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Urological Research

Enhanced Radioinduced Cytotoxicity of Cultured Human Bladder Cancer Cells Using 43 °C Hyperthermia or Anticancer Drugs

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Summary. Using a colony-forming technique and 2 human bladder cancer cell lines, T24 and KK-47, the enhanced radioinduced cytotoxicity in combination with 43 °C hyperthermia (HPT) or 4 anticancer drugs; bleomycin (BLM), cis-dichlorodiammineplatinum (II) (CDDP), mitomycin C (MMC), carbazilquinone (CQ), has been studied. In the series of both cell lines, the combination of 43 °C hyperthermia and irradiation resulted in exceedingly enhanced cytotoxicity. This was characterized by a marked decline of the slope of the radiation dose-survival curve as compared with slope in the combination of each of the anticancer drugs and irradiation. Among the 4 anticancer drugs, BLM was thought to be the most promising agent as a potentiator of the radiotherapy, on the basis of a remarkable decrease in the shoulder portion of the radiation dose-survival curve. The other 3 anticancer drugs showed a certain degree of potentiation of radiosensitivity.

Key words: Radioinduced cytotoxicity, Hyperthermia, Anticancer drugs, Bladder cancer.

There have been many reports suggesting that both a supranormal temperature and one of several anticancer drugs may be useful for cancer treatment by potentiating radiotherapy [8, 12]. In urological practice hot water perfusion with or without anticancer drugs in combination with irradiation has been used for bladder cancer therapy [3, 7, 15]. Among anticancer drugs, bleomycin (BLM), an antibiotic anticancer drug, has been most studied in vitro or in vivo in combination with irradiation [13, 14]. The synergistic cell killing effects from the combination of irradiation and hyperthermia or BLM, seem to rely upon a mechanism which affects DNA proteins in cells [10]. However, the cell killing effects enhanced by hyperthermia or anticancer drugs, including BLM, in combination with irradiation has not been examined in an in vitro cultured cell system.

The aim of the present study is to compare the cell killing effects from the single usage of irradiation, hyperthermia and several anticancer drugs. The combination of irradiation with either hyperthermia or several anticancer drugs using T24 and KK-47 cells derived from human bladder carcinomas also have been studied to find the most effective potentiator of radiotherapy.

Materials and Methods

Cells and Culture Conditions

Two continuous cell lines, T24 and KK-47, were derived from human transitional cell carcinomas of the bladder. T24 was obtained and originally described by Bubenik et al. [1], and was supplied by Dr. V. P. Collins, Department of Tumor Pathology, Institute of Pathology, Karolinska Hospital, Stockholm, Sweden. KK-47 was established in our department in 1977 and its biological and morphological properties are reported elsewhere [5].

The 2 cell lines were grown in Ham's F12 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 20 percent calf serum (Microbiological Associates, Md., USA) and streptomycin in 100 μg per ml. Three days after subcultivation, the cells, in an exponentially growing phase were used for this study.

Cell Preparations

The cells were gently trypsinized and counted, and 5 ml of single cell suspension containing 300 cells per ml was dispensed into each polyethylene test tube. Polyethylene test tubes were found to be the most suitable with the best cell freeing rate from the test tube surface by pipetting [12, 13].

Hyperthermia

The cells were exposed to 43 °C hyperthermia in a hot water bath (Hirasawa Co., Tokyo, Japan), in which the temperature was maintained to plus or minus 0.1 °C. After various times of 43 °C heating, 1 ml of the cell suspension and 4 ml of the fresh medium were transferred into triplicate tissue culture dishes, 60 x 15 mm (Falcon, Co., Cal., USA) and were cultured at 37 °C in a 5 percent CO_2 and 95

