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Radiosensitizing and repair-inhibiting properties of dipyridamole*

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Summary

Radioresistance and postirradiation repair of potentially lethal damage (PLD repair) are important factors underlying failure to control local disease in cancer. Dipyridamole (DP) is known as a modifier of the action of cytotoxic drugs. We therefore investigated DP as a potential radiosensitizer and inhibitor of PLD repair in X-irradiated Chinese hamster ovary (CHO) cells in vitro. Exposure to the drug alone resulted in a slight reduction of the clonogenic capacity of the cells. Preincubation for 18 h with 10 and 20 μ M DP in cells subcultured at low density, led to a significant radiosensitization. In confluent density-inhibited cultures, preincubation alone as well as pre- and postincubation with 20 μ M DP resulted in a significant inhibition of PLD repair. Dipyridamole and related compounds may thus be useful tools for modifying and investigating the response of mammalian cells to radiation.

Introduction

Dipyridamole (DP), a potent inhibitor of the nucleotide salvage pathway, has been used in vitro and in vivo to potentiate the cytotoxic activity of a number of anti-cancer agents including acivicin [10], adriamycin [16,23,30], cisplatin [19], etoposide [15,16,19], 5-fluoro-uracil [2,8,12,13,25], methotrexate [28,39], mithoxantrone [5], *N*-phosphonacetyl-L-aspartate [3,33] and vinblastine [16]. The mechanisms underlying these observations are currently not quite clear. However, DP is an inhibitor of cellular metabolism at various levels [36]. DP has an inhibitory effect on energy metabolism through a reduction in uptake of glucose, inorganic phosphate [21] and nucleosides [18,21,29]. DP acts also as an inhibitor of microtubule formation [14,26,32,34,35]. Since cellular processes for the repair of DNA damage depend on the presence of energy [6,7], its inhibitory effects on glucose and nucleoside uptake may contribute to enhancement of cytotoxic

treatment. Microtubule inhibitors, on the other hand, have been shown to block cells in the most radiosensitive phase of the cell-cycle, namely G₂/M [32]. For these reasons, we investigated effects of DP on radiosensitivity and cellular repair capacity in Chinese hamster ovary (CHO) cells X-irradiated in vitro.

Materials and methods

Cells. Chinese hamster ovary (CHO-K1) cells were maintained in opti-mem I (Gibco, Cat. No. 041-01985 M) supplemented with 10% Foetal calf serum (Gibco, Cat. No. 011-06290 M), and 1% penicillin-streptomycin solution (Gibco, Cat. No. 043-05140 H) under standard incubator conditions (humidified, 37°C, 5% CO₂).

Irradiation. The cells were irradiated at room temperature with an Oris IBL 637 cesium source at a dose rate of 83.3 cGy/min at several doses (3, 6, 9, 3 + 3 Gy).

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TABLE I
Survival of CHO-K1 cells irradiated 20 h after subculture at low density.

Dose (Gy)	SF radiation	SF (10 μ M) SF (20 μ M)		Respective	<i>p</i> -values
		Radiation + Dipyridamole			
3	61.2 \pm 6.8	50.8 \pm 14.9	47.9 \pm 5.1	0.02	0.0001
6	19.2 \pm 4.2	15.2 \pm 4.7	12.7 \pm 2.3	0.04	0.0001
3+3	29.3 \pm 6.4	21.4 \pm 8.4	21.7 \pm 2.6	0.04	0.01
9	3.3 \pm 0.2		2.2 \pm 0.1		0.03

Data shown in this table represent the means \pm 1 S.D. of six individual experiments; *p*-values were calculated using the paired *t*-test (two-sided). Left-handed *p*-values relate to comparison of SF (radiation) vs. SF (10 μ M); right-handed *p*-values relate to comparison of SF (radiation) vs. SF (20 μ M).

Drug. DP (Dipyridamole; 2,6-bis-(diethanolamino)-4,8-dipiperidino-pyrimido-(5,4-d)-pyrimidine, Boehringer Ingelheim) was dissolved once monthly in 0.01 N HCl to obtain a stock solution of 4 mM. From this stock solution, the drug was diluted to a concentration of 10 and 20 μ M.

