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Postirradiation Synthesis and Degradation of DNA in Various Tissues of Rats

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放射線을 照射한 흰쥐의 여러 가지 組織내의 DNA의 合成과 分解

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摘 要

放射線照射에 의해서 尿 및 血液內에 다량의 deoxycytidine (CdR)이 流出하는 것을 볼수가 있는데, 이것이 DNA의 合成이나 分解와 어떤 관련성이 있으며, 아울러 어느 臟器의 DNA가 放射線感受性이 큰지의 여부를 밝히기 위해서 400R의 X線을 흰쥐에 全身照射시켜, 간, 지라 및 흉선을 切除하여 均質化시킨 후, CdR-2-^{14}C을 써서 DNA의 合成率 및 分解率을 測定하였다.

DNA의 合成率은 흉선의 경우, 照射후 1~3日에 가장 심한 抑制現象이 나타났으며 5日후부터는 점차 回復됨을 볼 수 있었고, 한편 간과 지라의 경우는 抑制의 정도가 흉선에 비해서 적었으며 回復도 훨씬 빨리 이루어졌다. DNA의 分解率은 지라와 흉선의 경우 비슷하여서 照射후 1日에 영향이 極大로 나타났으며 回復도 아주 늦게 일어남을 볼 수 있었는데 반해서, 간의 경우는 分解도 덜 일어났으며 回復도 아주 빨리 일어남을 알 수 있었다.

이와 같은 결과는 각종 臟器의 放射線感受性의 차와 再生能의 차에 의해서 나타나는 현상임을 알 수 있고 CdR의 流出量의 增加는 放射線에 의한 DNA合成의 抑制와 DNA分解의 促進의 두가지 요인에 기인하는 것으로 추정되었으며, 전자의 경우는 흉선이, 후자의 경우는 지라와 흉선이 같은 정도의 重要性을 지니고 있음을 알 수 있었다.

INTRODUCION

Parizek et al. (1958) reported for the first time an increased excretion of deoxycytidine (CdR) in rat urine the first day after irradiation, with a linear dose-response in the range of 10 to 600R. Rotherham and Schneider (1958, 1960) have demonstrated that deoxyribosides are present in rat and mouse tissues and, indeed, that CdR is the major component found circulating in the blood with an apparent threshold. Guri et al. (1967) detected a normal excretion of CdR at levels similar to those reported by others (Rotherham and Schneider 1960, Kerciakes,

Saenger and Berry 1964) and confirmed previous studies indicating a significant increase in its excretion after X-irradiation, with a linear dose-response in the range of 10 to 400R. In the course of studies on the biochemical mechanisms involved in the radiation induced increase in urinary CdR excretion, Guri et al. (1968) also carried out the investigation on the plasma levels of CdR and found that the concentration of CdR is increased significantly in X-irradiated rats in comparison to controls.

Although several studies have led to the interpretation that the elevation in urinary CdR represents either a result of an interference in DNA synthesis and/or increased DNA degradation produced by radiation, the mechanisms have not been completely elucidated. The results obtained by Guri et al. (1967) strongly suggested that the radiation-induced loss of spleen DNA is a major factor in the urinary CdR elevation. Splenectomy was associated with a significant decrease in this response after irradiation, while the comparison of spleen DNA loss and urinary CdR increase revealed that at 100 to 200 R approximately 65 to 80 per cent of the urinary CdR could be attributed to the spleen.

In the present study postirradiation incorporation of CdR-2-^{14}C into DNA in the liver, spleen and thymus of the rats were investigated for the purpose of elucidating differential contribution of these tissues to the elevation of CdR levels in urine and plasma following moderate doses of X-radiation.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing from 96 to 146 g and from 2 to 3 months old, were used in these experiments. The animals were subjected to a single whole-body exposure of X-rays from a General Electric Maxitron 250 III Therapy unit. The dose received was 400 R and radiation factors were: 230 Kvp, 10 mA, Th II filter, approximately 18R/min at a distance of 50 cm.

The rats were sacrificed by exsanguination while anesthetized with ether at various time intervals after irradiation; the liver, spleen and thymus were excised, cleaned in ice-cold Krebs-Ringer phosphate buffer, pH 7.4 and homogenized in a glass tissue grinder.

The tissue homogenate was incubated with 0.1 μCi CdR-2-^{14}C in 10 mM glucose-Krebs-Ringer phosphate buffer, pH 7.4 in a Warburg flask at 37°C for 2 hours. In the degradation experiment, however, the same procedure as described for incubation experiment was employed except that the tissue homogenate was incubated without CdR-2-^{14}C.

