Free Rad. Res. Comms., Vols. 12-13, pp. 595-599
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TIME MODULATION EFFECT OF DIETHYLDITHIOCARBAMATE (DDC) ON RADIOSENSITIZATION BY SUPEROXIDE DISMUTASE (SOD) INHIBITION.

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Superoxide dismutase (SOD) is known to protect cells from the lethal effects of ionizing radiation by the dismutation of oxygen radicals. Diethyldithiocarbamate (DDC) is known inhibitor of SOD and may therefore be useful as a radiosensitizer. DDC however, is also a thiol radioprotector due to its ability to scavenge radiation induced free radicals. We have shown that DDC, if administered to tumours 1 hour prior to x-irradiation exerts a protective effect, whereas if administered 4 hours prior to irradiation, it radiosensitizes. This time modulation effect is not apparent after neutron irradiation where DDC protects in both situations. We have also examined the effect of DDC on the $LD_{50/30}$ in mice after total body irradiation.

KEY WORDS: Radiosensitization, radioprotection, superoxide dismutase, diethyldithiocarbamate.

INTRODUCTION

Diethyldithiocarbamate (DDC), a powerful copper chelator inhibits superoxide dismutase (SOD), one of the key enzymes involved in the defence against oxygen radicals. Many authors have suggested that this property can be used to sensitize tumour cells to the effects of ionizing radiation.[1-4]

DDC however, is also a thiol radioprotector due to its ability to scavenge radiation induced free radicals, and has led other authors to propose it as a radioprotector.[5,6]

We have previously shown[7] *in vivo* that DDC indeed inhibits SOD and that this inhibition (92% max) is sustained for up to 24 hours after DDC administration. We have also shown that DDC injected intratumourally can protect tumours if administered 1 hour prior to irradiation, yet sensitizes them if administered 4 hours prior to irradiaton. In this communication we report on tumour growth delay experiments following intratumoural DDC administration and neutron irradiation and the effect of DDC on the $LD_{50/30}$ of C57/Bl mice following total body irradiation.

METHODS

Tumours

A transplantable 3-methylcholanthrene induced rhabdomyosarcoma was induced

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into the left flanks of 6–8 week old BALB/c mice by innoculation with 0.1 ml cell suspension.

DDC Administration

Solutions of DDC (sodium salt) (Sigma St Louis) were prepared in water and injected intratumourally at a rate of 100 mg/kg or intraperitoneally at a rate of 500 mg/kg in the case of $LD_{50/30}$ experiments.

Total Body Irradiation

Seven to eight mice were placed in an acrylic plastic (perspex) box 15 × 15 × 2.5 cm. The box was closed with a 0.5 cm thick lid which served as build up. The mice were not restrained or anaesthetized. Irradiation was performed with a Picker V4M/60 cobalt unit emitting gamma rays of between 1.17 and 1.31 MeV at an SSD of 80 cm. 15 mice were used for each dose point. The $LD_{50/30}$ was calculated using the probit method of Finney (1971).[8]

Neutron Irradiation

Tumour bearing BALB/C mice were restrained on a perspex jig with elastic bands. The mice were briefly anaesthetized in ether prior to immobilization. Neutron irradiations were performed on the 66 MeV $p+ \rightarrow$Be neutron therapy isocentric unit of the

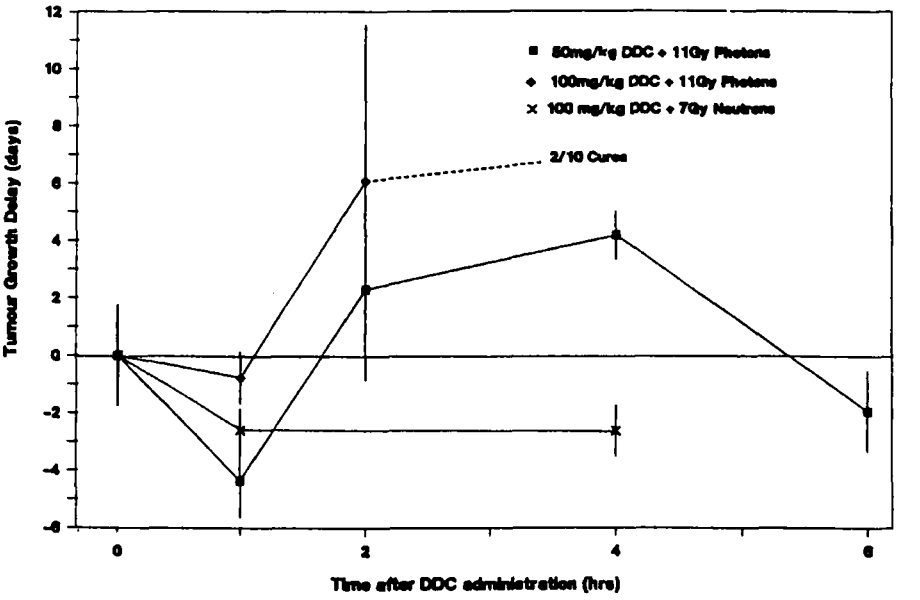

FIGURE 1 Tumour growth delay (mean ± SEM) after intratumoural adminstration of DDC followed after the hours indicated by 11 Gy x-irradiation or 7 Gy neutron irradiation. DDC was noted to protect tumours from radiation damage when injected 1 hour prior to x-irradiaiton, but sensitized when injected 2–4 hours after x-irradiation. DDC protected tumours against neutron irradiation when administered 1 or 4 hours prior to irradiation.

