

LOW-TEMPERATURE HYPERTHERMIA INDUCES THE FORMATION OF COLOR JUNCTIONS IMMEDIATELY AFTER IRRADIATION

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Introduction

Hyperthermia is used in combination with chemo- or radiotherapy as it is known to enhance the anti-cancer effects of both therapies. The enhanced effect of radiation after hyperthermia is the result of the interference of heat with the cells capability to deal with radiation-induced DNA damage. Recently there is much interest in the effect of hyperthermia at relatively low temperatures in the range of 40-42°C.

We compared radiosensitizing effects of 41°C hyperthermia on two human tumor cell lines which differed in sensitivity to heat.

Methods

41°C hyperthermia treatment was applied for 1h using a waterbath. Cells were irradiated using γ -rays from a ^{137}Cs source at a dose rate of about 0.7 Gy/min. For combined treatment radiation was applied immediately after heat.

Confluent cultures of RKO and SW-1573 cells were treated with hyperthermia and/or radiation. Clonogenic capacity, induction of chromosomal aberrations and of apoptosis were determined immediately after irradiation (immediate plating) and 24 hours after irradiation (delayed plating). Chromosomal aberrations, consisting of color junctions and fragments, were scored after inducing premature chromosome condensation (PCC) followed by fluorescent in situ hybridisation (FISH). With the PCC technique, chromosomal aberrations can be studied immediately after treatment. After lysing the cells in hypotonic PI-containing Nicoletti buffer for at least 24 hours, the induction of apoptosis was determined by analysis of the fraction of sub-G1 cells using FACS.

Results

41°C treatment led to a significant radiosensitization in the heat-sensitive RKO cells but not in the more resistant SW-1573 cells. Immediately after irradiation, the number of fragments was increased compared to control cells. When cells were incubated for 24 hours after irradiation, a decreased number of fragments and an increased number of color junctions appeared which indicates the rejoining of DNA double strand breaks. We show an increase in the number of color junctions immediately after combined treatment in both cell lines relative to radiation alone. This was an unexpected result as DNA damage repair after irradiation usually takes several hours. Hyperthermia did not lead to induction of chromosomal aberrations. Apoptotic fraction was slightly increased in RKO cells in the delayed plated cultures (4-10%) as compared to this fraction of RKO cultures directly after treatment (3-4%) and controls (2-3%). No apoptosis was detected in SW-1573 cells after these treatments.

Conclusion

The hyperthermia-induced radiosensitization in RKO cells was not accompanied by an increase in apoptotic cells. The appearance of an increased number of color junctions immediately after combined treatment in both cell lines suggests that an error-prone process of relatively fast DNA repair is stimulated by mild hyperthermia. This phenomenon is subjected to future studies.