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Differential protection of radiation-induced DNA single-strand breaks and cell survival by solcoseryl[1,2]

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Summary. V79 Chinese hamster cells were studied in vitro for modification of cobalt-60 gamma radiation effects by solcoseryl. This treatment did not modify cell survival but did protect against DNA single-strand breaks.
Key words. Radiation effects in vitro; cell survival; solcoseryl; actihaemyl; DNA single-strand breaks; radiation protectors.

Solcoseryl is a deproteinized extract of calf serum which has been shown to protect mice from experimental cleft palate formation[5], to promote wound healing[6] and to modify radiation response both in the clinic[7,17] and in experimental animals[8,12,16]. The mechanism(s) of action of this agent has not been determined. Solcoseryl's ability to improve wound healing may involve stimulated formation of granulation tissue or improvement of tissue oxygenation[18,19].

The present study tested the effect of solcoseryl in vitro using V79 cells. The ability of this agent to modify cell survival after cobalt-60 gamma irradiation was studied. In addition, the effect of solcoseryl on DNA single-strand break formation after irradiation was tested using the alkaline elution technique.

Cell preparation. V79-B310H Chinese hamster cells were cultured at 37 °C in a monolayer on 100-mm plates in MEM-10 (Gibco) containing 10% fetal calf serum, in a water-saturated atmosphere containing 5% CO_2. Cells were trypsinized (0.025% trypsin in PBS), at 37 °C for 10 min. A dilution of the suspension was counted by using a Coulter counter with appropriate corrections for coincidence.

Solcoseryl. Solcoseryl was obtained from the manufacturer as amber liquid in sterile 2-ml ampoules. Each ml contained 40 mg of the active material. Toxicity studies indicated that the addition of up to 60 mg/plate did not modify clonogenicity of this cell line. One million cells were diluted 1:3 with solcoseryl and media, which resulted in a final concentration of 13.3 mg/ml. The cells were treated for 30 min prior to and during irradiation.

Irradiation. A cobalt-60 gamma irradiator (AECL, Gamma Beam 650) at a dose rate of 10 Gy/min was used for these experiments. Radiation doses included 0, 2, 4, 6, 8, 10, 12 and 14 Gy. Both treated and control cells were irradiated, at the same time, in individual 15-ml sterile centrifuge tubes in an ice bath. Immediately after irradiation the cells were diluted and plated using standard colony forming techniques.

Alkaline elution. The alkaline elution technique of Kohn[9] has been described in detail elsewhere[10]. Briefly, 6–8 million cells were impinged onto a 47-mm diameter (0.8 µm pore size) polycarbonate filter. Cells were washed twice and lysed with 10 ml 2 M NaCl – 0.04 M EDTA – 0.2% sarkosyl (pH 12.1). Elution was performed in the dark with 0.1 M tetrapropylammonium hydroxide and 0.02 M EDTA (pH 12.1). The flow rate was 0.04 ml/min and fractions were collected every 90 min for 15 h. The DNA in each collected fraction and that remaining on the filter was assayed using a microfluorometric technique described in detail elsewhere[11]. The method has been demonstrated to accurately represent the kinetics of DNA elution when compared to techniques using radioactive methods[10]. The designation strand-scission factor (SSF) refers to a relative value determined as a result of the comparison of associated DNA elution curves. This value is used to characterize the relative number of DNA-strand breaks present. The SSF was determined from the relationship: $SSF = |\log((fx)/(fo))|$, where fo and fx are, respectively, the proportion of DNA retained on the filter after an eluted volume of 17.5 ml for the control and corresponding treated sample.

Figure 1 shows the survival of V79 cells irradiated with or without solcoseryl. The D_0's for these curves was found to be 2.4 Gy with no significant difference between treated cells and control. A correlation coefficient of 0.96 was obtained. It is apparent from these results that in vitro radiation protection under the conditions described is non-existent.

Figure 2 shows the effect of solcoseryl modification of the DNA single-strand breaks induced by gamma irradiation. Solcoseryl significantly reduced the SSF as compared to the untreated control groups for doses greater than 4 Gy.

In vitro cell survival data reported here do not correlate with improved animal survival reported after lethal irradiation[8,12]. These results suggest that the mechanism of radioprotection noted in animal lethality experiments may result from the capability of solcoseryl to modify the post-irradiation sequelae. Barth et al.[16] tested the effect of multiple injections of solcoseryl after irradiation with 6 Gy and found significant protection but less than reported by Bauer et al.[8,12]. These findings could possibly be explained by improved repair capabilities or stimulated proliferation of critical stem cells.

The ability of solcoseryl to reduce single-strand breaks in DNA after irradiation correlates well with similar results observed after treatment with a known radiation protec-

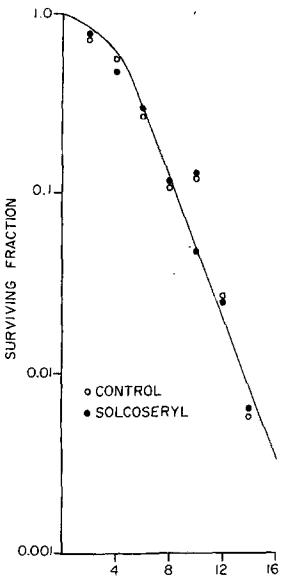

Figure 1. Survival of V79 Chinese hamster cells in vitro after cobalt-60 gamma irradiation. Closed circles represent cells treated with solcoseryl (13.3 mg/ml) prior to irradiation. Open circles represent untreated control cells.

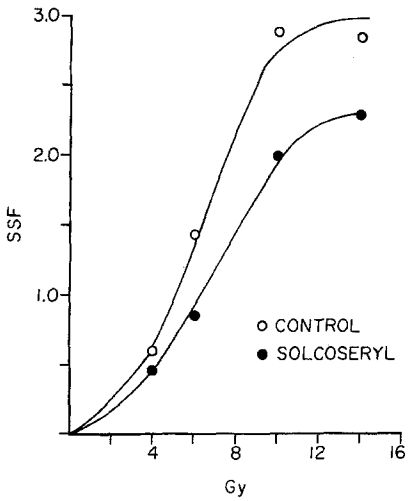

Figure 2. DNA single-strand breaks in V79 cells treated with solcoseryl (13.3 mg/ml); closed circles or untreated control cells (open circles). See text for description of Strand-Scission Factor (SSF).

tor[13]. The lack of correlation between this protection and cell survival is not surprising, inasmuch as it has been shown that double-strand DNA breaks apparently correlate better with cell lethality than do SSB[14]. The protective effect on SSB is not insignificant, however, because DNA damage of this type has been correlated with mutagenesis[15]. Further studies will be required to determine if the protection from SSB in DNA results in a modification of mutagenesis induced by radiation.

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Fine structure histochemical study of the distribution of dipeptidylpeptidase IV (DPP IV) in the meningeal lamellae of the rat

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Summary. DPP IV was localized in the meningeal lamellae of the spinal cord sheaths of the rat by light and electron microscopy. A membrane-bound reaction product of DPP IV was found in the internal, intermediate and external meningeal lamellae which delineated the CSF-filled meningeal spaces. The cells of the marginal glia displayed heterogeneous localization of the reaction product for DPP IV. DPP IV distribution in the spinal cord sheaths suggests its possible participation in the interactions of the meningeal cells with the neuropeptides in cerebrospinal fluid.
Key words. Dipeptidylpeptidase IV; histochemical localization; meningeal cells.