June, 1992

9-ß-Arabinofuranosyladenine Preferentially Sensitizes Radioresistant Squamous Cell Carcinoma Cell Lines to X-Rays[1]

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Running Title: Sensitization of Resistant Tumor Cells with Ara-A

Key Words: Ara-A, Squamous Cell Carcinomas, Radiotherapy, Radiation Resistance

Abbreviations: ara-A, 9-β-arabinofuranosyladenine;
PLDR, potentially lethal damage repair

[1]This research was supported by grant CA 42596 from the NCI, The Center for Radiation Therapy, a Faculty Research Award from the American Cancer Society; and the U.S. Department of Energy under Contract W-31-109-ENG-38 (JLS); and grant No. DE-FG02-88ER60661 (JLS).

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ABSTRACT

The effect of 9-ß-arabinofuranosyladenine (ara-A) on sensitivity to the deleterious effects of x-rays was studied in six squamous cell carcinoma cell lines. Three lines were relatively radioresistant, having D_0 values of 2.31 to 2.89 Gy, and the other three lines were relatively radiosensitive, having D_0 values of between 1.07 and 1.45 Gy. Ara-A (50 or 500 μM) was added to cultures 30 min prior to irradiation and removed 30 min after irradiation, and sensitivity was measured in terms of cell survival. The radiosensitizing effect of ara-A was very dependent on the inherent radiosensitivity of the tumor cell line. Fifty micromolar concentrations of ara-A sensitized only the two most radioresistant lines, SCC-12B.2 and JSQ-3. Five hundred micromolar concentrations of ara-A sensitized the more sensitive cell lines, SQ-20B and SQ-9G, but failed to have any effect on the radiation response of the two most sensitive cell lines, SQ-38 and SCC-61. Concentrations of ara-A as low as 10 μM were equally efficient in inhibiting DNA synthesis in all six cell lines. These results suggest that the target for the radiosensitizing effect of ara-A is probably related to the factor controlling the inherent radiosensitivity of human tumor cells. Therefore, ara-A might be useful in overcoming radiation resistance in vivo.

INTRODUCTION

The inherent radiosensitivity of tumor cells may play an important role in radiotherapy response. Squamous cell carcinoma cell lines derived from in-field radiation failures are more resistant to radiation than are cell lines derived from tumors prior to any radiation treatment (1-3), which suggests that radiotherapy selects for tumors that contain radioresistant cells. These tumor cell lines differ primarily in the initial slope of the survival curve.

Although the molecular basis for differences in radiosensitivity is still being investigated, we and others have reported that the more radioresistant cell lines (D_0 > 1.8 Gy) rejoin DNA and chromosome breaks faster than do radiosensitive (D_0 < 1.8 Gy) cell lines (4-7). The more resistant cell lines typically rejoin between 80% and 90% of induced DNA double-strand breaks within 1 h of irradiation, while the more sensitive cell lines require 2-3 h to rejoin a similar fraction of breaks. The difference between resistant and sensitive lines appears to be in the initial fast component of rejoining kinetics.

The nucleoside analog 9-ß-arabinofuranosyladenine (ara-A) has been reported to sensitize cells to the damaging effects of ionizing radiation (8-10). The mechanism for its activity is not clear, but it is usually assumed that the sensitization is related to the ability of ara-A to inhibit DNA strand break rejoining. Ara-A is an inhibitor of DNA polymerases α and ß (11-13). To further test the importance of DNA strand break rejoining as the major underlying cause of differences in human tumor cell radosensitivity, studies were initiated to examine the effect of ara-A on the radiation sensitivity of resistant and sensitive tumor cell lines.

