RADIOSENSITIZATION BY DIMETHYLBENZANTHRACENE, DIPHENYLCYCLOPROPENONE AND AMINOANTHRAQUINONES

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ABSTRACT — Radiosensitization of mice by dimethylbenzanthracene, diphenylcyclopropenone and aminoanthraquinones was investigated in a model where survival time after lethal radiation was scored. Survival time was shortened by nontoxic doses of the chemicals. The used *in vivo* system confirmed the radiosensitizing potential of dimethylbenzanthracene reported previously with *in vitro* studies. Moreover, radiosensitizing properties of diphenylcyclopropenone and aminoanthraquinones could be demonstrated. The sensitizing interaction of these chemicals with radiation adds a new facet to their toxicological spectrum and could, by enhancing radiation effects, influence estimates of risk. On the other hand, diphenylcyclopropenone or aminoanthraquinones deserve consideration as topical sensitizers in conditions where radiation is indicated to treat cutaneous malignancies.

KEY WORDS: Dimethylbenzanthracene, Diphenylcyclopropenone, Aminoanthraquinone, Radiation, Combined treatment.

INTRODUCTION

The investigation of combined exposures to radiation and chemicals has been considered important with regard to enhancement of radiation effects on carcinogenesis, mutagenesis and teratogenesis after prenatal irradiation (Unsclear, 1982 ; Streffer and Müller, 1984). The ability of chemicals to enhance the effects of ionizing radiation represents a generally neglected aspect of their toxicological spectrum. With regard to the ubiquitous risk of radiation exposure (e.g. radiodiagnostic interventions, radiotherapy in non-cancerous conditions, radionuclide pollution after nuclear accidents, prolonged space flight or

background radiation exposure), agents which sensitize to radiation should be recognized.

Prompted by the finding that drugs including allopurinol, indomethacin and riboflavin exhibit radiosensitizing properties (Floersheim, 1994), we directed our attention also to other chemicals which for biological or structural considerations qualified as potential radiosensitizers. One line of research allowed to detect radiosensitization by salts of the metals beryllium and indium and the rare earths cerium, lanthanum and scandium (Floersheim, 1995). In the present work, we report results obtained with dimethylbenzanthracene (DMBA), diphenylcyclopropenone (DCP) and 1- and 2-aminoanthraquinone (AAQ). The polycyclic hydrocarbon DMBA as a byproduct of incomplete combustion of coal, oil, tobacco and other hydrocarbons is known as

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an environmental pollutant with carcinogenic properties. DCP is used for immunological sensitization in dermatology and AAQ must be viewed in the context of quinone pharmacology.

As in the earlier work with drugs (Floersheim, 1994) and metals, radiosensitizing effects were studied *in vivo* using mice in which the modification of survival time after lethal irradiation was scored. This model is not a standard method for the evaluation of radiation modifiers, but the application of drugs at their $\frac{1}{2}$ LD₅₀ and serially reduced levels against the LD₁₀₀ radiation exposure has led to the discovery of valuable radioprotectors (Sweeney, 1979). Moreover, radiosensitization by indomethacin (Floersheim, 1994) disclosed with the model used in this study, was in accordance with the sensitization of tumor cells by indomethacin as detected by the conventional method based on the application of different radiation exposures (Furuta *et al.*, 1988). Also, the surmise that radiosensitization by the combined effects of a sensitizing agent and X-rays may be recognized both by the reduction of survival time after lethal radiation and by the survival curve of cells subjected to graded radiation doses, is supported by the fact that the sensitization to X-rays by DMBA observed in the survival time reduction model agreed with the sensitization observed when cells were exposed to DMBA and their surviving fraction was determined as a function of the X-ray dose (Chen *et al.*, 1990).