National Accelerator Centre in Faure, South Africa. A 20 × 20 cm field was used, and the mice were positioned such that only the tumour bearing leg was in the field. The jig was placed above 9 cm of perspex and a 20 × 20 × 2 cm closed perspex box containing a tissue equivalent solution was used as build up. The surface to axis distance was 150 cm. The dose to the tumours was 7Gy. Seven mice were used for each dose point. Tumour growth delay was assessed by measuring the orthogonal diameters of each tumour 3 times a week until a volume of 0.6 cm^3 was reached, whereafter the mice were sacrificed.

RESULTS

Figure 1 shows that tumours treated with DDC and neutrons grew faster than tumours treated with neutrons alone. Tumours treated with neutrons only took 12.76 ± 0.85 days to reach 0.6 cm^3, whereas tumours treated with DDC and neutrons to 10.16 ± 0.76 and 10.12 ± 0.91 days to reach 0.6 cm^3 for DDC administration 1 and 4 hours prior to irradiation respectively. The tumour growth delay for x-irradiation is presented for comparison.

Figure 2 shows that mice treated with DDC 1 hour prior to total body irradiation resulted in a moderate degree of protection (DMF = 1.2). There is no significant difference between the $LD_{50/30}$ of mice treated with DDC 4 hours prior to irradiation and mice treated with radiation only.

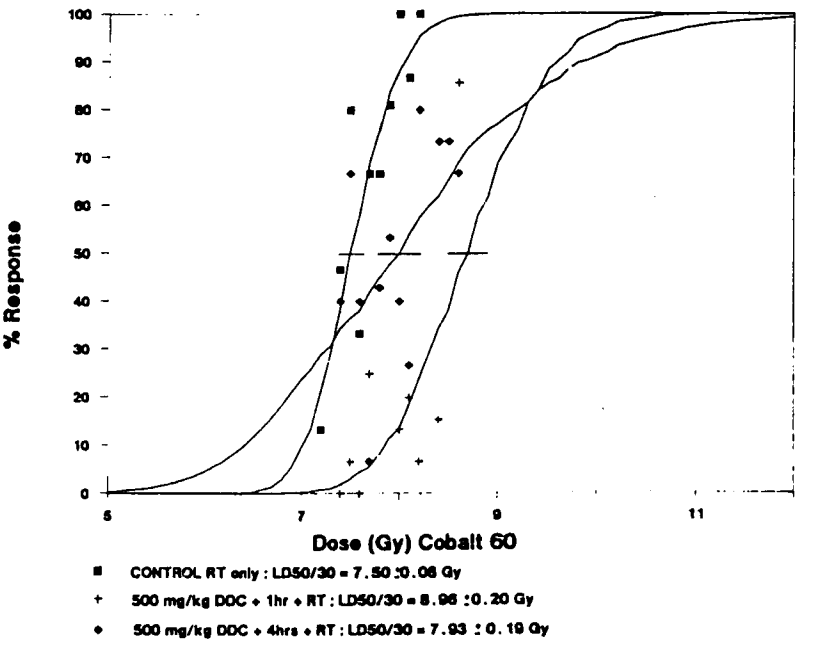

FIGURE 2 Isoeffect curves for lethality in mice after 500 mg/kg DDC administration 1 or 4 hours prior to γ-irradiation or irradiation only. (error bars represent mean ± SEM)

DISCUSSION

We have shown previously that the time of administration of DDC is critical to the response of tumours to x-irradiation.[7] When 50 mg/kg DDC was administered 1 hour prior to irradiation a clear radioprotective effect was seen. If however, 4 hours were allowed between DDC administration and x-irradiation, DDC acted as a radiosensitizer. When higher doses of DDC were used, some tumour cures were noted when the time gap was longer than 1 hour. Caven *et al.* (1976)[9] showed that DDC is rapidly excreted and the duration of action does not exceed 3 hours. This, together with our previous observation that SOD is significantly inhibited by DDC administration, led us to believe that DDC can radiosensitize tumours by SOD inhibition, but that if insufficient time is allowed for DDC metabolism to occur, this can be masked by a radioprotective effect.

It has previously been argued that chemical radioprotectors are more efficient in protecting tissues from low LET compared to high LET radiation. This could be due to greater influence of free radical scavenging and hydrogen ion donation because of a) LET differences in subcellular dose distributions or b) the degree to which the radiolysis of water affects cell killing by radiations of different qualities. Sidgestad *et al.* (1986)[10] showed that WR2721 was a more efficient protector against low LET radiation. We have shown that although DDC offers a small degree of protection to neutron irradiation, the time modulation effect demonstrated with x-rays is not evident. It would be important to note that there is a small gamma component to the Faure neutron beam and that this is highest at the edge of the field[11] where the tumours were situated for the irradiations.

We have shown in Figure 2 that DDC administered 1 hour prior to irradiation can protect mice against the lethal effects of total body irradiation to some degree, whereas when administered 4 hours prior to irradiation, its effect is less clear. There is certainly no suggestion of sensitization. Evans (1985)[12] showed that tumours were sensitized by DDC at the same time as showing a concommitant bone marrow protection (DMF = 2.1) in animals bearing a RIF sarcoma. Marklund *et al.* (1982)[13] showed that in general CuZn SOD appeared to be slightly lower in malignant cell lines as compared to normal cell lines.

Clearly a therapeutic gain by DDC is possible, however, the time modulation effect of DDC and its effect on other enzymes involved in the defence against oxygen toxicity such as catalase, glutathione peroxidase,[13] cytochrome oxidase[2] as well as its biphasic toxicity[14] and stimulation of stem cell proliferation[15] need to be explored further before any conclusions as to the therapeutic potential of DDC in human patients with cancer can be made.

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Accepted by Prof. G. Czapski