Subcultured cells. Exponentially growing CHO-K1 cells were seeded in 100 mm Falcon Primaria tissue culture dishes at the desired low density 20 h prior to X-irradiation. Two hours after seeding, DP was added at the concentrations of 10 and 20 μ M. Multiplicity due to proliferation prior to irradiation was excluded by observation.

Confluent cultures. CHO-K1 cells were grown to confluence in 60 mm Falcon Primaria tissue culture dishes. The medium was replaced by serum free fresh medium 24 h before irradiation. Preincubation with 20 μ M DP lasted 24 h. Cells were trypsinized and subcultured at low density in 100 mm dishes, either immediately following irradiation at a dose of 6 Gy (immediate subculture, yielding a survival fraction termed SF_i) or after 24 h confluent holding in the incubator (delayed subculture, yielding a survival fraction termed SF_d). To assess PLD repair, SF_d was compared to SF_i and expressed as a recovery ratio (RR).

Colony assay. Survival was measured with the routine colony forming capacity assay. The counting of the clones was done manually 14 days after seeding. The colonies were fixed in ethanol and stained with a dilution of Crystal Violet. Clones of more than 50 cells were considered survivors.

Data analysis. Statistical analysis was carried out with the Statview software package on Macintosh SE/30 PC using a paired *t*-test (two-sided).

Results

Subcultured cells. Clonogenic capacity of the cells was not affected at a 10 μ M concentration of the drug, but it was reduced by 18% if the cells were exposed to 20 μ M DP (*n* = 6; *p* = 0.008). Survival of X-irradiated cells was significantly reduced when preincubated with 10 and 20 μ M DP, as shown in Table I (*n* = 6). The shape of the dose-response curve was modified (Fig. 1) in that radiosensitization was primarily due to an increase in α (from 0.046 Gy⁻¹ without drug to 0.166 Gy⁻¹ with 20 μ M DP), while β remained almost unchanged (0.040 Gy⁻² without drug vs. 0.032 Gy⁻² with 20 μ M DP). Therefore, the α/β ratio increased from 1.15 to 5.19. Based on these values, we calculated estimates of enhancement of cell inactivation at low dose levels as is indicated in Table II. No effect on repair of sublethal damage was observed within the accuracy of the methods.

Confluent cultures. Clonogenic capacity of exposed cells (20 μ M) was reduced by 14% (*p* = 0.09). SF_i of confluent cultures was not affected by a preincubation

TABLE II
Enhancement of cell inactivation (ECI) by 20 μ M DP at clinical dose levels.

Dose (Gy)	1.0	1.5	2.0	2.5	3.0
ECI	2.18	1.87	1.65	1.48	1.23

Enhancement of cell inactivation (ECI) at lower dose levels was calculated from the experimentally determined values of α and β . ECI was calculated as follows: ECI = 1 - SF (radiation + 20 μ M DP) / 1 - SF (radiation).

Data at 3 Gy are experimental values. The data at lower doses have been extrapolated using the linear-quadratic model.

