AT8300393

OEFZS BER. No. 4235 BL-432/83 JULI 1983

Österreichisches Forschungszentrum Seibersdorf GesmbH

Unscheduled DNA synthesis in spleen cells of mice exposed to low doses of total body irradiation

Helga Tuschl
Rozika Kovac
Elisabeth Hruby

OEFZS Ber.No.4235 BL-432/83 Juli 1983

UNSCHEDULED DNA SYNTHESIS IN SPLEEN CELLS OF MICE EXPOSED TO LOW DOSES OF TOTAL BODY IRRADIATION

Helga TUSCHL
Rozika KOVAC
Elisabeth HRUBY

Arbeitsbericht
Proj.Nr.11208

Österreichisches
Forschungszentrum Seibersdorf
Ges.m.b.H.
Lenaugasse 10 A-1082 Wien

INSTITUT FÜR BIOLOGIE
Forschungszentrum Seibersdorf

UNSCHEDULED DNA SYNTHESIS IN SPLEEN CELLS OF MICE EXPOSED TO LOW DOSES OF TOTAL BODY IRRADIATION

SUMMARY

Unscheduled DNA synthesis was induced by UV irradiation of spleen cells obtained from C 57 Bl mice after repeated total body irradiations of 0.05 Gy ^{60}Co (0.00125 Gy/mice) and determined autoradiographically. An enhancement in the ability for repair of UV induced DNA lesions was observed in cells of gamma irradiated animals. While the amount of ^{3}H-thymidine incorporated per cell was increased, the percentage of labeled cells remained unchanged. The present results are compared with previous data on low dose radiation exposure in men.

KEY WORDS

DNA repair, low radiation doses, chronic radiation exposure, spleen cells, mice.

UNPROGRAMMIERTE DNA SYNTHESE IN MILZZELLEN VON C 57 BL MÄUSEN NACH CHRONISCHER BESTRAHLUNG

ZUSAMMENFASSUNG

Die unprogrammierte DNA Synthese wurde in den Milzzellen von C 57 Bl Mäusen nach Ganzkörperbestrahlung mit 0,05 Gy/d ^{60}Co autoradiographisch bestimmt. In den Zellen der gammabestrahlten Tiere konnte eine Zunahme der Fähigkeit zur Reparatur von UV Schäden der DNA beobachtet werden. Während der ^{3}H-Thymidineinbau pro Zelle signifikant erhöht war, blieb die Gesamtzahl der markierten Zellen unverändert. Die vorliegenden Tierexperimente werden mit früheren Untersuchungen an beruflich strahlenexponierten Personen verglichen.

SCHLÜSSELWORTE

DNA Reparatur, niedere Strahlendosen, chronische Bestrahlung, Milzzellen, Mäuse.

UNSCHEDULED DNA SYNTHESIS IN SPLEEN CELLS OF MICE EXPOSED TO LOW DOSES OF TOTAL BODY IRRADIATION

H. TUSCHL, R. KOVAC, E. HRUBY
Institut für Biologie, Forschungszentrum Seibersdorf,
A-2444 Seibersdorf, Austria

INTRODUCTION

Repeated exposure to various doses of gamma radiation or chronic inhalation of ^{222}Rn proved to exert a pronounced effect on the ability of peripheral lymphocytes to repair damage induced by a second insult, e.g. UV irradiation or Mitomycin C treatment (Tuschl et al.,1980a; Tuschl et al.,1980b; Tuschl et al., 1983). Persons regularly descending to the radioactive gallery of Badgastein (Austria) or employees of the Austrian Research Centre Seibersdorf exposed to low levels of ionizing radiation showed an enhanced UV induced unscheduled DNA synthesis (= UDS), when compared with unexposed controls (Tuschl et al., 1980b; Tuschl et al., 1983). On the other hand, the number of Mitomycin C induced sister chromatid exchanges decreased with exposure dose (Tuschl et al., 1983), again indicating an increase in the ability for the repair of DNA lesions.

Since interpretations of results obtained with studies in men cannot wholly exclude e.g. differences in life-long habits,

non-registered radiation exposure or contamination with chemical mutagens, animal studies were performed to ascertain the above mentioned data on radiation effects in men.

