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EFFECTS OF PACLITAXEL AND COMBINATION OF THE DRUG WITH RADIATION THERAPY IN AN *IN VIVO* MODEL OF ANAPLASTIC THYROID CARCINOMA

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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.**

Paclitaxel (Ptx) has been successfully used to treat different types of human cancers [1]. Among those, the possibility of its application in one of the most aggressive human tumors, anaplastic thyroid cancer (ATC), both as a monotherapy and in a combination with other anti-tumor agents was demonstrated [2–5]. Since Ptx mainly affects the microtubules thus blocking cell division, usage of additional agents that would damage DNA in tumor cells to cause genotoxic stress is a plausible modality. One of the agents widely used in combination with chemotherapy, is ionizing radiation (IR). It is known that Ptx enhances tumor cell radiosensitivity [6–9], possibly due to cell cycle arrest at G2/M phase in which the cells are considered to be more sensitive to radiation [8, 10]. The data obtained in our *in vitro* experiments showed that ionizing radiation significantly enhances proapoptotic effect of Ptx in anaplastic cancer cells [11]. The focus of this study was to elucidate the combined effect of low doses of Ptx and radiation on the *in vivo* growth of xenograft tumors developing from human undifferentiated thyroid carcinoma cells implanted in immunocompromised mice.

MATERIALS AND METHODS

Nude mouse xenograft model. Animal experiments described in this study were conducted in accordance with the principles and procedures outlined in the Guide for the Care and Use of Laboratory Animals of the Biomedical Research Center, Center for Frontier Life Science (Nagasaki University, Nagasaki, Japan). Human follicular undifferentiated carcinoma cells FRO were initially provided by J. A. Fagin (University of Cincinnati College of Medicine, Cincinnati, OH). Cells were grown in RPMI 1640 supplemented with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin (all reagents from Invitrogen Life Technologies, Paisley, UK) in a 5 % CO_2 humidified atmosphere at 37 ^{0}C. FRO cells (5 x 10^6 cells per animal) resuspended in RPMI 1640 were injected s.c. into both flanks of 8-week–old female BALB/c nu/nu mice (Charles River Japan, Tokyo), 9 animals per group. Tumor sizes were measured each alternate day with calipers, and tumor volumes were calculated according to the formula a^2 x b x 0.4, where a is the smallest tumor diameter and b is the diameter perpendicular to a. Treatment with Ptx and γ-irradiation started after the tumor size approached 100 mm^3. Ptx (10 mg/kg/day) diluted in Cremophor EL (Sigma, USA), ethanol and phosphate-buffered saline (PBS, pH 7.4) (1:1:1 v/v/v/) was injected intraperitoneally (i.p.) daily for 7 days. Animals from the control group received vehicle injections. For the exposure to γ-irradiation, anesthetized animals were fixed in stalls, and the tumors were locally exposed to a single dose of 5 Gy (Pony PS-3100SB, radiation source ^{137}Cs, 0.662 MeV, 1 Gy/min) 4 days after Ptx treatment had started. Tumor size was monitored for four more weeks during which the body weight, feeding behavior, and motor activity of each animal were used as indicators of general health.

To determine the effect of low doses of Ptx on advanced tumors, animals were treated with i.p. Ptx injections at a dose of 2.5 mg/kg/day daily for 7 days. Tumor size was measured for 18 consecutive days.

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Abbreviations used: ATC – anaplastic thyroid cancer; FBS– fetal bovine serum; IR – ionizing radiation; PBS – phosphate-buffered saline; Ptx – Paclitaxel.

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 mm^3) 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 mm^3 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).