To the incubation mixture was added 1.0 ml of ice-cold 0.5N perchloric acid and allowed to extract at 4°C for 30 minutes, then centrifuged at 2,000 rpm at 4°C for 15 minutes. The residue was recovered and washed with absolute ethanol containing 0.2 N potassium acetate and then centrifuged at 2,000 rpm at 4°C for 15 minutes. The residue thus obtained was again extracted with 1 ml of ethanol-ether mixture (3 : 1) at 50°C for 30 minutes and centrifuged at 2,000 rpm at 4°C for 15 minutes. The lipid-free residue was resespended in 1.0 ml of 0.1 N potassium hydroxide and incubated at 37°C for 18 hours. To the solubilized cell material was added 0.034 ml of 6 N hydro-

chloric acid and 1.0 ml of 0.5 N perchloric acid allowed to precipitate to form at 4°C for 30 minutes and centrifuged at 2,000 rpm at 4°C for 15 minutes. Then the residue, which contained DNA and protein, was resuspended in 1.0 ml of 0.5 N perchloric acid and heated at 90°C for 15 minutes, then cooled and centrifuged at 2,000 rpm at 4°C for 15 minutes.

The DNA-containing supernatant was then subjected to either one of the following procedures—i. e., counting of radioactivity or DNA quantitation. The diphenylamine reagent was prepared by adding 1.5 g of twice recrystallized diphenylamine and 1.5 ml of concentrated sulfuric acid to 100 ml of redistilled glacial acetic acid. 1.5 ml of supernatant was added to 3 ml of diphenylamine reagent and the incubation was made at 37°C for 18 hours. At the end of incubation, absorbance at 600 nm was read in a MPS 50L multipurpose recording spectrophotometer. For the counting of radioactivity of incorporated CdR-2-^{14}C into DNA, the supernatant was applied to a stainless steel planchet and allowed to dryness under an infrared lamp. The counting of radioactivity was made in a TGC-14 gas-flow type carbon counter.

RESULTS AND DISCUSSION

Incorporation Studies

Rate of incorporation of CdR-2-^{14}C into DNA in normal and X-irradiated rats at various time intervals postirradiation is shown in Fig. 1. In Fig. 2 is shown the rate of incorporation of CdR-2-^{14}C as percent of normal and the pattern of change is quite similar to that in Fig. 1. The high rate of incorporation observed in case of the thymus of normal rat might be attributed to the fact that the thymus used in the present study was obtained from the young, growing rat and that the mitotic activity of the thymus was higher than those of the liver and spleen.

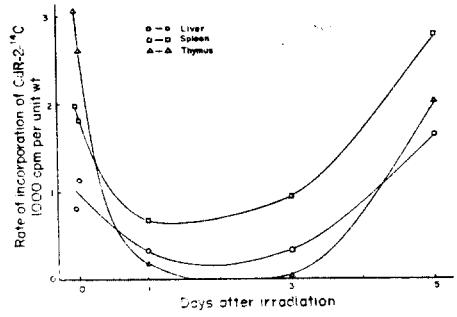

Fig. 1. The pattern of change in the rate of CdR-2-^{14}C incorporation into DNA in various tissues of rats following 400R total-body X-irradiation.

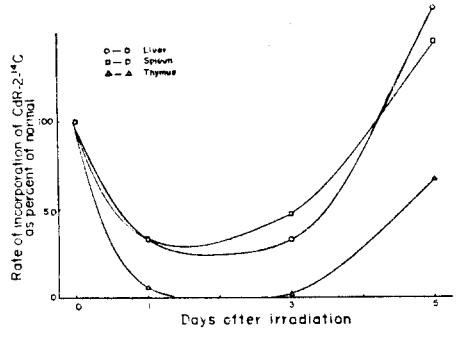

Fig. 2. The change in the rate of incorporation of CdR-2-^{14}C into DNA as percent of normal in various tissues of rats following 400R total-body X-irradiation.

During the first 3 days after irradiation, a marked decrease in the rate of incorporation in the liver, spleen and thymus was noted as well as a sharp decrease in total weight of the spleen and thymus as shown by the dry weight determination. Subsequ-

ent increase in the rate of incorporation occurs by day 5 for all the tissues. A fact to be noted was the difference in the rate of incorporation between the thymus and other tissues, indicating a profound difference in metabolic activity between these tissues.

As is evident from Fig. 2, the rate of CdR-2-^{14}C incorporation into DNA was markedly inhibited in the thymus at days 1-3, compared to the liver and spleen, though the decreasing pattern was quite similar. This finding is consistent with the fact that the synthesis of DNA in thymus cells is highly susceptible to inhibition by X-radiation, both in vitro and in vivo (Ord & Stocken 1961).

The rate of incorporation in the regeneration period increased up to about 1.5 times normal in case of the liver and spleen, whereas the rate of incorporation in the thymus remained about 0.7 times normal. This finding might be explained by the followings that the liver appears to be a very radioresistant organ and this is due, at least in part, to its large regenerative capacity. The white pulp of the spleen, the area of lymphocyte production, is rapidly depleted of lymphocytes after a moderate dose of radiation. Most of the cellular debris is removed by 24 hours after irradiation, and the spleen is frequently less than half normal size. On the other hand, 1 day after a moderate dose, most of the lymphocytes disappear from the thymus and regeneration occur in the thymus. A general pattern of change in the rate of incorporation observed in the present experiment agrees with that of Sugino et al. (1963) who observed a sharp decrease in the rate of incorporation of labeled pyrimidine nucleosides and ^{32}P into DNA in the regenerating thymus during the first 2 days after sublethal dose of total-body X-irradiation. In the regeneration period the increased rate of incorporation of labeled CdR lagged somewhat behind that of thymidine, but then rose to six times normal, reaching a peak at day 4 1/2. The differnce in the rate of increase in incorporation between the present results and those of Sugino et al. is attributable to the difference in the state of the thymus; the regenerating thymus recovers faster than normal thymus.