MATERIALS AND METHODS

C 57 Bl mice were used for the experiments. One group of mice were irradiated with a daily dose of 5 rad (0.125 rad/min) on four consecutive days, the other group was left without irradiation. Irradiation was performed by a Co^{60} facility, delivering a dose rate of 14 krad/hr. Two, 10 and 21 days after irradiation animals were sacrificed and the spleens removed asepticically. Spleen cell suspensions of 10^7 cells/ml were prepared in PBS, and after several washings one half of samples were irradiated at 254 nm UV at an incident dose rate of 1 J/m^2 sec for 20 sec. After irradiation ^3H-thymidine was added (10μCi/ml, spec.act. 80 Ci/mMol) and samples incubated at 37°C for 90 min. In the second series of experiments besides measuring 90 min repair replication, also 3 hours' repair was studied: irradiated samples were incubated for 90 min without ^3H-thymidine and for further 90 min in the presence of the radioactive precursor. After repair incubation, excess cold thymidine was added and cells prepared for autoradiography. Autoradiography was carried out with Kodak NTB 3 liquid emulsion.

Evaluation of autoradiograms

Unirradiated samples were used for the determination of S phases and background repair replication, due to accidental exposure or the foregoing total body irradiation. Such "background"

UDS could not be detected with the method used. For the measurement of UDS induced by in vitro UV irradiation, the number of labeled cells was determined by visual counting, while the incorporation of ^{3}H-thymidine per cell was calculated by the photometrical determination of the relative reflexion of silver grains using a Zeiss MPH 1 (values are arbitrery units of reflexion of silver grains).

RESULTS AND DISCUSSION

To determine the interindividual variation of UDS among the animals used, in our first experiment UDS was measured in four completely untreated mice (Table 1): no marked inter-individual variation was demonstrated.

Table 1: UDS in spleen cells of C 57 Bl mice after 20 J/m² UV.

animal no.	rel.reflexion of silver grains	percentage of labeled cells
1	17.7	61
2	18.0	65
3	21.5	66
4	20.0	67
	m = 19.3 ± 1.8	m = 64.8 ± 2.6

In the first series of experiments, 90 min repair incorporation after 20 J/m² UV irradiation of spleen cells was evaluated two and 10 days after total body gamma irradiation of 4x5 rad.

In both experiments (table 2 and 3), a significant increase in the amount of ^{3}H-thymidine incorporated during the first 90 min after UV irradiation was observed in gamma irradiated mice.

Table 2: UV induced UDS in spleen cells of C 57 Bl mice, two days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel. reflexion of silver grains/cell	percentage of labeled cells	percentage of S-phases
irradiated	1	11.8	70	2.5
	2	15.7	66	2.5
	3	16.9	70	2.7
	4	16.0	64	4.0
	5	14.1	42	3.2
		m = 14.9[a]	m = 62.4	m = 3.0
		s.d. = ±2.0	s.d. = ±11.7	s.d. = ±0.6
controls	1	13.1	63	2.5
	2	12.6	52	2.0
	3	10.0	37	3.0
	4	10.8	45	1.5
	5	11.6	37	3.0
		m = 11.6[a]	m = 46.8	m = 2.4
		s.d. = ±1.3	s.d. = ±11.0	s.d. = ±0.7

a) two tailed T-test: $P < 1.5\%$, difference significant

Table 3: UV induced UDS in spleen cells of C 57 Bl mice, 10 days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel. reflexion of silver grains/cell	percentage of labeled cells	percentage of S-Phase
irradiated	1	12.7	57	3.0
	2	11.3	53	3.2
	3	11.3	56	4.2
	4	11.6	46	4.2
	5	12.4	49	6.5
		m = 11.9[a]	m = 52.2	m = 4.2[b]
		s.d. = ±0.6	s.d. = ±4.6	s.d. = ±1.4
controls	1	9.3	52	2.2
	2	9.0	40	2.2
	3	12.0	57	2.5
	4	9.4	58	2.0
	5	-	-	-
		m = 9.9[a]	m = 52.2	m = 2.2[b]
		s.d. = ±1.4	s.d. = ±8.3	s.d. = ±0.2

a) two tailed T-test: $P < 5\%$, difference significant

b) two tailed T-test: $P < 0.5\%$, difference highly significant

In the second series carried out with a new delivery of mice, two days after 4x5 rad, the difference of repair capability was statistically not significant, though a higher incorporation was found in spleens of irradiated animals (table 4).