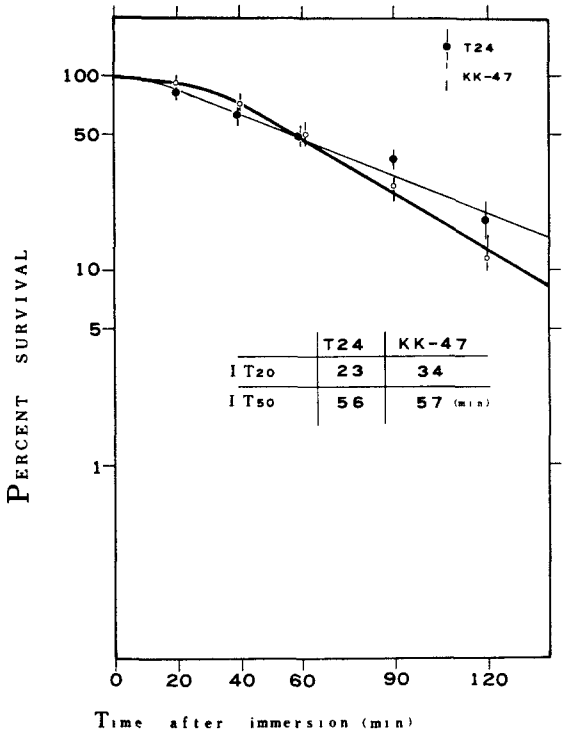

Fig. 1. Survival curves of T24 and KK-47 cells heated at 43 °C for various lengths of time. The *bars* indicate standard deviations of the mean for at least three independent experiments in the following figures

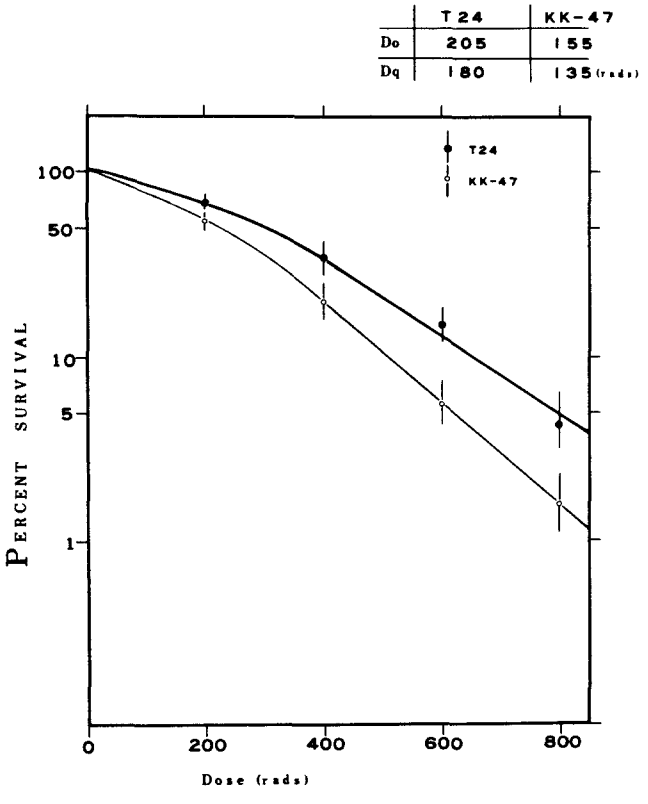

Fig. 2. X-ray dose-survival curves of T24 and KK-47 cells

percent air for 10 to 14 days. After cultivation, the number of colonies containing more than 50 cells were counted under a dissecting microscope. Heat response curves were generated and 20 percent and 50 percent growth inhibition times of the 43 °C temperature (IT_{20} and IT_{50}) were calculated from the response curve.

Irradiation

The cells were irradiated on a rotary platform at 37 °C. The X-ray unit provided 182 kv, potential at 15 milliamperes with a filtration of 0.5 mm Aluminium and 0.9 mm Copper; an average dose rate of 75 rads per min. As parameters of radioinduced cytotoxicity, Do and Dq values were adopted. Do indicates a radiation dose showing a 63 percent cell killing effect on an exponentially decreasing phase of the dose-survival curve, and Dq means a sublethal radiation dose defined by the shoulder portion of the dose-survival curve. From the survival curve, a 90 percent growth inhibition dose of radiation (IR_{90}) was computed.

Combination Treatment of Hyperthermia and Irradiation

The combination treatment of hyperthermia at the IT_{20} and IT_{50} values and irradiation was carried out. Following this combination treatment, the cells were freed and cultured as described above. Using the survival curves of these cells heated at the IT_{20} and IT_{50} values in combination with irradiation, Do, Dq and IR_{90} values in each hyperthermia series were computed.

Drug Sensitivity

A total of 4 anticancer drugs; bleomycin (BLM), cis-dichlorodiammineplatinum (II) (CDDP), mitomycin C (MMC) and carbazilquinone (CQ), were studied. A graded dose of each anticancer drug was added to each of the cell preparations in the test tubes. After a 2-h exposure of the anticancer drugs, the cells were centrifuged and washed with serum free Ham's F12 medium twice, and resuspended in 5 ml of the fresh medium. The mixture which consisted of 1 ml of the cell suspension containing 300 cells and 4 ml of the fresh medium was transferred into triplicate tissue culture dishes for incubation. The drug concentrations showing 20 percent and 50 percent growth inhibitions (ID_{20} and ID_{50}) as a function of increasing concentrations of the drugs were calculated from the dose-survival curves.

