studia biophysica, Berlin, Band 42, 1974, Heft 2, S. 105 - 110

Printed in the German Democratic Republic

SPLEEN DNA DAMAGE FOLLOWING IRRADIATION IN THE PRESENCE OF CHEMICAL RADIOPROTECTORS

D. SUCIU, Z. URAY, C. BANU
Oncological Institute, Cluj, Romania

The administration of radioprotective agents (AET, Luvatren, imipramine or sulphamethazine) has no influence on the early elimination of spleen deoxyribopolynucleotides after irradiation with 300 R. The spleen DNA fragmentation 24 hours after irradiation with 1000 R is also unprotected by administration of chemical radioprotectors.

Introduction

The deoxyribopolynucleotide content of lymphatic and hematopoetic tissues rises linearly and dose dependent after whole body irradiation with relative low doses (30–300 R). The cells from which the deoxyribopolynucleotides were released belong to a population sensitive to small radiation doses which is destroyed and eliminated from the tissue about 20 hours after irradiation /11–13/. At elevated radiation doses (1000 R) the DNA from the less sensitive cell population is fragmented to molecular weights of about 1.5×10^6 daltons. This process of DNA fragmentation is slower than the release of deoxyribopolynucleotides and could be well observed only 24–48 hours after irradiation /1, 15/.

The experiments to be reported were performed with the purpose of investigating the protective effect of some chemical radioprotectors (AET, Luvatren, imipramine and sulphamethazine) on the spleen DNA following the whole-body irradiation of mice and rats.

Materials and methods

Groups of 6 animals, RAP mice of 20–23 g were treated i.p. with 250 mg/kg body weight of AET (β, 2-aminoethylisothiouronium, bromide, hydrobromide) or 20 mg/kg body weight of luvatren (4′-fluoro-4/4′-hydroxy-4′-p-methyl-phenyl piperidinol/butirophenone) (LU). The animals were whole body X-irradiated (TUR II X-ray machine) in plastic cages (180 kV, 10 mA, filtered through 0.5 mmCu, FSD 40 cm, dose rate 36 R/min). Irradiation was carried out 15 min. after administration of AET and 30 min. after administration of LU. The control

groups were irradiated in the same conditions. In another experiment groups of 10 Wistar rats (100–120 g) received i.p. single doses of 300 mg/kg body-weight of AET, 50 mg/kg body-weight of imipramine (N-(γ-dimethylaminopropyl)iminodibenzyl) (IP) or 500 mg/kg body weight of sulphamethazine (2-sulphanylamido-4,6-dimethylpyrimidine) (SM). At 15 min. after administration of AET and 30 min. after administration of IP or SM the animals were X-irradiated with 1000 R. The control groups were irradiated in the same conditions. The animals were sacrificed 24 hours after irradiation.

The spleen deoxyribopolynucleotide content was determined following the indications of SKALKA et al. /12/. The tissue was homogenized in cold 0.14 M NaCl + 0.015 M Na citrate. The samples were centrifuged 15 min. at 5000 r.p.m. (0° C). The supernatant (I) was precipitated with perchloric acid to a final concentration of 0.2 N. After centrifugation for 10 min. at 2500 r.p.m. the supernatant was discarded and the precipitate was hydrolyzed in 0.2 N $HClO_4$ for 15 min. at 70° C. The amount of diphenylamine-positive substances was determined according to DE FRANCE and LE PECQ /3/. The precipitate fraction (II) initially obtained was hydrolyzed 16 hours in 0.2 N – KOH, then was treated with $HClO_4$ to a final concentration of 0.2 N and again hydrolyzed for 15 min. at 70° C. The diphenylamine reaction was carried out at 37° C for 24 hours. Colouration was read on a VSU-2P (Carl Zeiss Jena) spectrophotometer at 595 nm. The amount of deoxyribopolynucleotides was expressed as a percentage of the amount of DNA determined in fraction II.

The DNA extractions from rat spleen were done according to a procedure previously described /14/.

The viscosity measurements were made at DNA concentrations of 20–25 μg/ml in a 0.2 M NaCl solution at 25° C. The apparatus used was a capillary viscosimeter of the Ubbelohde type. The results were extrapolated to zero shear gradient and zero concentration as described by EIGNER et al. /7/. The molecular weights were calculated according to EIGNER and DOTY /6/.

Results

As may be seen from Fig. 1 the deoxyribopolynucleotide level in mouse spleen is an almost liniar function of the dose within exposure limits of 0–300 R. Similar results were obtained by SKALKA and MATYASOVA /13/. The data included in Tab. 1 show that after irradiation with 300 R the level of deoxyribopolynucleotide in the spleen of animals sacrificed at different time intervals rises to a maximum at 8 hours. It is also evident that administration of radioprotective agents has no

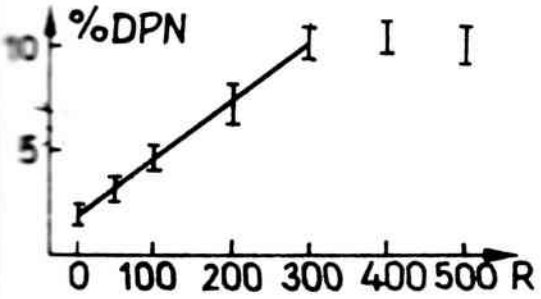

FIG. 1 The release of deoxyribopolynucleotides (DPN) from mouse spleen following irradiation with X-rays.

l Deoxyribopolynucleotide content of mouse spleen after irradiation with 300 R X-rays resence of radioprotectors[a]).