Table 4: 90 min UV induced UDS in spleen cells of C 57 Bl mice, two days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel. reflexion of silver grains/cell	percentage of labeled cells	percentage of S-phase
irradiated	1	19.2	60	5.7
	2	20.9	61	-
	3	19.2	69	7.2
	4	17.3	61	3.2
	5	24.3	64	2.7
		m = 20.2[a]	m = 63.0	m = 4.7[b]
		s.d. = ±2.6	s.d. = ±3.7	s.d. = ±2.1
controls	1	17.0	65	1.7
	2	23.0	58	2.0
	3	19.2	70	1.0
	4	16.0	59	1.2
	5	17.2	60	-
		m = 18.5[a]	m = 62.4	m = 1.5[b]
		s.d. = ±2.8	s.d. = ±5.0	s.d. = ±0.5

a) two tailed T-test: $P > 5\%$, difference not significant

b) two tailed T-test: $P < 5\%$, difference significant

Ten days later UDS was markedly enhanced by the repeated total body irradiations (table 5).

Table 5: 90 min UV induced UDS in spleen cells of C 57 Bl mice 10 days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel. reflexion of silver grains/cell	percentage of labeled cells	percentage of S-phases
irradiated	1	23.0	59	5.0
	2	22.1	61	2.5
	3	21.5	58	2.0
	4	17.4	57	5.7
	5	21.9	57	1.5
		m = 21.2 [a]	m = 58.4	m = 3.3
		s.d. = ±2.2	s.d. = ±1.7	s.d. = ±1.9
controls	1	16.7	57	8.5
	2	-	-	2.5
	3	15.3	57	2.0
	4	14.7	64	3.2
	5	15.0	57	1.7
		m = 15.4 [a]	m = 58.8	m = 3.6
		s.d. = ±0.9	s.d. = ±3.5	s.d. = ±2.8

[a] two tailed T-test: $P < 0.5\%$, difference highly significant

After a further period of ten days, no effect of gamma irradiation on the repair capability of spleen cells was observed (table 6).

Table 6: 90 min UV induced UDS in spleen cells of C 57 Bl mice 21 days after 4x5 rad, and in spleens of un-irradiated controls.

	animal no	rel.reflexion of silver grains/cell	percentage of labeled cells	percentage of S-phases
irradiated	1	32.2	69	2.7
	2	22.6	65	2.7
	3	-	-	-
	4	19.6	58	3.5
	5	22.0	70	6.0
		m = 24.1[a]	m = 65.5	m = 3.7
		s.d. = ±5.5	s.d. = ±5.4	s.d. = ±1.6
controls	1	15.6	61	2.7
	1	16.6	67	4.5
	3	15.3	69	-
	4	20.4	65	3.2
	5	21.5	69	4.2
		m = 17.9[a]	m = 66.2	m = 3.7
		s.d. = ±2.9	s.d. = ±3.3	s.d. = ±0.8

a) two tailed T-test: $P > 5\%$, difference not significant

The extremely high incorporation of ^{3}H-thymidine in animal No 1 seems to be an outlier - it might be caused e.g. by some kind of viral infection (Nishiyama et al., 1981) or any other insult of the immune system, leading to the production of juvenile cells (Spiegler et al., 1969).

When spleen cells were incubated for 3 hours after UV irradiation (tab. 7, 8), no marked difference between irradiated and unirradiated mice could be observed, thus indicating that repair replication might be enhanced only in the "fast" repair process of UV induced DNA damage.

Table 7: 180 min UV induced UDS in spleen cells of C 57 Bl mice two days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel.reflexion of silver grains/cell		animal no	rel.reflexion of silver grains/cell
irradiated	1	11.3	controls	1	12.0
	2	14.8		2	12.8
	3	-		3	12.6
	4	11.8		4	12.4
	5	13.7		5	14.8
		m = 12.9 [a]			m = 12.9 [a]
		s.d. = $\pm$1.6			s.d. = $\pm$1.1

a) two tailed T-test: $P > 5\%$,
difference not significant

Table 8: 180 min UV induced UDS in spleen cells of C 57 Bl mice 10 days after 4x5 rad, and in spleens of unirradiated controls.

