells in vitro with IR does not abrogate cell growth although moderate doses (1–2 Gy) can rather effectively induce apoptosis and transient growth arrest [11, 12]. At first, this effect of IR was confirmed in the in vivo experiments (Fig. 1, a). FRO cells transplanted into mouse flanks quickly formed tumors. Twenty days after implantation, tumor size exceeded 1000% of the initial (100 mm³) in the control animals. In line with the in vivo experiments, IR significantly (P < 0.05) reduced tumor size but did not prevent tumor growth. Treatment with Ptx was more effective (P < 0.05 as compared to irradiated group). From the 11th day after the beginning of Ptx treatment, we observed a highly significant reduction of tumor volume as compared to both the control and to the initial volume. At 20–29 days tumor volume was 0.8–1% of control. The combined treatment with radiation and Ptx showed the enhanced therapeutic effect (P < 0.05 as compared to the irradiated animals). Seven days after the beginning of treatment, tumor size was significantly decreased; after 20–29 days it was 0.3% of the control (P < 0.01), and in two animals the tumors completely regressed. The combination of irradiation and Ptx was slightly more effective than Ptx alone, but the difference was insignificant. In in vitro experiments the additive effect of both agents regarding caspase-3 activation and PARP cleavage was observed [11].

Next, after finishing this experiment, the control group was split into 1 control and 8 experimental animals which were subjected to the treatment with 4 times lower doses of Ptx (2.5 mg/kg/day). Fig. 1 b shows that the tumor in the control animal continued to grow up to 1700 mm³ during further 18 days whereas those in the animals receiving Ptx were evidently decreased. This observation indicates an efficiency of rather low doses of Ptx even in advanced tumors.

Examination of the extent of apoptosis in tumor tissues in situ in the advanced tumor group showed that after 11 days of treatment, Ptx effectively induced cell death, the intensity of which increased over the next 18 days (Fig. 2).
dominates over the mechanisms responsible for cell recovery and senescence.

Our in vivo data clearly show that treatment of anaplastic thyroid carcinoma with Ptx induces apoptosis in tumor tissue. Also, the use of moderate (1–5 Gy) doses of IR may be helpful for enhancement of Ptx effect on undifferentiated thyroid cancer xenografts, which confirms the data obtained for other tumors [6, 7]. In conclusion, the combination of Ptx with IR seems to be a promising modality for further preclinical and clinical trials for advanced thyroid cancer.

Fig. 2. Apoptosis induced by Ptx in tumor xenografts. Animals with advanced tumors were treated with daily i.p. Ptx injections at a dose of 2.5 mg/kg/day for 7 days. (a) Apoptotic cells in tumor tissue biopsies were detected at 11, 20, and 29 days, as described in Materials and Methods section, (x100). (b) Apoptotic index in the tumors. Data are mean ± SD. *P < 0.01 (ANOVA)

REFERENCES