MATERIALS AND METHODS:

Cell Lines. The six human squamous cell carcinoma cell lines used in this study were established from tumor biopsy specimens. Methods of establishment and characterizations of the cell lines have been published (1-5,14). Cell lines were maintained in media consisting of 72.5% Dulbecco's Modification of Eagle's Medium, 22.5% Ham's Nutrient Mixture F-12, 15% fetal bovine serum, 0.4 μg/ml hydrocortisone, 100 units/ml penicillin, and 100 μg/ml streptomycin. For all studies, cells of late (>40) passages were used.

Inhibition of DNA Synthesis Measurements. To determine the most effective concentrations of ara-A to use, the dose response for the inhibition of DNA synthesis was determined as described previously (15). Exponentially growing cells were prelabeled with 0.02 μCi/ml ^{14}C-thymidine (53.2 μCi/mmol) for 2 days. The radioactive medium was then removed, and the cells were washed twice in complete medium and then incubated an additional 4 h in a nonradioactive medium. Different concentrations of ara-A (0-500 μM) were added to each culture, and the dishes were incubated for 1 h at 37 °C. After this time, the cells were pulse-labeled with 1 μCi/ml ^{3}H-thymidine (20 Ci/mmol) for 10 min at 37 °C. The cells were then washed twice with 3 ml of ice-cold standard saline citrate (0.15 M sodium chloride, 0.015 M sodium citrate). Cells were harvested and split into two equal fractions. The DNA in each fraction was precipitated onto separate filters, and the ^{3}H and ^{14}C levels were determined by liquid scintillation spectrometry. The overall rate of DNA synthesis was estimated from the ratio of ^{3}H cpm (newly synthesized DNA) to ^{14}C cpm (total DNA). In each experiment, triplicate plates for each ara-A concentration were analyzed. The data presented are the mean of 2-4 experiments.

X-Ray Survival Analysis. Exponentially growing cultures of each cell line were x-irradiated as described previously (1-3). Briefly, cells were trypsinized from stock cultures, and between 500 and 40,000 cells were plated in 100-mm tissue culture dishes and allowed to enter exponential growth. Irradiation was carried out 18 h later with a 250-kV Maxitron operating at 26 mA at a dose rate of 1.07 Gy/min. All the cell lines had doubling times greater than 19 h (14); therefore, delaying irradiation for 18 h should not result in any significant increase in multiplicity. This was checked for each cell line. Ara-A (50 or 500 μM) was added to cell cultures 30 min before irradiation, kept on the cells during irradiation, and then removed 30 min after irradiation. Cells were irradiated at room temperature, but ara-A exposures were at 37 °C.

Cells were irradiated with doses of up to 7 Gy. After drug and radiation treatment, the cultures were incubated at 37 °C for 18-24 days, after which the cells were fixed and stained with crystal violet. Only colonies of more than 50 cells were scored as survivors. Both multitarget radiation survival parameters, D_0 and the extrapolation number (n), and linear-quadratic parameters, α and β, were determined by least squares regression analysis of all the data (2-6 experimental determinations).

RESULTS AND DISCUSSION

The six cell lines used in this study were derived from squamous cell carcinomas (head and neck) either prior to or after a course of radiotherapy. The characteristics of the cell lines have been previously described (1-5,14). The six cell lines vary in DNA content, all being hyperdiploid and most being near triploid or tetraploid. The cell doubling times range from about

19 h to more than 32 h. In general, the more radiosensitive cell lines (SQ-9G, SQ-38, and SCC-61) have longer doubling times and show a smaller proportion of their exponentially growing population in S phase. The more sensitive cell lines also have slightly reduced plating efficiencies.

To determine the most effective ara-A concentration, cells were exposed to 0-500 μM ara-A for 1 h, and then the thymidine uptake was measured. The results for four cell lines (SQ-20B, SCC-12B.2, SQ-38, and SCC-61) are shown in Figure 1. Each cell line had a similar response to ara-A over the 10-500 μM range. A 1-h exposure to 10 μM or greater was sufficient to reduce thymidine uptake to less than 10% of normal. There did not appear to be any difference between the cell lines in the relative efficiency with which ara-A was effective in suppressing DNA synthesis.

