MASTER

Progress Report

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Project Title: The Tumorigenic Action of Beta, Proton, Alpha and Electron Radiation on the Rat Skin

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Technical Scope Summary

Epithelial tumors are induced reproducibly in rat skin exposed to ionizing radiation, ultraviolet light, or polycyclic aromatic hydrocarbons. For these carcinogens, the comparative risk of tumor formation is being examined for clues to the generality of action and the mode of interaction of diverse carcinogens. Molecular lesions, such as, pyrimidine dimers and single strand breaks in DNA have been measured as a basis for assessment of cellular dose. Dose-response for single doses, time response for multiple doses and repair or recovery of oncogenic damage are major areas of current interest.

Progress Report

Abstract of Proposed Research

The induction of tumors in rat skin is being studied for various types, doses and dose rates of ionizing radiation and for various doses and wavelengths of ultraviolet light. These studies are designed to define as exactly as possible the dependence of tumor induction on dose of radiation and to establish to what extent the skin is capable of repairing or removing the damage that leads to cancer. Skin was exposed to Argon-40 ions in order to determine whether the tissue was capable of repairing the oncogenic damage produced by high LET radiation. A new finding that solar spectrum or germicidal ultraviolet light was capable of inducing skin tumors was investigated by measuring the formation of pyrimidine dimers in epidermal DNA for various doses, wavelengths and fractionation schedules. Whether ionizing radiation might produce damage that would render the skin more susceptible to the carcinogenic action of another agent, such as, a polycyclic aromatic hydrocarbon is being studied. The induction and repair of single strand breaks in epidermal DNA are being investigated in vivo. Single strand breaks are macromolecular lesions for which epidermal cells have an effective mechanism for repair or removal. Studies to define the lesion in DNA that is most closely associated with oncogenic action of a given carcinogen are proceeding by the use of specific sensitizers

that interact with DNA and alter its sensitivity to radiation and ultraviolet light.

Scientific Background and Recent Progress

Rat skin has proved to be a remarkably sensitive and reproducible model for studying the mechanism of radiation carcinogenesis and for formulating ideas that could lead to improved estimates of risk in radiation protection calculations. Studies of the shape of the dose-response curve (1), the effect of spatial distribution of dose (2, 3, 4), the effect of the time pattern (5, 6), and the importance of the density of energy deposition (7, 8) on the induction of skin tumors has led to important insights into radiation carcinogenesis. Whether tumor incidence is proportional to the amount of tissue irradiated was studied by irradiating rat skin in grid and sieve patterns of various pore sizes with electrons, protons and low energy X-rays. A consistent finding was that localized dose patterns were less oncogenic than uniform patterns for low linear energy transfer (LET) radiation, electrons (2) and X-rays (4), but not for high LET protons (9). It was estimated from geometrical considerations that the interaction distance between irradiated and unirradiated tissue was approximately 170 microns.

It was found that tumor yield was not dependent on the growth phase of hair follicles at the time of irradiation, although the growing follicle consisted of about ten times as many cells as

the resting follicle (12). When radiation penetrated sufficiently to reach the entire growing phase follicle, the number of tumors produced was not significantly greater than that observed in resting-phase skin. It was shown that radiation must penetrate to a depth of about 0.3 mm into the skin in order to induce tumors (3). The follicular stem cells may be the primary targets for oncogenesis, however, selective irradiation at 0.3 mm (13), the position of the stem cells, did not produce tumors. Secondary factors, such as gross tissue injury or epidermal depopulation may be required for full expression of the potential to induce cancer.

Skin is capable of repairing much of the radiation induced damage that leads to the formation of tumors (5, 6). Recovery of oncogenic damage from electron radiation occurred within 24 hours as demonstrated with split dose irradiation (5, 6). Our recent experiments indicate that the halftime of recovery or repair is between 2 and 3 hours (14, 15). A comparable degree of recovery was found for the carcinogenic effect of protons despite the fact that protons are more oncogenic than electrons (RBE about 2.0-2.4) (8).

