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The action of ionizing irradiation alone or in combination with cisplatin on malignant cells: investigations *in vitro*

KEYWORDS: Combined Modality Therapy; Tumor Cells, Cultured; Cisplatin; Radiation, Ionizing

ABSTRACT

The paper gives a brief review of the literature data based on in vitro investigations of cytotoxic action of cisplatin and irradiation, applied alone or in combination, to carcinoma cell lines and fresh tumor explants. Presented data, observed in the light of data obtained in clinics, indicate that in vivo some additional tumor destructive activity might be induced by applied therapy. Therefore examinations of the antitumor effect of some treatment need to be more complex and must include determination of the effects of applied therapy on set of parameters, which control tumor growth in vivo.

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INTRODUCTION

The aim to enhance the antitumor effect led many physicians to design various combined treatment modalities. Radiation therapy induces arrest in malignant cell proliferation and kills some of affected cells. For a long time combined external irradiation and brachytherapy has been considered as the standard treatment for locally advanced cervical cancers showing a special, notable success in the local control of this disease and improving the overall survival rate (1). *Cis*-diamminedichloroplatinum (*cis*-DDP, cisplatin), inducing tumor cell death mainly trough interaction with intrastrand DNA bases, has shown its powerful antitumor activity to various types of tumors, (2,3) both *in vitro* and *in vivo*. Combination of these two treatment modalities aimed to enhance local tumor control, relapse free survival and overall survival. Several clinical randomized trials assessing cisplatin delivered therapy concomitantly with radiotherapy have been conducted in United States and Europe. Results of some studies are in favor to treatment combinations although the optimal chemotherapeutic regimens remain to be defined (4-8). It must be mentioned that occasional absence of benefit of combined therapy of heavy advanced disease has been reported too (9,10).

INVESTIGATIONS *IN VITRO*

Investigations of the cytotoxic activity of combined treatment with gamma-ray irradiation and cisplatin in comparison to the cytotoxic effect of each treatment applied alone, *in vitro*, gave some opposite results. Several publications reported that cytotoxic effect of combined treatment is lower than treatments when radiotherapy or chemotherapy was applied alone. It was found (11,12) that pre-irradiation of cervix adenocarcinoma HeLa cells with multiple fractions of gamma rays (total dose of 15 Gy), did not change their sensitivity to gamma rays, but it increased the resistance to *cis*-DDP, that was long-lasting effect. The results suggest that caution is needed in medical application of radiation combined with chemical treatment. Similarly, Eichholtz-Wirth et al. (13) found that a low-dose fractionated gamma-irradiation (three cycles of 5 x 2 Gy) induces modest cisplatin resistance in HeLa cells. They explained that cisplatin resistance correlated with reduced expression of interleukin-1beta-converting enzyme (ICE)-related protease, a member of the ICE family with structural homology but different substrate specificity. It was suggested that cisplatin resistance in pre-irradiated HeLa-S3 cells is associated with alterations of a CPP32-linked apoptotic pathway, which is affected by the damage caused by cisplatin and not by irradiation. A considerable heterogeneity in cell survival among isolated resistant clones and in the propensity of the cells to enter apoptosis, associated with altered activation of the apoptotic pathway by members of the TNF family was reported in a later publication (14). The membrane receptor CD95 (Apo-1/Fas), which was up regulated immediately after cisplatin exposure in parental HeLa cells, was expressed at various levels in the resistant clones. There were also changes in the formation of the inhibitor protein I kappa B, which regulates the anti-apoptotic transcription factor NF kappa B. Since the response to radiation was not changed, it was suggested that changes in the activation of the caspase-dependent signaling cascade are involved in the pre-irradiated cell death pathway initiated by cisplatin but not by radiation damage. IkappaB/NF-kappaB mediated cisplatin resistance in HeLa cells after low-dose gamma irradiation was associated with altered silencer of death domain, SODD expression (15). Caney et al (16) reached almost the same conclusion that pre-exposure of human squamous carcinoma cells to low-doses of gamma-rays leads to an increased resistance to subsequent low-dose cisplatin treatment. In our experiments (17) HeLa cells were: irradiated by X rays with 2 Gy daily, during four days, and on the fifth day cell were additionally treated with 7 Gy. Cells in parallel samples were both incubated with 0.33μM *cis*-DDP or with 0.33μM *cis*-DDP, and irradiated. The higher cytotoxicity of combined treatment was seen only 24h after the cell treatment. Irradiation induced a direct toxicity to 17% of target HeLa cells. There was no direct toxicity of applied *cis*-DDP concentration, although cell

