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TRITIUM: A MODEL FOR LOW LEVEL LONG-TERM IONIZING RADIATION EXPOSURE

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SUMMARY: In 1971 a program was begun in the Medical Department at Brookhaven National Laboratory aimed at evaluating the somatic, cytogenetic and genetic effects of single and chronic tritiated water (HTO) ingestion in mice. This study serves not only as an evaluation of tritium toxicity (TRITOX) but due to its design involving long-term low concentration ingestion of HTO may serve as a model for low level long-term ionizing radiation exposure in general. Long-term studies involved animals maintained on HTO at concentrations of 0.3 μCi/ml, 1.0 μCi/ml, 3.0 μCi/ml or depth dose equivalent chronic external exposures to ^{137}Cesium gamma rays. Maintenance on 3.0 μCi/ml resulted in no effect on growth, life-time shortening or bone marrow cellularity, but did result in a reduction of bone marrow stem cells, an increase in DLM's in second generation animals maintained on this regimen and cytogenetic effects as indicated by increased sister chromatid exchanges (SCE's) in bone marrow cells, increased chromosome aberrations in the regenerating liver and an increase in micronuclei in red blood cells. Biochemical and microdosimetry studies showed that animals placed on the HTO regimen reached tritium equilibrium in the body water in approximately 17-21 days with a more gradual increase in bound tritium. When animals maintained for 180 days on 3.0 μCi/ml HTO were placed on a tap water regimen, the tritium level in tissue dropped from the equilibrium value of 2.02 μCi/ml before withdrawal to 0.001 μCi/ml at 28 days. The rate at which nonexchangeable tritium disappears from various subcellular components is much slower, varying among tissues.

INTRODUCTION: Since the first reports in the late 1890's of ionizing radiation effects on humans, there has been great concern as to the possible hazards from both single large and long-term low level radiation exposures to the human population. Although much has been learned, particularly concerning high dose effects, there are still a number of important unanswered questions concerning low level exposure in the range that might be approached in a nuclear energy economy. One primary source of radiation exposure in such an energy economy (particularly with the development of fusion technology) would be tritium, mostly as HTO. In order to examine the hazards, or lack thereof, of tritium in the environment, it is necessary to take a detailed look at a number of parameters. Since these have already been reviewed in detail by the author (1) the emphasis of this presentation will be to describe the TRITOX program at Brookhaven and its role as a model for low level long-term ionizing radiation studies.

On the following pages will be described the BNL TRITOX program outlined below.

BROOKHAVEN TRITIUM TOXICITY PROGRAM

I. Genetic and Reproductive Efficiency Studies
   A. Dominant Lethal Mutation Rate
   B. Cytogenetic Studies (bone marrow, regenerating liver, RBC's)
II. Somatic Effects
   A. Growth (Body Weight)
   B. Nonspecific Lifetime Shortening
   C. Bone Marrow Cellularity and CFU-S Content
III. Relative Biological Effectiveness (RBE)
   A. Comparison of HTO and ^{137}Cs Effects
IV. Biochemistry and Microdosimetry Studies
   A. Rate of Tritium Incorporation
   B. Site of Tritium Incorporation
   C. Rate of Tritium Disappearance
   D. Histone and DNA Turnover Studies
   E. Cellular Turnover Studies
V. Carcinogenesis
   A. Induction of Leukemia

MATERIALS AND METHODS: Mice Breeding and Maintenance. Except for leukemia determinations, all mice were Swiss Albinos of the Hale-Stoner-Brookhaven (HSB) strain.

Only brief descriptions of techniques will be presented at this time with citations given for more detailed descriptions.

Dominant Lethal Mutation Studies (DLM). Animals received from the colony at four weeks of age were divided into two groups, one of which was placed on HTO at one of three concentrations (0.3, 1.0, or 3.0 μCi/ml). The second group was placed on tap water to serve as controls. When the animals reached 8 weeks of age, they were bred within each experimental group, yielding a second generation which was maintained on the same regimen as their parents. The second generation animals were then assigned to one of four test groups as follows: Group I -- Males and females maintained on HTO, Group II -- Females on HTO, Males on tap water, Group III -- Males on HTO, females on tap water, and Group IV -- Males and females on tap water (controls). Breeding groups were arranged with one male placed with five females for a 5-day breeding period. Fifteen days after the midpoint of this breeding period, the females were sacrificed, the number of pregnant females noted, and the ovaries and the uterine contents examined for DLM's and statistical analyses made (2-4).