MATERIALS AND METHODS

Mice and irradiation: Inbred female C3H mice (G1. Bomholtgard, Ry, Denmark) weighing 22–24 g and fed a commercial pellet diet and tap water *ad libitum* were used throughout the experiments. Irradiation was performed with a cobalt 60 γ -ray source (Gammatron) at the dose rate of 0.15 Gy/min and a target distance of 80 cm. We applied 10.5 Gy which was the radiation LD₁₀₀. With this dose, the mice succumbed at about 10 to 12 days, indicating haematological toxicity and death by bone marrow failure. For the irradiations, panels of twelve mice were kept in a perforated plexiglass chamber. Dosimetry was performed with a strontium-calibrated ionization chamber. Irradiations were done at the

same time of the day.

Drugs: 7,12-Dimethylbenzanthracene (DMBA), diphenylcyclopropenone (diphencyclone, DCP) and 1- and 2-aminoanthraquinone (AAQ) were purchased from Fluka AG, Buchs (Switzerland). For each agent the LD₅₀ was determined. In the experiments with DCP and AAQ, the highest tested dose was the $\frac{1}{2}$ LD₅₀. With DMBA, 0.87 mg/kg was the $\frac{1}{8}$ LD₅₀. No lethality was observed when the highest tested doses of the chemicals were given alone. The lower doses were administered at serially twofold dilutions. The agents were injected by the intraperitoneal route 30 or 120 min before the irradiation.

The tested chemicals were applied in a solvent consisting of 12.5% dimethylsulfoxide (DMSO) in propylene glycol. The agents were injected intraperitoneally in a volume of 0.1 ml/10 g body weight. Accordingly, the mice received with each drug administration also 1.25 ml DMSO/kg body weight. Controls received 0.1 ml/10 g body weight of the solvent. The solvent alone did not modify the survival of lethally irradiated mice as compared to distilled water.

Sensitizing activity: Survival of the mice was followed daily and the experiments were terminated at day 30. The mean survival time was entered in the Tables.

Statistical evaluation: Statistical significance of the differences in mean survival time between treated groups and the respective controls were expressed according to the two sided *t*-test.

RESULTS

Table 1 shows that DMBA at the dose of 0.87 mg/kg applied 30 min prior to irradiation reduced the survival time of the mice from 10.2 ± 2.0 days (controls) to 4.3 ± 1.0 days. With 0.44 mg/kg applied at –30 min, survival time was 4.7 ± 1.6 days. If the two doses were applied 120 min before the irradiation, no significant modification of the mean survival time was seen.

Pretreatment with DCP (Table 2) applied at 30 or 120 minutes before irradiation in the doses of 37 mg/kg, 18.5 mg/kg and 9.2 mg/kg consistently shortened the mean survival time (range 3 to 6 days) as compared to the control value of 10.1 ± 1.7 days. No modification of survival time

Table 1. Mean survival time of mice (n=12) irradiated with 10.5 Gy and pretreated with dimethylbenzanthracene.

Dose (mg/kg)	Applied at (min)	Mean survival time (days $\pm$ S. D.)	P
Controls	-30	10.2 $\pm$ 2.0	
0.87	-30	4.3 $\pm$ 1.0	<0.01
0.87	-120	11.1 $\pm$ 7.2	N. S.
0.44	-30	4.7 $\pm$ 1.6	<0.01
0.44	-120	12.5 $\pm$ 8.6	N. S.

S. D. : standard deviation

N. S. : not significant

Table 2. Mean survival time of mice irradiated with 10.5 Gy and pretreated with various doses of diphenylcyclopropenone.

Dose (mg/kg)	Applied at (min)	n	Mean survival time (days $\pm$ S. D.)	P
Controls	-30	18	10.1 $\pm$ 1.7	
37	-30	12	3.0 $\pm$ 0.9	<0.01
37	-120	12	4.7 $\pm$ 0.8	<0.01
18.5	-30	6	5.8 $\pm$ 3.8	<0.01
18.5	-120	6	6.0 $\pm$ 2.9	<0.01
9.2	-30	6	6.0 $\pm$ 3.0	<0.01
9.2	-120	6	4.5 $\pm$ 1.4	<0.01
4.1	-30	6	12.2 $\pm$ 0.6	N. S.
4.1	-120	6	10.5 $\pm$ 10.1	N. S.