The temporal aspects of tumor induction in rat skin are well documented (10). Tumors begin to appear after a latent period of about 20 weeks, and they continue to appear at a reasonably constant rate for up to 90 weeks which is a substantial portion

of lifetime. The guarantee time or minimum latent period does not depend significantly on dose except possibly at very high doses.

The growth and proliferation rates of early and late tumors were similar and exhibited no dependence on time of appearance. Late tumors apparently need more time for development than early tumors, but once they are large enough to be detected (about 1.0 mm diameter), they grow and progress indistinguishably from the early tumors (10, 11). A micro-tumor detection technique has provided additional information for the analysis of tumor growth rates, and suggests that the late-appearing tumors are probably new events and not simply the slowly-growing portion of the tumor population. Other experiments have indicated that a carcinogenically-sensitive subpopulation of cells exists in the skin (3).

Radiation may have created conditions favorable for the development of tumors rather than having caused them directly. This raises the question of whether weak carcinogens, such as, ultraviolet radiation or low doses of chemical carcinogens may be strongly carcinogenic on skin previously irradiated with relatively low doses of radiation.

The possible promoting effect of ultraviolet radiation (UVR) on rat skin irradiated with ionizing radiation has been studied. UVR and ionizing radiation are both complete carcinogens when

administered separately in rat skin and high doses of UVR causes edema, erythema, and scaling with subsequent eschar formation. Tumor induction in albino rat skin was compared for single or multiple doses of erythemal (275-375 nm) UVR following single doses of electron radiation. The tumors induced by electrons were primarily squamous or basal cell carcinomas and were clearly distinguishable, both grossly and histologically, from UVR-induced tumors. The majority of the latter were keratoacanthomas.

The marked difference in the histological features of the tumors may reflect differences in the oncogenic targets of the two radiations. In rats exposed to both UVR and electrons, the incidence and types of tumors indicated that the effects of the two agents were generally additive and independent except for delay in the appearance of electron-induced tumors during UVR exposure. For a given total dose, multiple exposures to erythemal UVR induced fewer tumors than single exposures, however, multiple exposures produced a severe scaling on the skin surface which could have reduced the amount of UVR reaching the basal cells.

Rats of various ages were exposed to low voltage X-rays in order to determine the age dependence of the dose response curves for tumor induction (2). Newborn, 28, 58, 100, 200, and 350 day old rats were exposed to a range of doses between 500 and 5000 rads. The time from irradiation to appearance of first

tumor (latent period) varied between 15-24 weeks but was not strongly age dependent.

The dose required to produce maximum tumor yield increased with age, whereas the maximum tumor yield itself (tumors per rat) decreased with age. The rats became progressively more resistant to the oncogenic effect of the radiation with age. An increase in radioresistance with age was observed in our previously reported studies on skin tumor in rats irradiated with electrons or protons at 28 and 58 days of age, and supports the general conclusion that several types of radiation become less oncogenic with increasing age.

The carcinogenic potential of ionizing radiation in conjunction with a chemical carcinogen is of practical importance in simulating environmental conditions. Because the tumor response for ionizing radiation is well established in rat skin, the interaction with other carcinogens can be studied with respect to equivalent doses to produce the same response. Temporal additivity can be analyzed by comparing the individual temporal incidence curves for the different carcinogens with the incidence curve obtained with combined treatment.

A combined treatment experiment has been initiated in which the dorsal skin of rats was irradiated with doses of 750, 1500, 2250 or 3000 rads of electrons followed by weekly applications 20 μ g, 100 μ g or 500 μ g dimethylbenz(a)anthracene (DMBA) in

acetone (22). The tumor growth and appearance kinetics are consistent with the Blum-Druckrey reciprocity formulation, i.e., $dT^n = \text{constant}$, where d is DMBA dose rate, T is time to tumor appearance, and n is a constant that must be evaluated empirically. The combined radiation and DMBA treatment groups exhibited tumor yields which were approximately equal to the sum of the yields induced by the two treatments separately. That is, the two carcinogens appeared to act independently of each other and pre-treatment with radiation did not alter the susceptibility of skin to subsequent carcinogenesis by DMBA.