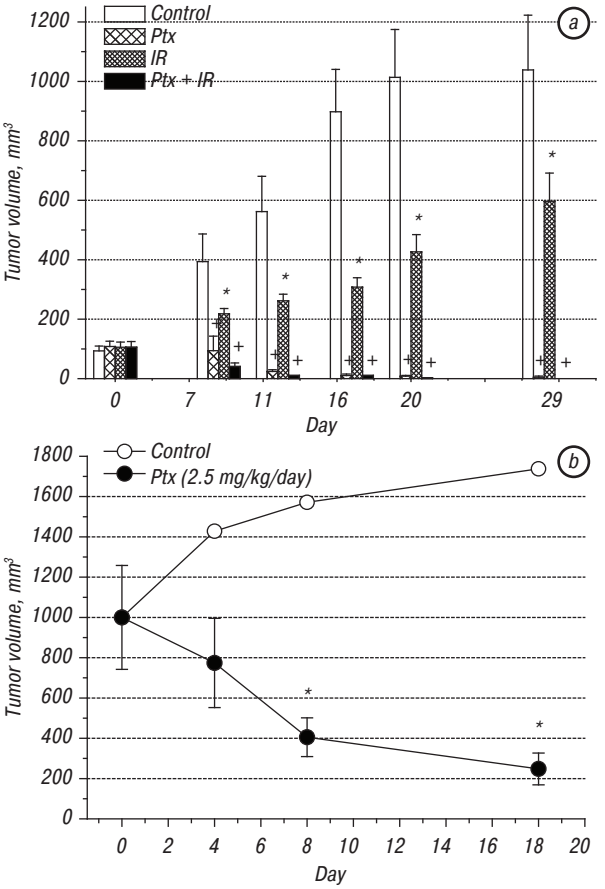

Fig. 1. Effect of IR and Ptx on the growth of tumor xenografts *in vivo. (a)* Effect of IR and Ptx as of monoagents and in combination. Ptx was injected i.p. at a dose of 10 mg/kg/day for 7 days. For IR-monotherapy and combination therapy, animals were exposed to a single dose of 5 Gy, 4 days after Ptx treatment had started. Data are presented as mean ± SD of 9 tumors. *$P < 0.05$ *vs.* control group (Mann — Whitney test); + $P < 0.05$ *vs.* irradiated group (Kruskal — Wallis test). (*b*) Effect of low doses of Ptx on advanced tumors. Animals were treated with Ptx i.p. injections at a dose of 2.5 mg/kg/day for 7 days. Data are presented as mean ± SD, n = 1 for the control group, n = 8 for the treatment group. *$P < 0.05$ *vs.* n = 9 at day 0 (Kruskal — Wallis test)

Despite the combined effect of Ptx and radiation in various tumors have been under investigation for a long time, the mechanism of radiosensitization is not fully understood. It is known that exposure to IR activates a complex system of sensors, mediators, signal transducers, and effectors. The latter two categories include checkpoint kinases, CHK1 and CHK2 (transducers), and p53 and Cdc25A-C (effectors) [13]. Their activities undergo changes under the action of both agents. The response of tumor cells to Ptx does not necessarily require the intact p53 and related signaling mechanisms [14 for rev.] as demonstrated, in particular, by its high cytotoxicity in ARO cells (initially assumed to be anaplastic thyroid carcinoma cell line but recently reclassified into colon carcinoma) with inactive *TP53* [2]. In this study, the potentiation of cytotoxic effect of the combination therapy (Ptx and radiation) in tumors originating from FRO cells with wild-type p53 may perhaps be attributed in part to the activation of p53-dependent signaling pathways. In this case, the augmentation of apoptosis most likely