S. D. : standard deviation

N. S. : not significant

Table 3. Mean survival time of mice irradiated with 10.5 Gy and pretreated with 1- and 2-aminoanthraquinone.

Treatment	Dose (mg/kg)	Applied at (min)	n	Mean survival time (days $\pm$ S. D.)	P
Controls		-30	18	12.4 $\pm$ 2.6	
1-aminoanthraquinone	10	-30	6	5.7 $\pm$ 2.7	<0.01
do.	10	-120	6	8.8 $\pm$ 10.4	N. S.
do.	5	-30	6	5.2 $\pm$ 1.6	<0.01
do.	5	-120	6	9.8 $\pm$ 9.9	N. S.
2-aminoanthraquinone	90	-30	6	5.2 $\pm$ 0.4	<0.01
do.	90	-120	6	5.5 $\pm$ 0.8	<0.01
do.	45	-30	6	4.8 $\pm$ 1.2	<0.01
do.	45	-120	6	5.5 $\pm$ 1.6	<0.01

S. D. : standard deviation

N. S. : not significant

occurred with the dose of 4.1 mg/kg DCP.

A decrease of survival time was also seen by pretreatment with 1- and 2-AAQ (Table 3). With 1-AAQ, survival time was diminished only after administration of the agent 30 min before irradiation. 2-AAQ decreased survival time with both doses of 45 or 90 mg/kg applied either 30 or 120 min before irradiation from a control value of 12.4 ± 2.6 days to approximately 5 days.

DISCUSSION

All three agents shortened significantly the survival time of mice irradiated with the lethal dose of 10.5 Gy. The effect occurred with relatively small doses, namely, with 0.44 mg/kg of DMBA ($1/16$ LD₅₀) and 9.25 mg/kg DCP, corresponding to $1/8$ of the LD₅₀. With 1- and 2-AAQ, the smallest tested doses were 5 and 45 mg/kg, respectively (each $1/4$ LD₅₀). All three agents were applied to the mice in a vehicle that delivered 1.25 ml DMSO/kg. DMSO exerts radioprotection at 2.5 ml/kg, but no effect is detectable at 1.25 ml/kg (Floersheim, 1993). DMBA (0.87 mg/kg and 0.44 mg/kg) and 1-AAQ did only accelerate radiation lethality if applied at -30 min, but not at -120 min. We assume that plasma concentrations of these agents after administration at -120 min were not sufficiently high at the time of irradiation to provide the sensitizing effect.

Additional studies are needed to determine whether the observed combined effects of chemicals and radiation are synergistic. It is unlikely that the radiation agonist effects are due to additive toxicities because (a) the chemical agents studied were well tolerated if applied alone and (b) a large array of other chemicals tested primarily at analogous nontoxic doses ($1/2$ LD₅₀) failed to accelerate radiation death. This fact points to a specific sensitizing interaction of the active compounds with radiation and makes it unlikely that the data merely reflect increased toxicity of the agents in irradiated animals. The reduction of the survival time by pretreatment with DMBA, DCP and AAQ is compatible with the assumption that the death pattern in the irradiated mice was changed from the hematological (bone marrow) to the gastro-intestinal type death. However, in a similar experimental set-

up where allopurinol, indomethacin and riboflavin accelerated radiation death (Floersheim, 1994), no histological support for this contention was obtained.