growth was totally inhibited. The cytotoxic action of combined treatment was 52%; survived cells were giants. However, 10 days after the end of cell treatment in flasks with cells treated with *cis*-DDP the number of cells was reduced to almost 1/3 of the number in control samples. Number of cells in flasks treated with irradiation alone was negligible low in comparison to controls or to samples with *cis*-DDP treated cells. Moreover it was 2.7 times lower from the number of cells where combination of *cis*-DDP and irradiation was applied. Therefore, results from this experiment obtained 10th days after the end of treatment(s) did not show any enhancement in toxicity of combined *cis*-DDP and irradiation.

Better results were reported by Britten at al (18). They have also found that concomitant administration of cisplatin reduced the clinically relevant radiosensitivity in the majority (11 out of 19) of the human cervical tumor cell lines. Radiosensitization was observed only in 4 out of 19 cases, and in other 4 out of 19 cell lines there was no significant change in radiosensitivity. However, the sum of the independent cell killing by radiation and cisplatin was approximately twofold higher than after radiation alone. They suggested that concomitant cisplatin/radiotherapy regimens might result in a higher level of local tumor control, but primarily through additive toxicity and not through radiosensitization. In order to clarify the mechanisms of cell resistance in relation to combined treatment few experiments were done by Kato et al. (19). Investigating mechanisms involved in the irradiation-induced cisplatin resistance in various head and neck squamous cell carcinomas (HNSCC) cell lines, Kato et al. reported that different molecular determinants contribute to sensitivity of cells to cisplatin and radiation, including glutathione (GSH), and activation of nuclear factor-kappaB (NF-kappaB), a transcription factor that regulates cytoprotective genes. GSH and NF-alphaB can contribute independently to cisplatin and radiation sensitivity of human HNSCC.

Mentioned discrepancies between *in vivo* and *in vitro* results led experimental oncologists to more detailed studies. It was recognized as a critical point that appropriate targets must be chosen. Thus a new experimental approach appeared and papers reported synergistic effect of radiotherapy and chemotherapy to malignant cells. Monk et al. used an *in vitro* assay (20) to determine the synergistic effects of irradiation and chemotherapy on human cervical carcinoma cell lines and fresh tumor explants. *In vitro* response to irradiation was determined for 4 cell lines and 26 fresh tumor explants in an agar-based assay. Cells were exposed to increasing doses of radiotherapy with or without cisplatin. Cell suspensions were cultured for 5 days, with [3H] thymidine added on day 3 and proliferation was measured. Heterogeneous radiotherapy dose-response relationships in the *in vitro* assay were demonstrated. Explants were more sensitive to radiotherapy than cell lines. Unlike cell lines, fresh tumor cells consistently displayed sensitivity to synergist action of radiotherapy and chemotherapy. The synergy between radiotherapy and inhibitor of glutathione biosynthesis, butation-sulfoximin suggests that glutathione depletion may enhance the effect of radiotherapy too. Clinical trials to evaluate this assay are indicated.