One hour exposures to 50 or 500 μM ara-A had only small effects on plating efficiency. In some cases, the lower concentration increased plating efficiency slightly. The plating efficiencies of the unexposed tumor cell lines ranged from 20-50%. The plating efficiencies for cells exposed to 50 μM ara-A for 1 h ranged from 8-50%, while for cells exposed to 500 μM ara-A, the plating efficiency ranged from 4-20%. The largest reduction in plating efficiency was seen in SQ-9G cells exposed to 500 μM ara-A, where plating efficiency was reduced from 30% to about 4%. There was no relationship between the reduction in plating efficiency seen with ara-A and either inherent radiosensitivity or the percentage of the cells in S phase. Previous investigators had suggested that the target for ara-A toxicity is S phase cells (8). The lack of correlation between sensitivity to ara-A and the proportion of cells in S phase is likely due to the short 1-h ara-A exposure times.

The effect of ara-A exposure on x-ray sensitivity is shown in Figure 2 and summarized in Table 1. Fifty micromolar concentrations of ara-A significantly increased radiosensitivity in SCC-12B.2 and JSQ-3, the two most radioresistant cell lines studied. This concentration of ara-A had no effect on the x-ray survival of any of the other four cell lines. Increasing the ara-A concentration to 500 μM was successful in sensitizing SQ-20B and SQ-9G to x-rays. It had no effect on SQ-38 or SCC-61. A plot of the relationship between the enhancement ratio ($D_0^{\text{no drug}}/D_0^{+\text{ drug}}$) and inherent radiosensitivity (D_0) is shown in Figure 3. Even though the data are limited in number, linear regression analysis reveals a significant ($p < .05$) relationship between these two parameters. Thus, ara-A preferentially sensitizes inherently more radioresistant cells than more sensitive cells to x-rays.

These results seem, at first glance, to be at odds with a previous report by Chavaudra *et al.* (10), who reported no relationship between inherent radiosensitivity and ara-A radiosensitization. The work by Chavaudra *et al.* (10) examined six very different tumor cell lines. They included two melanomas, one neuroblastoma, one colorectal adenocarcinoma, and one nephroblastoma. In our study, we limited ourselves to a single histological type, head and neck squamous cell carcinomas. Perhaps the relationship between inherent radiosensitivity and sensitivity to ara-A effects is consistent within histological subtype, and would not be seen when comparisons are made between different tumor cell subtypes. Alternatively, the effect we report might be related to the short 1-h ara-A exposure time used as compared with the longer 7-h time used by Chavaudra *et al.* (10).

The basis for the selective radiosensitization of the more resistant cells is unknown. The DNA synthesis inhibition studies suggest that ara-A is able to enter and affect polymerase action in each cell line to a similar degree. The ara-A metabolite has been reported to be rapidly

degraded, so levels of intracellular ara-A should decline soon after removal from the medium. It is possible that the tumor cell lines differ in their ability to degrade the ara-A metabolite. However, residual intracellular ara-A would be expected to affect plating efficiencies, and there is no relationship between the reduction in plating efficiency seen with ara-A treatment and the ara-A-mediated radiosensitization of these tumor cell lines.

The more radioresistant cell lines have a greater proportion of their population in S phase. S phase cells are thought to be a target for ara-A toxicity. The difference between the cell lines in S phase percentage is small (14), however, and the ara-A exposure time is only 1 h. It seems unlikely therefore that the greater proportion of S phase cells in resistant cell lines would explain the specificity of the ara-A effect.

It has been reported that ara-A inhibits the repair of potentially lethal damage (8,9). This raises the possibility that the differential effects reported here are due to differences between the cell lines' potentially lethal damage repair (PLDR) abilities. However, measurements of PLDR in these cells reveal that the radioresistant cell lines do very little PLDR, while the more sensitive cell lines do much more (1,2, unpublished observations). Therefore, differences in PLDR ability cannot explain the differential effect.

