DEOXYCYTIDINE STUDIES IN X-IRRADIATED RATS

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A number of investigators have reported increased excretion of deoxycytidine (dCT) in the urine of rats following total body exposure to ionizing radiation doses ranging from 10 to 800 r. Deoxycytidine, or cytidine desoxyribose, as it is often called, is one of the major pyrimidine nucleoside components of deoxyribonucleic acid. Peak values in the urine excretion of dCT occur soon after radiation exposure (0-24 hours, although high levels have been noted in the 0-12 hour post-irradiation period), drop sharply during the 24-48 hour period, and by 48-72 hours have almost returned to pre-irradiation control values. The present study was carried out in an attempt to obtain information on the metabolism of deoxycytidine by rats. Control rats and also x-irradiated rats were used since increased deoxycytidine-uria appears to be specific for ionizing radiation. Other stresses to rats, as trauma, burns, and formaldehyde injections do not result in increased amounts of Dische positive substances in the urine.

METHODS

Control and x-irradiated rats were injected in the tail vein with 2 uc of ^{14}C-dCT. Expired air and excreta were collected and analysed for periods up to 48 hours. The rats were then sacrificed and ^{14}C-dCT activity analysed in the various tissues by liquid scintillation techniques.

SLIDE 1. We see the various experimental groups used in the study. Group 1 is a control group receiving ^{14}C-dCT only. Groups 2,3 and 4 all received total body x-irradiation of 700 r and ^{14}C-dCT given at various times prior to and following the radiation exposure period. In Group 2 the ^{14}C-dCT was given 2 to 3 minutes prior to the radiation exposure. In Group 3 the ^{14}C-dCT was given 1 1/2 to 2 hours post exposure and in Group 4 the ^{14}C-dCT was given 18 to 20 hours post irradiation exposure

These times were chosen based on the prior findings of our group and other investigators of the early excretion of deoxycytidine following radiation exposure. The ^{14}C-dCT was obtained from Schwartz Bioresearch Corporation (10.6 mc/mmole specific activity, 10 uc/ml) and its purity was verified by paper chromatography using as the horizonal solvent Butanol: acetic acid: water (1120:30:30) and was found to be approximately 99% pure. Female rats, approximately 2 months old were used in the present study and were kept in metabolism cages for the collection of urine and feces and also for expired air.

SLIDE 2. Shows the metabolism set-up used for the study. The expired air collection was measured continually by an ionization chamber and vibrating reed electrometer to a recorder.

SLIDE 3. Shows a closer view of the metabolism cage for housing the rat for excreta collections.

SLIDE 4. We see typical curves obtained for each of the groups. Each curve is an average of data on four rats. In all cases there was early rise in the expired $^{14}CO_2$ activity, rising to a maximum at 1 1/2 to 2 hours and then decreasing gradually. The rise in the curve for the irradiated rats appears to be quicker and more sharply ~~in the irradiated rats~~ than in the control rats. The total $^{14}CO_2$ expired in the air by the rat in the observed period was calculated as follows: the total area under the curve (millivolts times minutes) times the conversion factor (1.78 times 10^{-3} uc/millivolt) times the flow of air through the metabolic cage and ionization chamber (cc/minute). The volume of the ionization chamber used in this study was 1000 cc.

SLIDE 5. This slide shows the $^{14}CO_2$ activity recovered in expired air of normal and x-irradiated rats injected with ^{14}C-deoxycytidine. For the control rats receiving ^{14}C-dCT, approximately 45.2% of the injected ^{14}C activity was recovered in the expired air. In the rats receiving ^{14}C-dCT just prior to radiation exposure and in a short time interval following the radiation exposure the recovery was less, approximately 31-32% of the injected activity. In the group receiving ^{14}C-dCT

18-20 hours post irradiation, the value rises again to 40.6% of the injected ^{14}C activity, still lower (at a significant level) with respect to the control group. This data probably does not indicate a greater utilization of dCT by the irradiated rats, but suggests that the x-irradiation has altered the deoxycytidine pool size in the body to an extent that the metabolism of the ^{14}C injected deoxycytidine is also altered. The fact that the percent of injected ^{14}C activity is increasing and approaching the control group might indicate that at this time interval, 18-20 hours post irradiation exposure, the dCT pool size in the body is approximately back to normal. This appears to coincide with the information obtained on previous studies by our group and by other investigators that at approximately 24 hours post irradiati exposure there is a sharp drop in the deoxycytidine levels in the excreted urine of t x-irradiated rats.