Radiosensitizing effects of DMBA in an *in vitro* system involving the survival of X-irradiated C3H mouse embryo-derived cells have been described (Chen *et al.*, 1990). It was shown that DMBA progressively removed the shoulder of the X-ray survival curve and abrogated the survival sparing from dose fractionation. It was thought that DMBA-formed bulky adducts with DNA are a form of DNA damage operationally equivalent to X-ray damage. Accordingly, DMBA seems to act as an indirect carcinogen in concert with ionizing radiation. Metabolic differences associated to *in vitro* vs. *in vivo* conditions must be considered to explain that sensitization occurred at intervals of not less than four hours as compared to 30 min in our experiment.

The finding that DMBA, DCP and AAQs reduce survival time in lethally irradiated mice may also provide clues as to the type of radiation damage involved. Since DMBA removes in cultured mammalian cells the shoulder of the dose-response curve (Chen *et al.*, 1990), a similar shoulder modifying effect of DCP and AAQs may be considered. On the other hand, sensitizing chemicals modifying the slope of the dose-response curve such as misonidazole and metronidazole were also tested in our experiments and failed to reduce survival time in lethally irradiated mice (data not shown). One may thus construe that shoulder modifiers can be distinguished from slope modifiers by their effect in the survival time reduction model where shoulder modifiers are active in contrast to slope modifiers. To provide more evidence for this possibility, *in vitro* studies with DCP and AAQs are needed as performed by Chen *et al.*, (1990) with DMBA. Of practical relevance with regard to radiation hazard may then be that while e.g., with misonidazole, sensitization is lost under progressive radiation fractionation (Denekamp and Stewart, 1978), shoulder modifiers maintain on the contrary their sensitizing potential in fractionated radiation exposure (Chen *et al.*, 1990).

DCP is an electrophilic and chemically highly reactive agent. No experimental evidence is available to explain its radiosensitizing mecha-

nism. One could imagine that DCP adds chemically to and blocks endogenous nucleophilic groups (such as thiols) with radioprotecting potential. On the other hand, DCP could afford an enhancing effect on radicaloid species produced by radiation.

DCP is used as a contact allergen for immunotherapy by repeated application to the scalp of patients with alopecia areata (van der Steen *et al.*, 1991). It is not mutagenic in the Ames test (Stute *et al.*, 1981) and teratogenicity and organ toxicity could not be detected in the hen's egg test or in the mouse teratogenicity assay (Happle, 1985; Happle, 1986). No indications for genotoxic effects or oncogen activation have been materialized. As a rare side effect, erythema multiforme-like eruptions have been described (Perret *et al.*, 1989).

In contrast to other tested quinones including anthraquinone, benzoquinone, duroquinone, quercetin, and ubiquinone (Coenzyme Q₁₀) which were inactive, 1- and 2-AAQ enhanced the effect of radiation in mice. It is not clear why AAQs act as radiosensitizers. Electrophilic properties and a stabilization of the quinoid form by 1- and 2-amino groups may have a rôle. Anthraquinones are used as laxants and quinoid molecules have carcinogenic potency. Anthraquinones participate in redox cycles in which semiquinone intermediates are oxidized to quinones with synchronous reduction of O₂ to O₂⁻ (Cerutti, 1985) which is likely to act additively with ionizing radiation. In line with radiosensitizing properties of quinones are also considerations of quinone cytotoxicity which tend to focus on redox activation by oxidoreductases and concomitant production of semiquinone superoxide and hydroxyl radicals (Wolff and Spector, 1987). In a proposed model of radiation sensitization by quinones, the catalytic rôle of the quinoid structure in the electron transport system and particularly of compounds able to react with free radicals produced by a one-electron oxidation process was stressed (Simic and Hayon, 1972).

This preliminary work limited to the study of survival time should be followed by the investigation of other parameters such as mechanism of action, plasma levels and pathological findings. The enhancement of radiation injury by the tested chemicals illustrates an unorthodox aspect

of toxicology and adds an additional facet to their toxicological spectrum. The presented results indicate the necessity to study more subtle and delayed forms of tissue damage through the radiation-enhancing effects of DMBA, DCP and AAQ.