Combination Treatment of Anticancer Drugs and Irradiation

Drug exposures at the ID_{20} and ID_{50} values were performed simultaneously with irradiation. From the radiation dose-survival curves in combination with the drug exposures, Do, Dq and IR_{90} values were computed.

Results

Thermosensitivity

The surviving fractions of the cells exposed at 43 °C heating for 20 to 120 min are shown in Fig. 1. The IT_{20} and IT_{50} in T24 cells were 23 min and 56 min, respectively, and those in KK-47 cells were 34 min and 57 min, respectively. These IT_{20} and IT_{50} heatings were employed in combination with irradiation to compare the cell killing effect of the combination of the 4 anticancer drugs and irradiation.

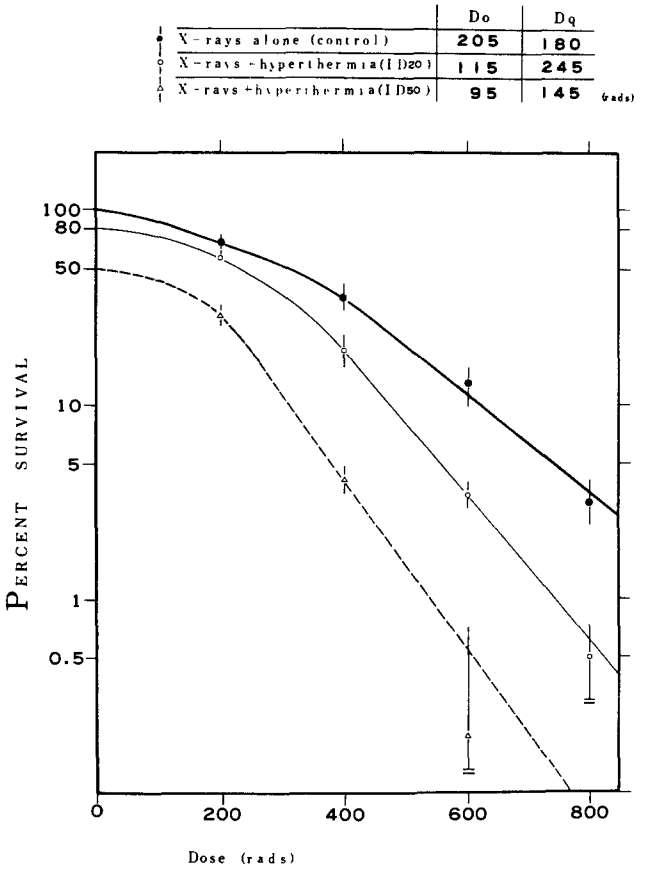

Fig. 3. Survival curves of T24 cells after the simultaneous treatment with X-rays and hyperthermia (IT_{20} or IT_{50}) at 43 °C

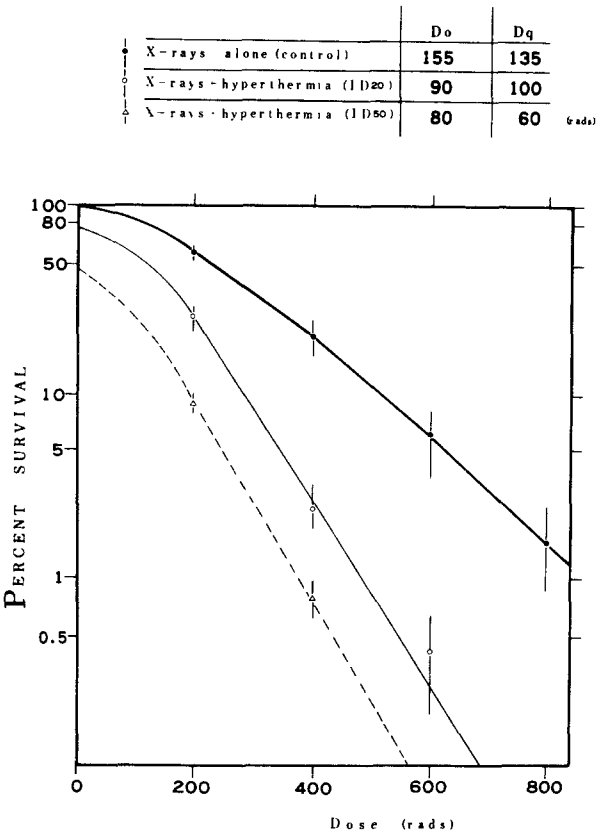

Fig. 4. Survival curves of KK-47 cells after the simultaneous treatment with X-rays and hyperthermia (IT_{20} or IT_{50}) at 43 °C

Radioinduced Cytotoxicity

The dose-survival curves of T24 and KK-47 cells exposed to a graded dose of irradiation are shown in Fig. 2. The radioinduced cytotoxicity of T24 cells quoted by Do and Dq were 205 rads and 180 rads, respectively. Those of KK-47 cells were 155 rads and 135 rads, respectively. T24 cells were slightly radioresistant compared with KK-47 cells.