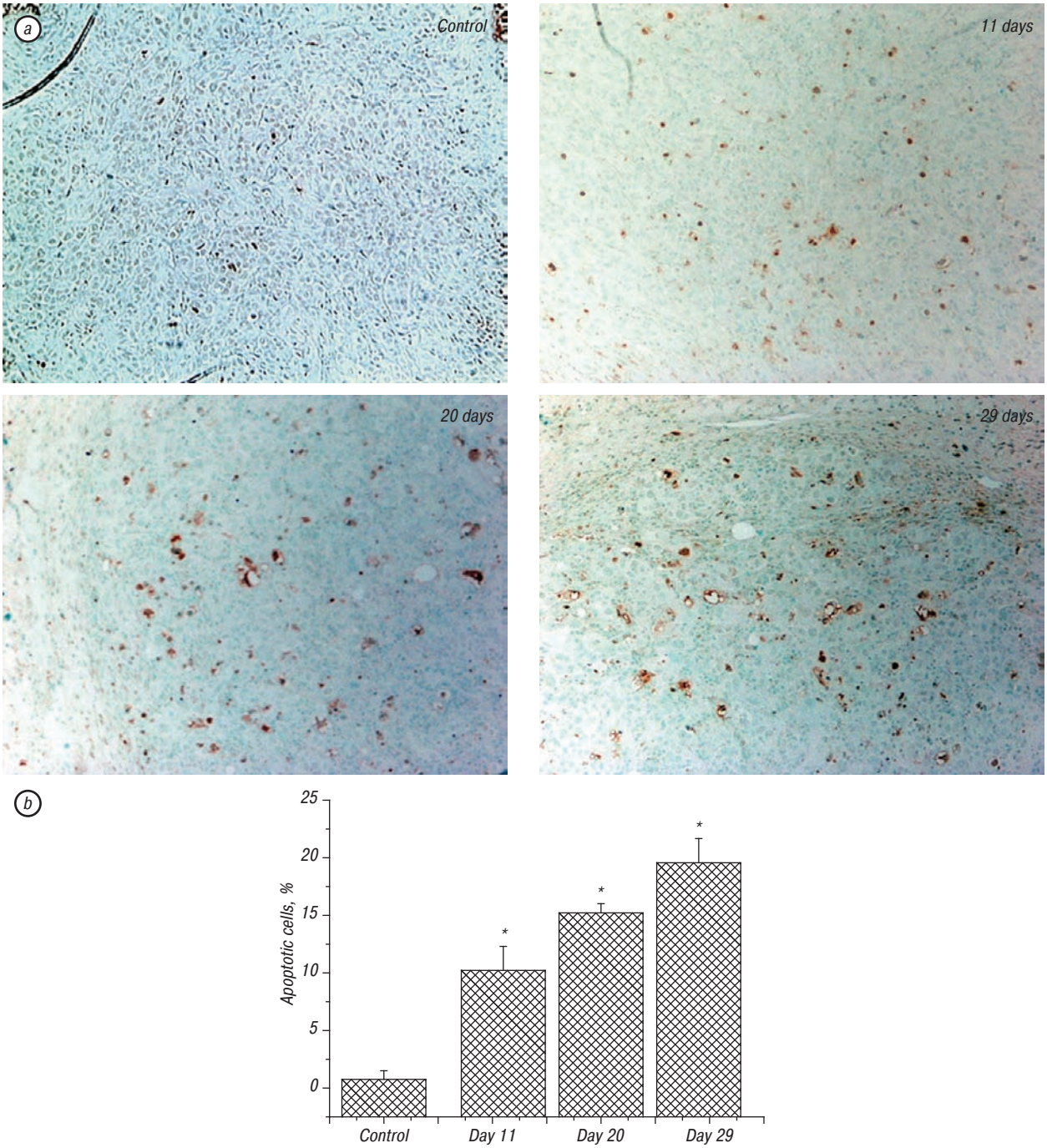

Fig. 2. Apoptosis induced by Ptx in tumor xenografts. Animals with advanced tumors were treated with daily i.p. Ptx injections at a dose of 2.5 mg/kg/day for 7 days. (*a*) Apoptotic cells in tumor tissue biopsies were detected at 11, 20, and 29 days, as described in Materials and Methods section, (x100).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

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.

REFERENCES

1. **Kingston DGI.** The shape of things to come: Structural and synthetic studies of taxol and related compounds. Phytochemistry 2007; **68**: 1844–54.

2. **Pushkarev VM, Starenki DV, Saenko VA, *et al*.** Molecular mechanism of the effects of low concentrations of taxol in anaplastic thyroid cancer cells. Endocrinology 2004; **145**: 3143–52.

3. **Xu G, Pan J, Martin C, *et al*.** Angiogenesis inhibition in the in vivo antineoplastic effect of manumycin and paclitaxel against anaplastic thyroid carcinoma. J Clin Endocrinol Metab 2001; **86**: 1769–77.

4. **Ain KB, Egorin MJ, DeSimone PA.** Treatment of anaplastic thyroid carcinoma with paclitaxel: phase 2 trial using ninety-six-hour infusion. Collaborative Anaplastic Thyroid Cancer Health Intervention Trials (CATCHIT) Group. Thyroid 2000; **10**: 587–94.

5. **Yeung S-C J, She M, Yang H, *et al.*** Combination chemotherapy including combretastatin A4 phosphate and paclitaxel is effective against anaplastic thyroid cancer in a nude mouse xenograft model. J Clin Endocrinol Metab 2007; **92**: 2902–9.

6. **Dey S, Spring PM, Arnold S, *et al.*** Low-dose fractionated radiation potentiates the effects of paclitaxel in wild-type and mutant p53 head and neck tumor cell lines. Clin Cancer Res 2003; **9**: 1557–65.

7. **Spring PM, Arnold SM, Shajahan S, *et al.*** Low dose fractionated radiation potentiates the effects of taxotere in nude mice xenografts of squamous cell carcinoma of head and neck. Cell Cycle 2004; **3**: 479–85.

8. **Toiyama Y, Inoue Y, Hiro J, *et al.*** The range of optimal concentration and mechanisms of paclitaxel in radio-enhancement in gastrointestinal cancer cell lines. Cancer Chemother Pharmacol 2007; **59**: 733–42.

9. **Zhang AL, Russell PJ, Knittel T, *et al*.** Paclitaxel enhanced radiation sensitization for the suppression of human prostate cancer tumor growth via a p53 independent pathway. Prostate 2007; **67**:1630–40.

10. **Safran H, Rathore R.** Paclitaxel as a radiation sensitizer for locally advanced pancreatic cancer. Crit Rev Oncol/Hematol 2002; **43**: 57–62.

11. **Pushkarev VM, Starenki DV, Popadiuk ID, *et al.*** The effect of combined action of Taxol and ionizing radiation on thyroid anaplastic cancer cells. Rep Ukr Acad Sci 2009; №5: 209–12 (in Ukrainian).

12. **Pushkarev VM, Starenki DV, Yamashita S, *et al.*** The enhancement of cytotoxic action of Taxol on thyroid anaplastic cancer by γ-irradiation. Rep Ukr Acad Sci 2010; № 3: 190-3 (in Ukrainian).

13. **Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S.** Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 2004; **73**:39–85.

14. **Tronko MD, Pushkarev VM, Popadiuk ID, *et al.*** The intracellular mechanisms mediating action of taxanes in tumor cell. J AMS Ukraine 2010; **15**, №3: 408–23 (in Ukainian).