The most likely explanation for our observation is that the effect of ara-A is related to the previously reported differences in DNA double-strand break repair (4-6). The radioresistant cell lines rejoin DNA and chromosome breaks faster than do the more sensitive cell lines. In general, it is the initial fast component of rejoining that is more pronounced in resistant cells and reduced in sensitive cells. Perhaps this fast component is also the target for the ara-A sensitization.

Whatever the mechanism, these results raise the possibility that ara-A might be useful clinically to preferentially sensitize radioresistant tumor cells. As there is evidence for inherent

radioresistance being a component of radiotherapy failure (1-3), ara-A treatment might be able to improve local tumor control.

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FIGURE LEGENDS

Fig. 1. Inhibition of DNA synthesis in human tumor cell lines exposed to ara-A for 1 h. Open circle, SCC-12B.2; open triangle, SQ-20B; filled circle, SCC-61; filled triangle, SQ-38.

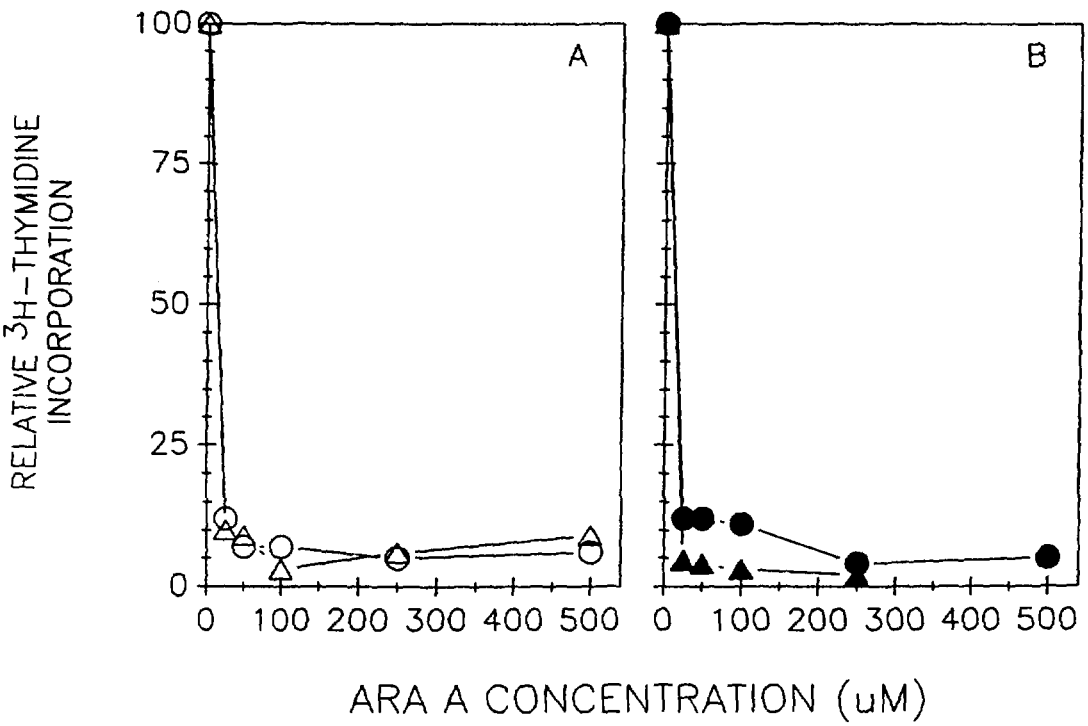
Fig. 2. Effect of a 1-h exposure to 50 μM (open circle) or 500 μM (filled circle) ara-A on x-ray sensitivity. The results are normalized to the unirradiated, ara-A-treated response. The solid line is the x-ray response in the absence of ara-A.