Combination Treatment of Hyperthermia and Irradiation

The survival curves of these cells exposed to 43 °C hyperthermia and irradiation are shown in Figs. 3 and 4. As listed in Table 1, the marked decrease of Do were obtained in the combinations of heatings (HPT) at the IT_{20} and IT_{50} values and irradiation in the series of both cell lines. The decreases of Do in T24 and KK-47 cells in combination with heating at the IT_{20} value reached 44 percent and 42 percent, respectively, and in combination with heating at the IT_{50} value reached 54 percent and 48 percent, respectively. The decrease of Dq was remarkable in KK-47 cells, and there was a decrease of 26 percent in combination with heating at the IT_{20} value and a 56 percent decrease in combination with heating at the IT_{50} value.

Chemosensitivity

The ID_{20} and ID_{50} concentrations of BLM, CDDP, MMC and CQ for a 2-h exposure are listed in Table 2. The survival curve represented an exponential decrease in MMC and CQ, while it had an upward concavity in BLM. There was no substantial difference of the chemosensitivity between the 2 cell lines treated with each of the drugs.

Combination Treatment of Anticancer Drugs and Irradiation

Changes of the parameters Do and Dq, of radiation dose-survival curve in combination with each of the drugs are listed in Table 1. The irradiation at the IR_{90} value decreased in combination with hyperthermia treatments or anticancer drugs are listed in Table 3. On the basis of reductions of the IR_{90} value, BLM showed a remarkable enhancement of radioinduced cytotoxicity. The amounts of the reduction of the IR_{90} value in combination with the ID_{20} and ID_{50} doses of BLM in T24 cells were 21 percent and 48 percent, respectively, and those in KK-47 cells were 26 percent and 51 percent, respectively.

Table 1. Parameters of radioinduced cytotoxicity (Do, Dq) affected by combination use of hyperthermia (IT_{20} or IT_{50}) or anticancer agents (ID_{20} or ID_{50})

	T24							
	ID_{20} (or IT_{20})				ID_{50} (or IT_{50})			
	Do	Reduction of Do (%)	Dq	Reduction of Dq (%)	Do	Reduction of Do (%)	Dq	Reduction of Dq (%)
X-rays alone (control)	205		180					
HPT	115	44	245	−36	95	54	145	19
BLM	170	17	140	22	130	37	115	36
CDDP	150	27	240	−33	110	46	240	−33
MMC	130	37	220	−22	115	44	230	−28
CQ	200	2	65	64	195	5	60	67
	KK-47							
	ID_{20} (or IT_{20})				ID_{50} (or IT_{50})			
	Do	Reduction of Do (%)	Dq	Reduction of Dq (%)	Do	Reduction of Do (%)	Dq	Reduction of Dq (%)
X-rays alone (control)	155		135					
HPT	90	42	100	26	80	48	60	56
BLM	155	0	45	67	155	0	0	–
CDDP	140	10	105	22	120	23	110	19
MMC	110	29	150	−11	105	32	125	7
CQ	135	13	180	−33	130	16	110	19

Table 2. ID_{20} and ID_{50} of anticancer agent for a 2-h exposure in T24 and KK-47 cells

	T24	
	ID_{20}	ID_{50} (μg/ml)
BLM	1.1×10^{-1}	2.4×10^{-1}
CDDP	3.0×10^{-1}	6.5×10^{-1}
MMC	1.0×10^{-1}	1.5×10^{-1}
CQ	1.2×10^{-3}	4.0×10^{-3}
	KK-47	
	ID_{20}	ID_{50} (μg/ml)
BLM	2.2×10^{-1}	5.1×10^{-1}
CDDP	5.0×10^{-1}	1.2
MMC	1.6×10^{-1}	2.5×10^{-1}
CQ	2.6×10^{-3}	6.7×10^{-3}

Discussion

The combination of irradiation and hyperthermia showed an enhanced cell killing effect compared with the combination of irradiation and each of the 4 anticancer drugs. The hyperthermia enhancement of the radioinduced cytotoxicity was characterized by a prompt decrease of Do in both cell lines. The degree of the enhancement by hyperthermia resulted from both the temperature and the sequence of these 2 modalities. It was observed that hyperthermia beyond 43 °C followed by irradiation (pre-heating) showed an enhanced cell killing effect exhibiting a more marked decrease of Do than hyperthermia beyond 43 °C after irradiation (post-heating), which has been interpreted as a primary enhanced cell killing effect of hyperthermia [4, 13].