Cells become increasingly unable to repair radiation-induced lethal damage as the LET of the radiation is increased. Whether the loss of capacity to recover or repair would also apply to tumor induction was investigated by irradiating rat skin with argon nuclei. Rat skin was irradiated with $^{40}\text{Argon}$ ions at an energy of 470 Mev/nucleon (19). These irradiations were performed at the Lawrence Berkeley Laboratory heavy-ion linear accelerator (HILAC) and Bevatron in combination (BEVALAC) (32). Dorsal skin flaps were irradiated with single and fractionated exposures to the plateau region of the Bragg ionization curve (average LET = 115 kev/ μ at doses between 50 and 1266 rads. Four hundred animals were irradiated in 20 dose groups as indicated in the protocol. The animals were observed for 90 weeks. The tumor yield was greatest in the 950 rad dose group. The tumor

yield increased with a dose exponent of 1.2 up to 950 rads and declined slightly at the highest dose. The latter decline was presumably the result of cell or tissue destruction due to acute radiation lethality. No evidence of recovery when the radiation dose was split or fractionated was observed.

The oncogenic dose response of albino rat dorsal skin to single exposures of ultraviolet radiation (UVR) has recently been characterized at two wavelengths (16, 17). Erythema UVR (275-375 nm, spectrum) was found to produce a maximum tumor yield of five tumors per rat at 70 weeks after a dose of 12.5×10^5 ergs/mm². Whereas germicidal wavelength UVR (254 nm, monoenergetic) produced a maximum tumor yield of three tumors per rat at 70 weeks after a dose of 25×10^5 ergs/mm². By comparison of the two dose response curves, it was estimated that the erythema UVR was about (10 times) more effective than the germicidal UVR per unit dose for tumor induction.

In order to determine the wavelength dependence of UVR penetration in epidermis, the transmission of both UVR wavelengths through a single layer of rat epidermis was measured. The transmission of erythema UVR to the basal cell layer of the epidermis was between 7 and 15% of surface dose, and the transmission for germicidal UVR was between 1.5 and 3% of surface dose. Therefore, the effective UVR dose to the basal cell layer was about five times greater for erythema than germicidal UVR for

equivalent surface exposures.

The majority of tumors observed in these experiments were epidermal tumors diagnosed as keratoacanthomas. About 15-20% of the observed tumors were exophytic in nature with keratinization. A small percentage of the tumors regressed spontaneously.

To our knowledge, the tumor data represent the first definitive dose response information for single UVR exposures. The production of tumors by single doses presents a unique opportunity to study early biochemical effects following a single UVR exposure without the complicating problems such as hyperplasia, desquamation and ulceration encountered in chronic exposures. In addition, the oncogenic dose response data at two UVR wavelengths allows comparison with the biochemical effects under two different conditions. Investigation of UVR induced damage and repair to polynucleotides will be discussed in detail in the following section.

The question of split dose recovery for UVR oncogenesis is of interest because the dose response relation is linear, whereas for ionizing radiation the dose response relation is approximately quadratic. Therefore, UVR oncogenesis should exhibit no split dose recovery as has been observed for ionizing radiation.

Damage and subsequent repair processes of cellular DNA are believed to be related to the cellular reaction to ultraviolet

light and ionizing radiation. Recently these processes have been implicated in the carcinogenic response (25, 26) and several experiments have been undertaken to detect DNA damage and repair following in vivo exposure (27).