A potential useful aspect of noncarcinogenic radiosensitizers concerns their topical application to enhance the efficacy of radiotherapy in skin cancers such as basalioma, spinalioma and melanoma as well as in non cancerous skin conditions including mycosis fungoides, Sézary syndrome and other T-cell lymphomas. Local or intralesional DCP or AAQ may deserve to be tested as adjuncts to cutaneous radiotherapy in only partially radiosensitive skin disease.

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REFERENCES

- Cerutti, P. A. (1985): Prooxidant states and tumor promotion. *Science*, **227**, 375-381.
- Chen, C. C., Wells, R. L. and Elkind, M. M. (1990): Additivity of cytotoxic damage due to dimethylbenz(a)anthracene and X-rays. *Radiat. Res.*, **123**, 44-48.
- Denekamp, J. and Stewart, F. A. (1978): Sensitization of mouse tumors using fractionated X-irradiation. *Br. J. Cancer*, **37**, 259-263.
- Floersheim, G. L. (1993): Radioprotective effects of calcium antagonists used alone or with other types of radioprotectors. *Radiat. Res.*, **133**, 80-87.
- Floersheim, G. L. (1994): Allopurinol, indomethacin and riboflavin enhance radiation lethality in mice. *Radiat. Res.*, **139**, 240-247.
- Floersheim, G. L. (1995): Modification of radiation sensitivity by salts of the metals beryllium and indium and the rare earths cerium, lanthanum and scandium. *Radiat. Res.*, **141**, 314-319.
- Furuta, Y., Hunter, N., Barkley Jr. T., Hall, E. and Milas, L. (1988): Increase in radioresponse of murine tumors by treatment with indomethacin. *Cancer Res.*, **48**, 3008-3013.
- Happle, R. (1985): Immunologische Behandlung der

- Alopecia areata. *Hautarzt*, **36**, (Suppl. 7), 108–111.
- Happle, R. (1986): Alopecia areata and Alopecia totalis: Pathogenese und topische Immuntherapie mit Diphencyprone. *Therapiewoche*, **36**, 2706–2716.
- Perret, C. M., Steijlen, P. M. and Zaun, H. (1989): Erythema multiforme-like eruptions: a rare side effect of topical immunotherapy with diphenylcyclopropenone. *Dermatology*, **180**, 5–7.
- Simic, M. and Hayon, E. (1972): A model of radiation sensitization by quinones. *Int. J. Radiat. Biol.*, **22**, 507–511.
- Streffer, C. and Müller, W. U. (1984): Radiation risk from combined exposures to ionizing radiations and chemicals. *In Advances in Radiation Biology*, Vol. 11 (Lett, J. T., Ehmann, U. K. and Cox, A. B., eds.), Vol. 11, pp. 173–210, Academic Press, New York.
- Stute, J., Hausen, B. M. and Schulz, K. H. (1981): Diphenylcyclopropenone-ein stark wirksames Kontaktallergen. *Dermatosen*, **29**, 12–15.
- Sweeney, T. R. (1979): A survey of compounds from the antiradiation drug development program of the US Army Medical Research and Development Command. Walter Reed Army Institute of Research, Washington, DC.
- UNSCEAR (1982): Biological effects of radiation in combination with other physical, chemical or biological agents. *In Ionizing radiation: sources and biological effects*. United Nations Publications Sales No. E.82, IX.8, pp. 727–773.
- van der Steen, P. H. M., van Baar, H. M. J., Perret, C. M. and Happle, R. (1991): Treatment of alopecia areata with diphenylcyclopropenone. *J. Am. Acad. Dermatol.*, **24**, 253–257.
- Wolff, S. P. and Spector, A. (1987): Pro-oxidant activation of ocular reductants. 2. Lens epithelial cell cytotoxicity of a dietary quinone is associated with a stable free radical formed with glutathione *in vitro*. *Exp. Bye Res.*, **45**, 491–803.