On the other hand, post-heating showed more increased cell killing at a temperature below 43 °C. This effect was characterized by the decrease of Dq and thought to be a retardation of recovery from the cell damage induced by irradiation due to the combined low grade hyperthermia [4]. The diverse interaction between the high grade and low grade hyperthermia provided by 43 °C as a border line suggested the existence of 2 independent cell killing mechanisms [4, 16]. Our previous paper [12] showed the cell killing effect of irradiation in combination with 43 °C hyperthermia, in which there was no difference between the cell killing effects of the heating before and after irradiation unless treatment intervals of the 2 modalities exceeded one hour. Moreover, 43 °C hyperthermia is a critical level for bladder cancer patients treated by an in-

Table 3. IR_{90} values affected by combination use of hyperthermia (IT_{20} or IT_{50}) or anticancer agents (ID_{20} or ID_{50})

	T24			
	ID_{20} (or IT_{20})	Reduction of IR_{90} (%)	ID_{50} (or IT_{50})	Reduction of IR_{90} (%)
X-rays alone (control)	625			
HPT	470	25	305	51
BLM	495	21	325	48
CDDP	560	10	420	33
MMC	495	21	415	34
CQ	490	22	365	42
	KK-47			
	ID_{20} (or IT_{20})	Reduction of IR_{90} (%)	ID_{50} (or IT_{50})	Reduction of IR_{90} (%)
X-rays alone (control)	510			
HPT	285	44	190	63
BLM	375	26	250	51
CDDP	395	23	305	40
MMC	385	25	290	43
CQ	440	14	320	37

travesical perfusion therapy without anesthesia [15]. For this reason, 43 °C hyperthermia or drug exposure combined simultaneously with irradiation was used in this study.

The advantage of hyperthermia in combination with irradiation was advocated because relatively radioresistant S phase cells are the most sensitive to hyperthermia, and radioresistant hypoxic cells situated in a large tumor mass can be destroyed by irradiation in combination with hyperthermia [2, 8]. Because of the development in temperature measurement which is used in local hyperthermia treatment, several cancers may be controllable by the combined modality of irradiation and hyperthermia [6, 17].

As for anticancer drugs being utilized in combination with irradiation, BLM has been reported as one of the most promising drugs in in vitro and in vivo studies [9, 14]. The mechanism of the synergistic cell killing by BLM in combination with irradiation has been explained in terms of injury to DNA or to nucleoproteins. The degree of the synergism by these 2 agents largely depends on the degree of temperature and time sequences of both treatments [9, 13]. According to our data, BLM was found to be the most synergistic among the 4 anticancer drugs tested in combination with irradiation. The potentiation of radioinduced cytotoxicity by BLM was characterized by a marked decrease of Dq in KK-47 cells. This result agrees with the observations by Midander et al. [9]. While T24 showed a significant decrease of both Do and Dq on the radiation dose-survival curves in combination with BLM, CDDP and MMC showed a considerable decrease of Do in both cell lines at their ID_{20} and ID_{50} values. CQ showed a marked decrease of Dq in T24 cells and a mild decrease of Do in KK-47 cells.

Many workers have discussed whether the combined effects of hyperthermia and some anticancer drugs are additive or synergistic on the radiation dose-survival curves [4, 8, 12]. Murthy et al. [11] reported that the synergism of radioinduced cytotoxicity was defined by means of a reduction of Do on the radiation dose-survival curve, and the combined effect of irradiation followed by a low grade hyperthermia below 43 °C, which resulted in a decrease of Dq, was thought to be a potentiation in an additive mode. In the present study, all but BLM combined with irradiation in KK-47 cells induced the decrease of Do and Dq in both cells lines. The change of these parameters was similar for the 4 anticancer drugs, each having a different degree of synergism in combination with irradiation. Based on the present results, hyperthermia and BLM may be quite useful in combination with radiotherapy for bladder cancer. The other 3 anticancer drugs might be potentiators of radiotherapy.

Further investigations are needed to obtain a better combination modality including irradiation, hyperthermia and several anticancer agents.

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