Cytogenetic Studies. Regenerating Liver Studies - Since the liver in the adult mouse is not very mitotically active, individual cells tend to accumulate injury during continuous radiation exposure. This damage will be expressed as chromosome aberrations if the cells are stimulated into division by partial hepatectomy. To evaluate such effects in the regenerating liver, animals were maintained on 0.3 and 3.0 μCi/ml HTO and tap water beginning at weaning and continuing until sacrifice after approximately 90, 330, 530, and 700 days. At these intervals the animals underwent partial hepatectomy followed after 54 hours by chromosome analysis using previously published methods (5).

Bone Marrow Cell Evaluation for Sister Chromatid Exchanges (SECs). Before and at selected times during HTO ingestion mice were given BrdUrd infusions for 24 hours using the technique of Schneider et al (6). Two hours before sacrifice by CO_2 inhalation, the animals received an injection of colchicine. Bone marrow was then removed from the tibia and femur and evaluated for induction of SECs as previously described (7).

Micronuclei Evaluation in Red Cells. Since these were the first determinations on HTO ingesting animals using the micronuclei test, animals were maintained on somewhat higher HTO concentrations (3.0, 7.5, 15.0, and 30.0 μCi/ml) beginning at weaning and continuing for 5 to 6 weeks. At the end of this ingestion period, blood samples were drawn and red blood cells evaluated for micronuclei as described by Tice (8).

SOMATIC EFFECT STUDIES

Growth and Nonspecific Life-time Shortening -- Two hundred male animals were maintained on 3.0 μCi/ml together with age matched tap water controls throughout their lifetimes. Animals were weighed monthly and examined weekly for gross changes in appearance. Cages were checked daily for deaths. Dead animals were autopsied and evaluated for any gross abnormalities.

Bone Marrow Cellularity and Stem Cell Content -- The leg bone marrow (femur and tibia) was analyzed for total cellularity, relative number of hematopoietic stem cells (CFU-S/60,000 bone marrow cells) and total number of CFU-S per leg. Harvesting of the bone marrow was done using a quantitative grinding technique (9). The stem cell quantitation was done using the spleen colony assay (10,11).

Relative Biological Effectiveness (RBE) Studies -- For a number of years there has been considerable disagreement concerning the assignment of a correct RBE or "Q" value for tritium exposure in the form of HTO. In order to obtain data which might help in resolving this question, comparisons were made between animals maintained on HTO and those receiving a continuous (22 hrs/da) external equal dose rate gamma exposure to ^{137}Cesium gamma rays. Comparisons of all measured parameters were made between animals maintained on 3.0μCi/ml of HTO and equivalent gamma exposures, and using a smaller gamma source, between animals maintained on 0.3 μCi/ml of HTO and animals receiving an equivalent dose rate gamma exposure.

Biochemistry and Microdosimetry Studies.

Rate of Tritium Incorporation -- A number of determinations were made to determine radiation dose delivered to tissues of interest on an activity/gram basis as well as on the basis of tritium incorporation into specific subcellular fractions. The activity/gram and

tritium content in specific subcellular constitutents were done by previously described tecniques (12-14).

The rate of tritium disappearance from tissues, cellular and subcellular components was determined in the same manner on animals maintained on HTO for 180 days followed by maintenance on tap water.

Carcinogenesis

Induction of Leukemia -- The question of induction of leukemia by continuous ingestion of HTO is being investigated in mice of the CBA/CABNL strain. At three and nine months of age, animals received either a single injection of HTO or begin continuous ingestion of 3.0 µCi/ml for periods which would result in integrated whole body doses of 50, 100, 200, or 300 rads. These exposures were chosen to be equivalent to other animals receiving the same exposures in the form of a single 250 kVp, 100 rad/min x-ray exposure. Animals are observed throughout their lifetime for development of leukemia.

This aspect of the TRITOX program is part of a larger leukemia study being done in collaboration with E.P. Cronkite.

RESULTS: Summaries of data including statistic analyses for animals maintained on 3.0 µCi/ml and 1.0 µCi/ml have been published (11,15). In summary, these studies showed that when both male and female breeding partners are maintained on 3.0 µCi/ml, a significant reduction in viable embryos ($p < .0001$) and a significant increase in early deaths ($p < .01$) is apparent. Similarly, when only the females are maintained on 3.0 µCi/ml a significant reduction ($p < .01$) in viable embryos is found.

When both breeding partners are maintained on 1.0 µCi/ml a significant ($p < .01$) reduction in viable embryos is found. In all other cases for animals ingesting 3.0, 1.0 or 0.3 µCi/ml, no significant effects are observed.