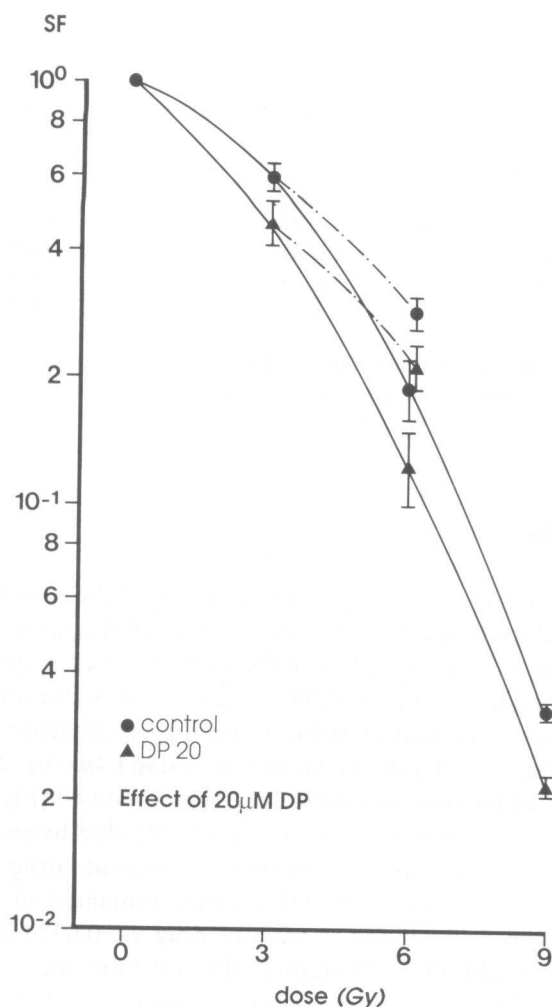


Fig. 1. Dose-response curve of subcultured X-irradiated CHO-K1 cells, preincubated with 20 μ M compared to subcultured X-irradiated controls. Dashed lines represent results from split-dose experiments.

with 20 μ M DP for 24 h ($p = 0.82$). However, there was a significant effect on PLD repair at 6 Gy. RR was reduced from 1.5 ± 0.13 for controls to 1.24 ± 0.21 ($p = 0.015$) for preincubation alone and to 1.06 ± 0.23 ($p = 0.029$) for combined pre- and postincubation. The experiment has been repeated five times independently. This reduction of RR represented an inhibition of PLD repair by 48% (preincubation alone) and 88% (pre- and postincubation), respectively. Postirradiation exposure alone to the same concentration (20 μ M) did not alter repair capacity of CHO-K1 cells ($p = 0.93$).

Discussion

DP has been shown to have promising activities in experimental and clinical oncological practice. DP acts as an enhancer of drug toxicity against malignant cells [1-5,8,10,12,13,15,16,19,23,25,28,30,33-36,39]. Various cellular and subcellular targets are involved. Transport systems such as nucleoside transport [1,4,18,21,29], glucose and inorganic phosphate transport [21] are inhibited. DP modifies intracellular triphosphate levels [21] and cellular energy supply. The activity of the salvage pathway of DNA synthesis is reduced [3,10,12,23,33,36]. Ultrastructure (microtubule formation) and related activities such as cellular mobility (directional migration and capacity of invasion and metastases), are inhibited by dipyridamole [14,26,34,35]. Furthermore, DP prolongs doubling time and causes cell-cycle redistribution of the CHO-K1 cells with accumulation of the cells in G2 + M, the most sensitive part of the cell-cycle (Coucke et al., oral communication, Association Française de Cytométrie en Flux, Bordeaux, France, 1990).

Preincubation with 10 and 20 μ M DP resulted in a statistically significant reduction of the survival of X-irradiated CHO-K1 cells subcultured at low density. At radiation dose levels currently used in clinical practice (1-25 Gy), the calculated values of the enhancement of cell inactivation (ECI) ranges from 2.18 to 1.65 (Table II). In confluent, density inhibited conditions, DP was a potent inhibitor of PLD repair.

Several mechanisms may underly radiosensitization and inhibition of repair by DP, namely, its reduction of energy levels available for repair [6,7,18,21,29,32] as well as its inhibitory effect on microtubule formation [14,26,32,34,35]. However, additional mechanisms are likely to be involved since a number of other analogues of nucleotides have been shown to have similar effects on irradiated cells, such as, for example, bromo-deoxyuridine, iodo-deoxy-uridine and 6-thioguanine [11,22,24]. These drugs are known to get incorporated in DNA and by this way increasing the frequency of strand breaks. Modifying intrinsic radioresistance and repair of potentially lethal damage could be of clinical importance. Both factors are potential causes of failure in radiotherapy [9,17,20,27,31,37,38]. Our findings therefore suggest that DP or a related compound may one day become a useful modifier for radiotherapy.

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