SLIDE 6. Shows the blood and urine ^{14}C levels in normal and x-irradiated (700 r) rats injected with ^{14}C deoxycytidine. As evidenced by the low levels of ^{14}C-dCT in the blood in the control group, it appears that the deoxcytidine rapidly disappears from the blood following its intravenous injection into the tail vein. The blood level in the irradiated rats was considerably higher for all irradiated groups. The ^{14}C levels in the urine were also considerably higher in the irradiated groups than in the control group. No large differences in the blood & urine ^{14}C level were seen between the irradiated rat groups. These studies for urine are interesting in that the results do not parallel the respiratory findings of the previous slide. Instead of returning to normal values as happened in the respiratory study, the urine and ~~blood~~ levels increased and remained high for all of the irradiated groups. The time-periods involved here post-irradiation perhaps do not provide an explanation based on the possibility of irreversible kidney damage.

SLIDE 7. Shows the distribution of the ^{14}C activity in tissues of normal rats injected with ^{14}C-dCT. On the left we have plotted the total activity as per cent of the administered dose and on the bottom we have listed the various organs. It is seen that there is considerable amount of ^{14}C activity in the small and large intestines. High levels are also seen in the thymus, muscle, spleen and liver. There is little activity in the kidney, blood, heart, and brain.

SLIDE 8. Shows some of the tissue ^{14}C levels in normal and x-irradiated rats injected with ^{14}C-dCT. The values here for the control, that is the ^{14}C-dCT only group, is the same as those shown on the previous slide. Irradiation plus the injection of dCT at various time intervals pre-and post-irradiation exposure does no seem to have any effect on the ^{14}C activity in the small intestine, which is surprising since the small intestine is considered to be very radiosensitive. The ^{14}C level in the large intestine does decrease in the irradiated group. The ^{14}C level in the thymus of the irradiated group appears to be at a maximum for the group injected with ^{14}C-dCT just prior to radiation exposure, slightly lower in the group injected 1 1/2 to 2 hours post-irradiation exposure, and even lower approximating the control level in the group given ^{14}C-dCT 18 to 20 hours post-irradiation period. These changes seem to some extent to correlate with the type of phenomena observed for the expired air data.

SLIDE 9. We see the tissue ^{14}C levels in normal and x-irradiated rats injecte with ^{14}C-dCT in three other tissue samples. Again we see the levels of ^{14}C activity in the control group, the level being slightly higher in the spleen and the liver, and the kidney being much lower than either of the spleen or of liver. The level in the spleen in the 700 r irradiated group given dCT just prior to radiation exposure is the same as that of the control group; however, the other two irradiated groups show levels significantly lower than either of the first two groups. For the liver tissue, the ^{14}C levels are higher (at a significant level) in all of the irradiated groups than in the control group. The same applies for the kidney sampl where the levels in the irradiated groups are all significantly higher than the

control group. The results of these experiments definitely indicate the metabolism of deoxycytidine is significantly different in normal and irradiated rats. It is difficult to compare the normal and irradiated groups, because of the well-known phenomena that after radiation the amount of free nucleosides such as deoxycytidine are increased in the body. Therefore it appears that the specific activity factor (the pool size) is crucial in the interpretation of the data. Some preliminary studies on the importance of the "pool size" factor were carried out, in which the deoxycytidine "pool size" was increased by injecting various milligram amounts of stable dCT at the same time the radioactive dCT was injected.

SLIDE 10. Shows the effect of deoxycytidine pool size on expired air activity in ^{14}C deoxycytidine injected rats, Here the control group is shown again as having 45.2% of the injected ^{14}C activity appearing in the expired air. The results indica that the dCT metabolism isvery sensitive to the pool size factor, because a very slight elevation of pool size, even 5 milligrams, produced alterations of the expire air ^{14}C activity. The expired air activity was 14.2% of the injected ^{14}C activity for the group given 5 milligrams of stable dCT, this value decreased slightly further to 13.1% of the injected ^{14}C activity for the group receiving 20 milligrams of stable dCT, and for the group given 40 milligrams of dCT the expired air ^{14}C activity was 5.5%. For comparison the expired air ^{14}C activity is also given on thi slide for the group given 700 r and then ^{14}C-dCT 1 1/2 to 2 hours post-irradiation. This level is 31.2% of the injected ^{14}C activity and is appreciably higher than for the group given 5 milligrams of the stable dCT. The results here indicate perhaps that the increase in pool size for the 700 r x-irradiated animals is somewhere around 2 to 2 1/2 milligrams of dCT. This also correlates well with the data we have found for the dCT excreted in urine by rats given 800 r total body irradiation. We are presently carrying out experiments involving the use of even lower levels of stable dCT.