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ADJUVANT ANTIPROLIFERATIVE AND CYTOTOXIC EFFECT OF ALOIN IN IRRADIATED HeLaS3 CELLS

A. Nićiforović, M. Adžić, B. Zarić, V. Vučić and M. B. Radojčić

VINCA Institute of Nuclear Sciences
P.O. Box 522, Belgrade, Serbia

Abstract

Naturally occurring phytoanthracycline, aloin, was used to radiosensitize HeLaS3 human cervix carcinoma cells. The results indicated that the cytotoxic adjuvant effect of aloin was synergistic with IR at all drug concentrations and comparable to the cytotoxicity of 5-10Gy IR alone. Radiosensitization of HeLaS3 cells was achieved by 60μM aloin which reduced IC_{50} dose of IR from 3.4- to 2Gy. The cell damage by both agents compromised cell capacity to conduct programmed cell death by apoptosis, and led to the synergic cytotoxic cell death by necrosis.

Introduction

Ionizing radiation (IR) induces dose and time dependent inhibition of HeLaS3 human cervical carcinoma cell growth due to perturbation of cell-cycle and induction of apoptosis [1]. The cell proliferation was inhibited for 50% (IC_{50}) at 3.4Gy, while 50% cell cytotoxicity (EC_{50}) was not attainable within the dose range of 2-10Gy. These cellular effects correlated with increased activity of mitochondrial antioxidant enzyme Mn-superoxide dismutase (MnSOD), which was not coupled with the respective catalase (CAT) activity. The cytosolic CuZn-superoxide dismutase activity decreased [2]. Since IC_{50} dose is rather high the aim of the presented study was to test the adjuvant phytoanthracycline, aloin, which possess less undesirable side-effects in comparison to other currently used drugs. Aloin is shown to cause perturbation of HeLaS3 cell-cycle, and increase percentage of cells in S phase and apoptosis [3]. Since aloin diminishes tumour metastatic potential induced by IR-triggered MnSOD overexpression [4], and since IR and aloin share, at least in part, a common pathways in the mechanism of their action, it is of interest to investigate their possible synergic activity in HeLaS3 cells.

Experimental Procedure

HeLaS3 cells were irradiated with 2-, 5- or 10Gy of gamma-rays from a ^{60}Co source at the dose rate of 20Gy/h. Different concentrations of aloin (20-100μM) were used to treat cells immediately after irradiation. The cell growth and viability were determined by the trypan blue exclusion assay. For cell cycle analysis, the cells were fixed in ice-cold 70% ethanol, treated with RNAse-A, incubated with propidium iodide and analyzed by a FACS Calibur flow cytometer. Cell cycle distribution was determined using ModFIT software. Statistically significant differences were evaluated using one-way analysis of variance (ANOVA) and the Tukey *post-hoc* test. $p<0.05$ was considered significant.

Results and Discussion

The adjuvant effect of aloin (20-100μM) was tested on HeLaS3 cells irradiated with standard clinical fraction dose of 2Gy and cell viability (% of viable cells, *Figure 1A*) and viability index (ratio of viable cells in treated *vs.* control sample, *Figure 1B*) were followed. The results clearly indicated that the cytotoxic adjuvant effect of aloin was synergistic with IR at all drug concentrations and comparable to the cytotoxicity of 5-10Gy IR alone. Moreover, 60μM aloin as adjuvant reduced IC_{50} dose of IR from 3.4 to 2Gy.

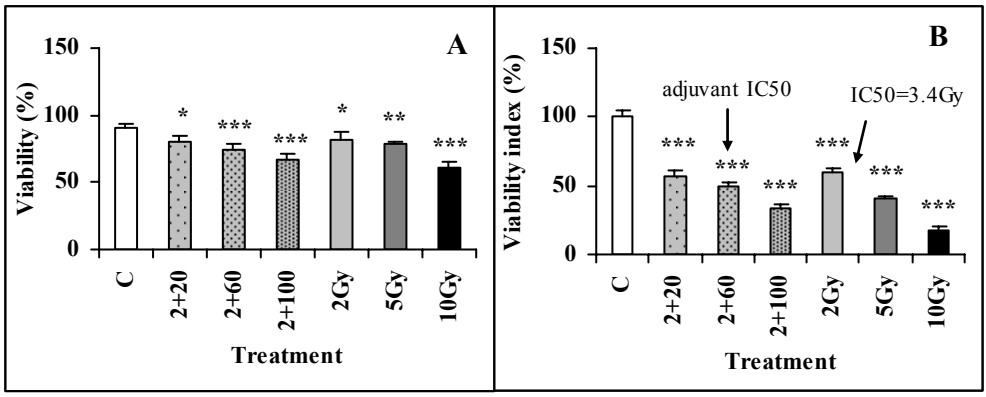

Fig. 1. Combined effect of IR (Gy)+aloin (μM) or IR alone on HeLaS3 cell viability (A) and viability index (B). Data are presented as mean ± SD (n=6)