The measurement of UVR induced excision repair DNA synthesis was attempted using the sparse labelling radioautographic technique. This involves injecting either intradermal or intraperitoneal H^3 -Thymidine immediately following UVR irradiation and subsequent skin biopsies taken one hour post-irradiation. Radioautographs of thin sections from the biopsies exhibit sparse labelling if DNA repair synthesis has occurred, because only small segments of newly synthesized DNA will be labelled. The cells in S-phase are heavily labelled and easily distinguishable from sparsely labelled cells. In our experiments using erythemal UVR (275-375 nm) in the dose range of 1.58×10^5 to 25.2×10^5 ergs/mm² or germicidal (254 nm) UVR in the range of 0.65×10^5 to 26.0×10^5 ergs/mm², we found no evidence of sparse labelling above background in non S-phase cells in the dorsal skin of albino rats. This is a carcinogenic dose for both erythemal and germicidal radiations and a dose at which DNA repair synthesis has been demonstrated in hairless mouse epidermis. Both wavelengths of UVR produced a general decrease in H^3 -Thymidine uptake in the labelled S-phase cells, indicating an inhibition of cellular DNA synthesis. The inhibition of DNA synthesis caused by erythemal

UVR was observed at 1 and 5 hours post-irradiation and to a lesser degree at 48 hours.

Pyrimidine dimers have been associated with UVR oncogenesis for several reasons. The clinical syndrome, xeroderma pigmentosum is characterized by hypersensitivity to solar radiation and a high incidence of multiple carcinomas. The cells from these individuals are more sensitive to UVR in vitro than normal cells (23) and lack the ability to excise pyrimidine dimers (24).

Tumor formation in fish has been linked to UV induced pyrimidine dimers (31) by means of in vivo photomonomerization enzymes. Post UVR treatment with visible light (310-440 nm, monomerizing wavelength) produced a decrease in tumor incidence, indicating that pyrimidine dimer reversal results in decreased levels of oncogenic damage.

An assay system for in vivo detection of pyrimidine dimers in epidermal tissue has been developed by Pathak (33) and modified by Bowden, et al. (34). Epidermal DNA is labelled with ^3H -TdR by painting on the dorsal skin or ID injection. The DNA is extracted, deproteinized, washed, hydrolyzed and chromatographed on Whatman WA-2 Ion-Exchange chromatography paper. The labelled thymine monomers and pyrimidine dimers are detected by liquid scintillation counting of the chromatograph.

Pyrimidine dimer formation has been assayed in vivo in the oncogenic UVR dose range for both erythema and germicidal

wavelengths in the present study. The rate of production of pyrimidine dimers was found to be 0.026% per 10^5 ergs/mm² for germicidal UVR and 0.18% per 10^5 ergs/mm² for erythematous UVR, where the percentage refers to the proportion of thymine detected in dimer form.

Proposed experiments involving UVR induced DNA lesions and their repair include the measurement of pyrimidine dimer removal at various times after irradiation which has been suggested by the work of Bowden, et al. (34). The formation of DNA interstrand crosslinks by 8-methoxypsoralen will also be studied in conjunction with oncogenic photosensitization.

Experiments are being initiated to measure single strand breakage of DNA induced by carcinogenic doses of ionizing radiation. The technique used is DNA strand unwinding procedure of Rydberg (36) as modified by Sheridan and Huang (37). The assay is based on the increasing rate of strand separation of DNA duplexes in alkali for molecules with increasing numbers of single strand scissions. The rate of strand unwinding is determined from the fraction of DNA that is double stranded after various alkali treatment times. Single stranded DNA is distinguished from double stranded DNA by treatment with S₁ nuclease.

This method can detect single strand breaks at doses as low as 200 rads and with a sensitivity of one break per 10^9 daltons. We are adopting this procedure for use with rat skin epidermal

cells exposed to ionizing radiation in vivo. Initial results indicate that single-strand breaks in epidermal DNA are produced in proportion to the radiation dose, and that these breaks are repaired in such a way that the persistence halftime is between 13 and 15 minutes.

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