	Controls	AET[b])	LU[b])
ours	7.80 ± 0.65 (4)	9.63 ± 1.12 (2)	10.20 ± 0.81 (2)
ours	10.37 ± 0.38 (6)	13.67 ± 1.25 (5)	12.86 ± 0.62 (4)
ours	19.87 ± 0.54 (3)	15.31 ± 0.92 (3)	17.70 ± 1.40 (2)
ours	4.78 ± 0.27 (2)	6.27 ± 0.43 (2)	5.41 ± 0.17 (2)

results are expressed as percentage of deoxyribopolynucleotides in the DNP complex. means, the standard deviations of the means and the number of determinations are en.

ntrol groups were only injected with AET or LU; the spleen deoxyribopolynucleotide ntent of these animals was about 2 %, essentially the same as for the untreated and rradiated animals (Fig. 1).

t on the deoxyribopolynucleotide level 2–20 hours after irradiation. The ts summarized in Tab. 2 show that the administration of radioprotective nts has no effect on the spleen DNA fragmentation 24 hours after whole-body iation of animals with a large dose (1000 R) of X-rays.

TAB. 2 Viscosity data[a] of the rat spleen DNA molecular weight after irradiation with 1000 R X-rays in the presence of radioprotectors.

DNA samples	$[\eta]$ (dl/g)	Mw x 10^{-6} (daltons)
Controls	32.3 – 36.2	4.6 – 5.5
X-rays	12.4 – 14.2	1.55 – 1.75
AET + X-rays[b]	12.4 – 22.3	1.55 – 2.6
IP + X-rays[b]	12.85 – 22.0	1.6 – 2.6
SM + X-rays[b]	11.5 – 20.0	1.45 – 2.3

a) The results are the limit values of four viscosity measurements, two for each DNA sample obtained from the two groups of animals used in each experiment.
b) Control groups were only injected with AET, IP or SM; the spleen DNA molecular weight of these animals was about 5 x 10^6 daltons.

Discussion

The effect of protection against radiation injury in living animals produced by AET /4/, LU /16/, SM /9/ and IM /17/ has been well established. It was also demonstrated that solutions of DNA /8/ and the DNA from cell cultures /10/ is relatively well protected against ionizing radiations in the presence of radioprotectors. On the other hand, it was shown that AET had no effect, at least 48 hours, on the weight loss of spleen, small intestine, thymus, total white-cell count, marrow count and body-weight of mice irradiated with 900 R /2/. From the data included in Tab. 1 it is evident that the elimination of the spleen deoxyribopolynucleotides in mice irradiated with 300 R is not protected by administration of radioprotectors. This means that the radioprotective agents used in our experiment do not protect the spleen cells against pycnotic death which already occurs within the short interval of several hours after moderate doses of radiation. The administration of radioprotectors has no effect either on the fragmentation of spleen DNA 24 hours after irradiation with 1000 R (Tab. 2). The results found in this experiment support a previous assumption /2/ that unknown recovery mechanisms are protected

by administration of radioprotective agents and in this manner lethality is reduced. Since the number of hematopoetic stem cells is very low and their localization is unknown /5/ a direct evaluation of a radioprotective effect on DNA from this cells is impossible. However, it is well known that few days after irradiation under protection with radioprotective agents the weight of hematopoetic and lymphatic organs increases as compared with the unprotected animals and finally an increased survival of the protected animals is observed.

References

/1/ BAUER, R.D., LORING, W., KURNIK, N.B., Radiation Res. *26* (1965) 507
/2/ DAQUISTO, M.P., ROTHE, W.E., BLACKBURN, E.W., Int. J. Rad. Biol. *4* (1961) 33
/3/ De FRANCE, P., Le PECQ, J.B., Path. Biol. *9* (1961) 2341
/4/ DOHERTY, D.G., BURNETT, W.T., Proc. Soc. Exptl. Biol. Med. *89* (1955) 312
/5/ DUPLAN, J.F., FUHRER, J., Compt. Rend. *160* (1966) 1142
/6/ EIGNER, J., DOTY, P., J. Mol. Biol. *12* (1965) 549
/7/ EIGNER, J., SCHILDKRAUT, C., DOTY, P., Biochim. Biophys. Acta *55* (1962) 13
/8/ KOLLMANN, G., SHAPIRO, B., MARTIN, D., Radiation Res. *31* (1967) 721
/9/ LAZÁNYI, A., URAY, Z., HOLAN, T., JAGAMOS, M., Naturwissenschaften *53* (1966) 502
/10/ SANTUCCI, F., LEDOUX, L., Compt. Rend. *157* (1963) 1127
/11/ SKALKA, M., MATYÁŠOVÁ, J., J. Intern. J. Rad. Biol. *7* (1963) 41
/12/ SKALKA, M., MATYÁŠOVÁ, J., CHLUMECKÁ, V., Fol. Biol. *11* (1965) 113
/13/ SKALKA, M., MATYÁŠOVÁ, J., Fol. Biol. *11* (1965) 378
/14/ SUCIU, D., MUSTEA, I., Bull. Soc. Chim. Biol. *52* (1970) 1123
/15/ SUCIU, D., URAY, Z., MUSTEA, I., studia biophysica *20* (1970) 131
/16/ URAY, Z., FARCASAN, M., MANIU, M., Agressologie *11* (1970) 357
/17/ URAY, Z., HOLAN, T., Naturwissenschaften *53* (1966) 552

Введение рентгеночувствительных агентов /АЕТ, имипрамин, сульфометазин или лувatрен/ не влияет на выделение дезоксирибополинуклеотидов из селезенки в результате облучения 300 р. фрагментация ДНК из селезенки через 24 часа после облучения 1000 р также является незащищенной вследствие введения рентгеночувствительных агентов.

Eingegangen am 18.10.73
Dr. D. SUCIU, Institutul Oncologic Cluj, Str. Republicii Nr. 34 - 36, Cluj, Romania