Flow-cytometric analysis of PI stained HeLaS3 cells indicated perturbation of the cell cycle *i.e.* significant decrease of cells in G1 phase (*Figure 2*) which was followed by the increased arrest of cells in S phase and increased apoptotic cell death (subG0/G1 peak). The adjuvant effect of aloin was observed predominantly in the S phase of cell cycle, at all drug concentrations. Although, adjuvant effect of aloin on cell apoptosis was not observed, aloin was still effective cytotoxic agent leading to decreased viability of survived cells most probably by necrosis [2].

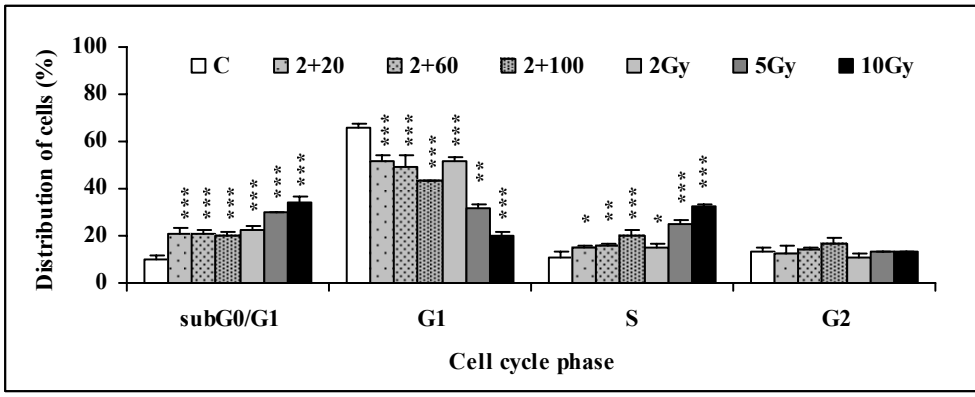

Fig. 2. Cell cycle distribution of HeLaS3 cell treated with IR (Gy)+aloin (μM) or IR alone. Data are presented as mean ± SD (n=3)

The synergic effect of aloin+IR on cell viability and in S-phase cell cycle arrest indicated that both agents may share a common regulatory pathway. Since, $HeLaS_3$ cells constitutively posses enhanced activity of MnSOD, and since both IR or IR+aloin caused its further elevation (which was not followed by the respective CAT activity) both agents caused H_2O_2 accumulation (data not shown). Increased concentration of this toxic ROS may lead to their synergism in cytotoxicity. High H_2O_2 concentration could also be the reason for down-regulation of CuZnSOD observed in either IR or IR+aloin treated cells (data not shown). Decreased CuZnSOD activity could contribute to decreased cell proliferation, emphasizing the importance of CuZnSOD in HeLaS3 cell growth [5]. In addition to that, increased MnSOD and H_2O_2, *via* Cip1 (p21) and cyclines D3 inhibition, may lead to the S phase cell cycle arrest. Although, IR or aloin alone increased subG0/G1 peak, their synergic effect on cell apoptosis was not observed. This result indicates that the mechanism of apoptosis induction by IR and aloin are probably different. Due to that, simultaneous cell damage by both agents could compromise cell capacity to conduct programmed cell death by apoptosis, and instead may lead to the synergic cytotoxic cell death by necrosis. The synergism of IR+aloin induced cell cytotoxicity is reflected in reduction of IC_{50} dose to 2Gy by 60μM aloin.

Conclusion

The adjuvant effect of aloin in irradiated HeLaS3 cells is reflected in the reduction of IC_{50} cytotoxicity dose from 3.4Gy to 2Gy in the presence of 60μM aloin. Its radiosensitizing effect is achieved through up-regulation of MnSOD activity leading to accumulation of toxic H_2O_2 This toxic ROS, either directly or through down-regulation of CuZnSOD activity, causes decrease in cell proliferation. Thus, through the altered redox balance, IR+aloin, compromises HeLaS3 cell capacity to conduct programmed cell death by apoptosis, and instead lead to the synergic cytotoxic cell death by necrosis.

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