Fig. 3. Plot of the relationship between the enhancement ratio ($D_0^{\text{no drug}}/D_0^{+\text{ drug}}$) and inherent radiosensitivity for 50 μM (open circle) and 500 μM (filled circle) ara-A treatments.

Table 1 *Effect of ara-A on radiation sensitivity*

Survival curve parameters were determined by least-squares regression analysis (nd - not determined).

Cell Line	Ara-A Dose (μM)	D_0 (Gy)	n	α (Gy^{-1})	β (Gy^{-2})
SCC-12B.2	0	2.66	2.1	0.204	0.154
	50	1.81	1.4	0.364	0.023
	500	nd	nd	nd	nd
JSQ-3	0	2.63	1.7	0.154	0.022
	50	2.11	1.2	0.403	0.007
	500	nd	nd	nd	nd
SQ-20B	0	2.39	1.4	0.319	0.008
	50	2.35	1.6	0.146	0.032
	500	1.39	1.2	0.729	0.002
SQ-9G	0	1.46	1.4	0.479	0.023
	50	1.79	1.2	0.336	0.029
	500	1.28	0.7	0.920	0.022
SQ-38	0	1.46	1.8	0.330	0.042
	50	2.53	1.8	0.150	0.024
	500	1.74	0.9	0.556	0.002
SCC-61	0	1.07	1.8	0.566	0.044
	50	1.53	1.8	0.296	0.044
	500	1.39	1.6	0.437	0.034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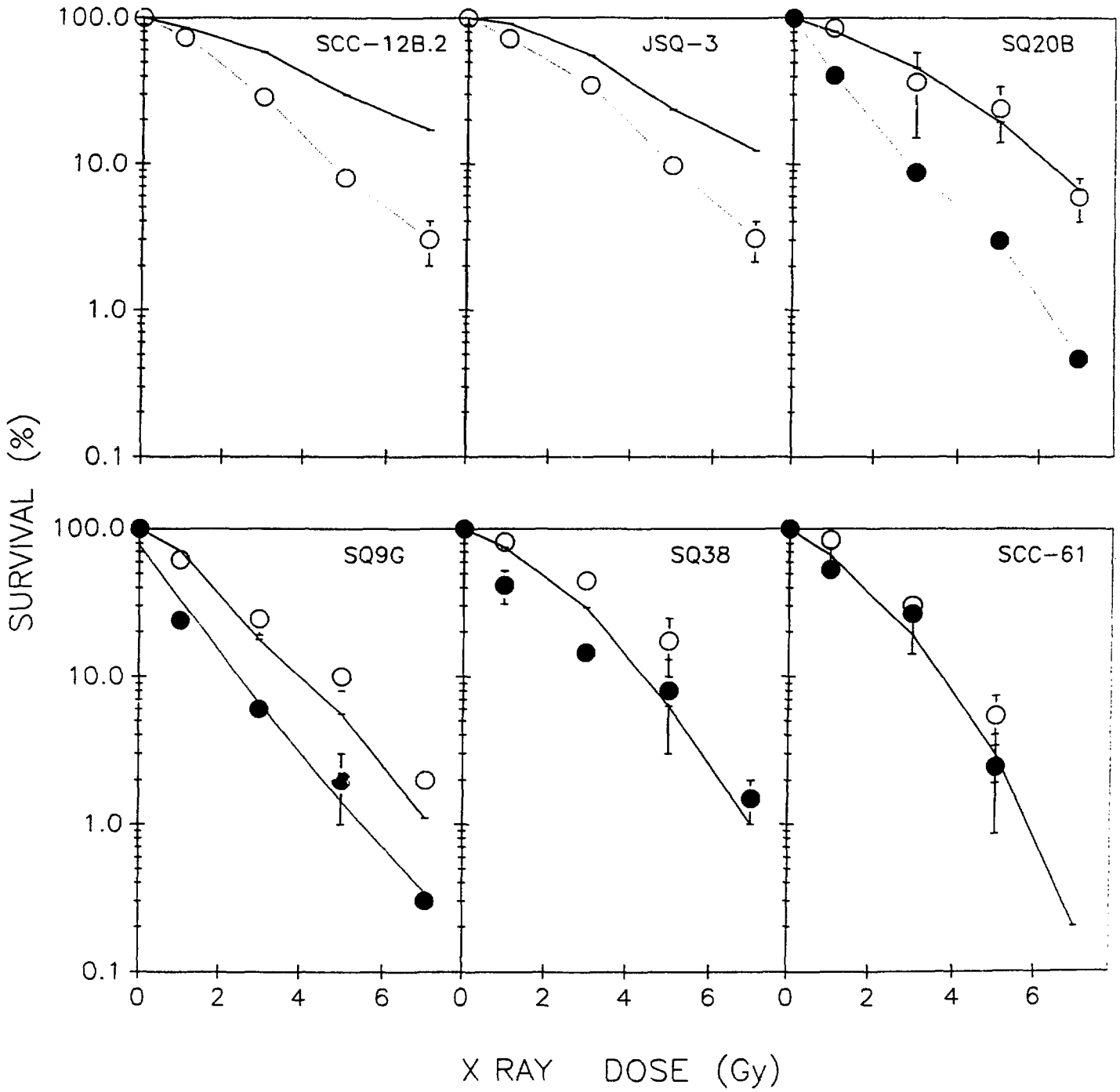
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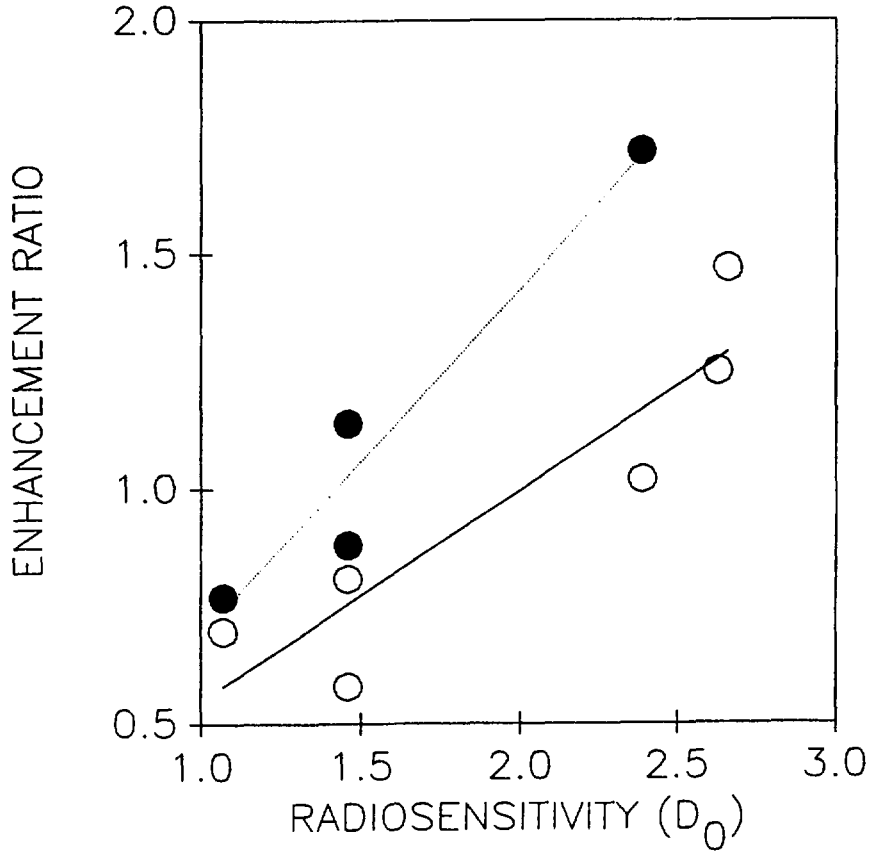
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