Degradation Studies

The DNA contents in the liver, spleen and thymus in normal and irradiated rats at various time intervals after irradiation are shown in Fig. 3. The DNA content is expressed in terms of mg per 100 mg dry tissue. In Fig. 4 are shown changes in the amount of DNA as percent of normal to observe general pattern of change. The DNA content in unit weight for various tissues is variable from tissue to tissue. The highest content was observed for the thymus, then the spleen. A marked decrease in the DNA content in the thymus and spleen was observed at day 1 after irradiation. The regene ration period starts from day 3, and at day 5 the liver recovered to normal levels. The results of Sugino et al. (1963) showed an abrupt decrease in the content of DNA in the regenerating thymus at day 1 and the tendency remained by day 3, followed by a gradual increase thereafter.

From the incorporation and degradation studies, general pattern of change could be divided into two periods, the radiation reaction period and regeneration period.

The radiation reaction period is characterized by cellular death with the result

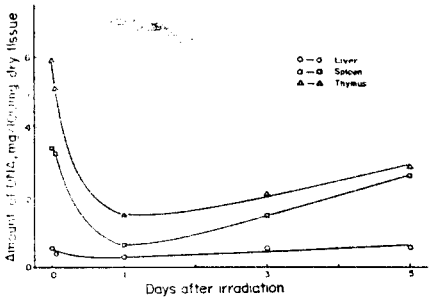

Fig. 3. The pattern of change in the amount of DNA in various tissues of rats following 400 R total-body X-irradiation.

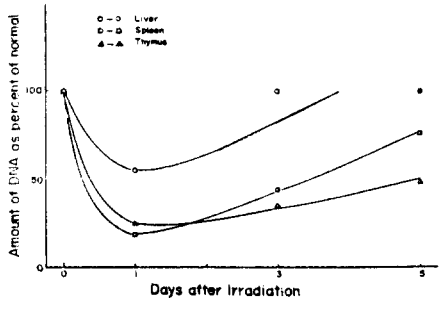

Fig. 4. The change in the amount of DNA as percent of normal in various tissues of rats following 400 R total-body X-irradiation.

of decrease in the weight of the organs and decrease in the DNA content. During this same period, the rate of the synthesis of DNA, expressed in terms of DNA labeled after 2 hours of incorporation of CdR-2-^{14}C, was greatly decreased approximating a zero level for the thymus and 30 percent for the liver and spleen. As far as this specific period is concerned, the change in the rate of incorporation of CdR-2-^{14}C into DNA showed nearly the same pattern for the liver and spleen, and the change in the amount of DNA showed the same pattern for the spleen and thymus.

During the regeneration period, abruptly increasing pattern of precursor incorporation into DNA can be recognized, particularly for the liver and a lesser degree for the thymus. This marked increase in the rate of incorporation of CdR-2-^{14}C occurs during the initial phase—that is, the period of days 4 to 5. This difference also is due to the radiosensitivity and the differential regenerative capacity of various organs as indicated in the incorporation experiments. The results of this experiment indicate that the spleen and thymus are important sites in the enhanced CdR excretion. Moreover these data strongly suggest that the radiation-induced losses of spleen DNA and thymus DNA are the major factor in the elevated CdR level as well as the radiation-induced inhibition of CdR incorporation into DNA in the thymus.

SUMMARY

The effect of 400 R total-body X-irradiation on the rate of deoxycytidine-2-^{14}C (CdR-2-^{14}C) into DNA and on the degradation of DNA has been studied in the liver, spleen and thymus of the rat.

The postirradiation period can be divided into a radiation reaction period followed by a regeneration period. During the period of radiation reaction, which consists of days 1-2, markedly decreased CdR-2-^{14}C incorporation into DNA of each organ is observed. Rate of incorporation of labeled precursor in the thymus shows the most profound decrease, whereas those in the liver and spleen show similar decrease when expressed as percent of normal. The change in the amount of DNA as percent of normal exhibits a sim-

ilar pattern in all organs, but the rate of decrease is larger in the spleen and thymus compared to that in the liver.

The period of regeneration as judged by the incorporation experiment appears day 4 to 5, which consists of the second phase of the regeneration period. The second phase is highlighted by a markedly increased rate of CdR-2-^{14}C incorporation and by a slow and continued increase in the amount of DNA in all organs. The regeneration occurs faster in the liver and spleen than in the thymus which is the most radiosensitive of the all.

The findings of the present experiments are strongly suggestive of the fact that the radiation-induced loss of spleen and thymus DNA as well as the radiation-caused inhibition in the CdR incorporation into DNA of the thymus are the important factors in the elevated levels of CdR in the urine and plasma.

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