Another approach to resolve mentioned discrepancy was based on the fact that a key event in cervical carcinogenesis is the disruption of p53 tumor suppressor pathway by human papillomavirus (HPV) E6 gene. Koivusalo et al. (21) have studied the effect of irradiation and *cis*-DDP on the induction of this tumor-suppressor pathway. They found that regardless of the HPV type in SiHa (HPV 16+), CaSki (HPV 16+), HeLa (HPV 18+), and UT-DEC-1 (HPV 33+) cell lines, cisplatin activated a p53 reporter and reduced the HPV E6 mRNA. After irradiation alone, a decrease in HPV E6 mRNA levels and an activation of the p53-reporter were detected in SiHa, CaSki, and HeLa cells. Concomitant platinum treatment and irradiation led to poly(ADP-ribose) polymerase cleavage as a sign of caspase-3 activation and apoptosis.

RECOMMENDATIONS FOR FURTHER INVESTIGATION

Observed discordance between laboratory and clinical data led experimental oncologist to great dilemmas. Results from clinical studies are promising, and may be that overall parameters which control tumor cells death are not yet determined. In some *in vitro* experiments, originally radiation-insensitive HeLa cells were used as an inappropriate target cell model. Experiments were done in nutrition medium supplemented with bovine serum albumin and not with human sera. The use of fresh tumor explants is suggested if the predetermination of malignant cells sensitivity for individual tumor to specific treatment is expected. It might be possible that some new indirect cytotoxic mechanisms could contribute to enhance antitumor activity. Results obtained by Monk et al. (20) reporting the synergistic effect of combined treatment (cisplatin and irradiation) on fresh tumor explant are along with proposed hypothesis.

Generally, questions that are frequently set up to experimental oncologists are related to the translation of laboratory results (based on the model systems developed for *in vitro*, or for *in vivo* studies) to the clinic. Therefore, if the straight answer for the some treatment modality is expected, then the proper choice of the experimental set up must be done. Examinations *in vitro*, which need to be done generally include: the proper choice of target tumor cells (the best choice are patient's tumor cells); use of autologous sera and patient's peripheral blood mononuclear cells (PBMC) in the experiment, determination of the total treatment cytotoxicity (direct or reproductive), or and the type of inhibition of tumor cell growth (apoptosis or necrosis).

Limitations in the experimental ability to set up the patient's tumor cells in culture and to grow them in the presence of autologous serum, and financial shortage, led many investigators to do their experiments on tumor cell lines as target models. The presence of patient's serum and of PBMC in cell culture is frequently omitted, and the changes of the metastatic potential of treated tumor cells is only rarely determined. This gives some incomplete set of data. Knowing all insufficiency of this approach experimental oncologist must give the result with reliable great limitations. Examinations of the antitumor effect *in vivo* on animal tumors, or better on human tumor xenografts could give the most reliable results, but data on the antitumor cellular immunity, which directly correlate with patient's response, could not be obtained from these experiments.

It seems that straightforward prediction of the patient's response to therapy is almost impossible to get. Good results of combined treatment in relation to irradiation alone, seen in some patients (4-8), seems not to be related only with direct toxicity of applied combined treatment to malignant cells. Therefore, is a question whether combined therapy (irradiation and cisplatin) contribute to the enhancement of the patient's antitumor immunity? When irradiation or cisplatin induce death of some malignant cells, the apoptotic bodies could be removed by phagocytes whose relative number and their phagocytic capacity sharply increases in irradiated patients with CA-PVU during and at the end of radiotherapy (22). If cisplatin affects (enhances) the mode of antigen presentation to immune system, this could led to the better activation of the antitumor immune response to patient's own tumor antigens, and subsequently to the tumor shrinkage. In every case, determination of the patient's immune response to autologous tumor antigens, or of change in tumor susceptibility to be immunologically destroyed, could give the correct answer regarding the effects of combined irradiation and cisplatin therapy on immunological control of patient's tumor growth.

CONCLUSION

In conclusion, presented data on antitumor effects of irradiation and cisplatin applied alone or in combinations against malignant tumor cells *in vivo* and *in vitro* pointed that determination of solely cytotoxic effect of applied treatments *in vitro*, could not be the end of a translational research. Examinations need to be more complex and must also include determination of the effects of applied therapy on other parameters, which control tumor growth *in vivo*.

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