Cytogenetic Studies

Regenerating Liver Studies - Animals maintained on 3.0 µCi/ml for 90, 330 and 530 days exhibited a significant increase in the number of abnormal cells in the regenerating liver as compared to animals maintained on nontritiated water. The level of chromosome damage following the HTO ingestion was similar on a per rad basis to that seen in Chinese hamster livers after protracted ^{60}Co gamma exposure or internally deposited ^{144}Ce, an energetic beta emitter.

Similar effects were not seen in animals maintained on 0.3 µCi/ml HTO.

Bone Marrow Cells - The SCE levels in leg bone marrow cells of mice maintained on 3.0 µCi/ml HTO for 28 to 261 days were always higher than those in age matched control groups. The range of SCEs per cell was from 2.00 to 4.03 for HTO animals and 1.70 to 2.81 for control animals. Using a one-way analysis of varience and covarience, the probability that the mean of all the control data is different from that of the exposed animals is less than 0.0001. Details of this study are reported elsewhere (7).

Micronuclei Studies in Red Blood Cells - The results of the micronuclei tests are still somewhat preliminary. However, it is apparent that in animals maintained on 30.0 µCi/ml HTO for 5 to 6 weeks beginning at an age of 3 weeks, there is a significant increase in micronuclei ($p < .01$). A slight but not significant increase was noted in animals maintained on 15.0 µCi/ml, however in those slides analysed to date, no effect was seen in animals on 3.0 or 7.5 µCi/ml HTO. These studies are currently being repeated; however, it seems fair to say that at the highest concentration there is a significant increase in micronuclei.

Somatic Effects

Growth (body weight) and non-specific lifetime shortening - Continuous ingestion of 3.0 µCi/ml of HTO or equivalent external gamma exposure caused no measurable effect on growth as measured by body weight or longevity.

Bone Marrow Cellularity and CFU-S Content - There was no measurable effect on the total number of leg bone marrow cells in any of the animals maintained on 0.3-3.0 µCi/ml HTO or receiving equivalent continuous external gamma ray exposures. In contrast, there were measurable reductions in the number of CFU-S. In both of these groups the CFU-S depression continued with some variability throughout the lifetime of the animals. Similar effects were not measurable in the 0.3 µCi/ml animals. Details of this study have been previously published (16).

Relative Biological Effectiveness (RBE)

In all studies completed to date, there was no significant difference ($p<.01$) between animals ingesting HTO and animals receiving equivalent external ^{137}Cs gamma ray exposures. However, for several of the parameters measured the effects were somewhat greater for the HTO animals, although not significantly so. This could be interpreted as an indication that the RBE or Q value for HTO may be slightly greater than 1 but less than 2 for those parameters measured in this study.

Biochemistry Microdosimetry Studies

When animals were placed on an HTO regimen, tritium concentrations in body water and soft tissues rapidly approached equilibrium levels (17). When removed from the HTO regimen the tritium level in tissues dropped rapidly. In animals maintained on 3.0 μCi/ml which reach an equilibrium level of 2.02 μCi/ml in soft tissues, following withdrawal, the tritium level drops to 0.07, 0.01 and 0.001 μCi/ml by 7, 14 and 28 days. The rate at which nonexchangeable tritium disappears from brain and liver histones follows a significantly different pattern with liver histones exhibiting a half-life of 117 days and brain histones 159 days. The tritium activity in liver and brain show that brain data points fit a straight line, in contrast to the liver where the data points form a curved line indicating the presence in the liver of two-cell populations with distinctly different turnover times. The initial specific activity in liver DNA of 0.90 dpm which we previously reported (11) is in good agreement with an expected value of 0.89 calculated on the basis of previous reports (18). Further details of these studies and others related to cellular and subcellular component turnover have been published (11-14).

Carcinogenesis

Studies on the induction of leukemia are still in progress with no definitive results as yet.

DISCUSSION: The continued increase in use of fission reactors for power generation adds to the world inventory of tritium. With the development of fusion power reactors, the amount of tritium involved will be significant. It is apparent that at levels as low as 33 times the MPC for HTO, measureable effects can be seen in mice. It is also apparent that results of studies as described supply valuable basic information concerning cellular and subcellular component turnover.

As predicted on the basis of established principles of radiobiology, continuous exposure to tritium beta rays from HTO ingestion to levels of equilibrium, at which time the major portion of the exposure is due to the unbound tritium, results in measurable effects on several animal systems. However, the importance of position of incorporation of tritium into molecules of biological importance has not been well defined nor has the low dose portion of the dose response curve for several effects of interest.

The use of chronic ingestion of HTO as a model for studying the effects of general low level long-term whole body radiation exposure has merit as long as proper consideration is given to differences in energy deposition at the microdosimetry level.

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