	animal no	rel.reflexion of silver grains/cell		animal no	rel.reflexion of silver grains/cell
irradiated	1	17.9	controls	1	18.0
	2	16.1		2	-
	3	18.1		3	17.4
	4	18.2		4	17.9
	5	20.9		5	16.7
		m = 18.2[a]			m = 17.8[a]
		s.d. = ±1.7			s.d. = ±0.6

a) two tailed T-test: $P > 5\%$, difference not significant

This "fast" process is known to be linear to incubation time and not rate-limited, while the "slow" UV repair (3-18 hours after irradiation) is rate-limiting. These data very well correlate with results obtained on the measurement of UV induced ^{3}H-thymidine incorporation into the chromatin of ^{222}Rn exposed probands (Klein et al., 1983): During the first five minutes after UV irradiation an enhanced uptake of ^{3}H-thymidine into core and spacer region of chromatin was observed in lymphocytes of exposed persons; three hours later no difference between exposed and unexposed controls could be found.

In general, the results of the present investigations very well agree with previous experiments on ^{222}Rn and gammaexposed

persons (Tuschl et al., 1980b; Tuschl et al. 1983). Furthermore, they indicate that the enhancement of UV induced UDS is really attributable to the radiation exposure, and cannot be ascribed to other parameters of individual life-habits or individual genetic dispositions for the repair of DNA damage.

As already discussed with these previous experiments on exposed persons (Tuschl et al., 1980b; Tuschl et al., 1983), the observed enhancement of UV induced UDS by protracted irradiation could be due to some kind of "adaptation" process, an inducible DNA repair, known to occur in bacteria and mammalian cells after treatment with alkylating agents (Karran et al., 1982; Kaina, 1982). Furthermore, a number of co-factors is necessary to dissociate DNA from histones and to render it susceptable to nuclease action. One of these co-factors may be poly(ADP-ribose), modifying the supercoiling of DNA. The most effective activators of poly(ADP-ribose)polymerase are DNA strand-breaks. The latter could be expected to arise by repeated gamma irradiation. In ^{222}Rn persons (Altmann, 1980) poly(ADP-ribose)synthesis was found to differ in its amount from unexposed controls.

No statistically significant difference in the percentage of labeled cells, i.e. cells carrying out UDS, could be observed with exposed and unexposed mice. In some cases total body irradiation led to an increase in the number of S-phases; this increase could not be correlated with the total amount of UDS in the animal concerned (e.g. tab. 4 - animal no 3). Furthermore, similar increases in S-phase numbers were obtained with untreated mice (e.g. tab. 5 - animal no. 1). But

from the present results it cannot be ruled out that a shift within the lymphocytic cell population in favour of juvenile cells, having higher repair capacity, could contribute to the enhancement of UDS (Spiegler et al., 1969), although it does not seem very likely to be the only cause for such an enhancement. Rosetting tests of peripheral lymphocytes of occupationally exposed persons did not reveal any difference of B/T cell ratios after low gamma doses (Tuschl et al., 1983). Since gamma doses used in the present investigations were much higher, further experiments will have to clarify a possible involvement of different lymphocyte subpopulations in the observed changes of DNA repair capability.

In any case, the present study demonstrates that repeated exposure to low doses of ionizing radiation can modify the ability to repair DNA lesions induced by a second insult, e.g. UV irradiation. This increase in repair efficiency seems to be maximum about 10 days after the last total body irradiation and decreases to normal values three weeks post irradiation.

REFERENCES

ALTMANN, H., 1980, Z.Phys.Med. 6, 314.

KAINA, B., 1982, Mut.Res. 93, 195.

KARRAN, P., STEVENS, S. and SEDGWICK, B., 1982, Mut.Res. 104, 67.

KLEIN, W., KOCSIS, F. ALTMANN, H., GÜNTHER, R., EGG, D. and SANDRI, B., 1983, OEFZS-Bericht No. A0379, BL-409/83.

NISHIYAMA, Y. and RAPP, E., 1981, Virology 110, 466.

SPIEGLER, P. and NORMAN, D., 1969, Radiat.Res. 39, 400.

TUSCHL, H. and KOVAC, R., 1980, DNA-Repair and Late Effects (Publ. NRC Israel) 305.

TUSCHL, H., ALTMANN, H., KOVAC, R., TOPALOGLOU, A., EGG, D. and GÜNTHER, R., 1980, Radiat.Res. 81, 1.

TUSCHL, H., KOVAC, R. and ALTMANN, H., 1983, Health Physics (in press).

OEFZS-Berichte
Verleger, Herausgeber und Hersteller:
Österreichisches Forschungszentrum Seibersdorf Ges.m.b.H.
Redaktion: Univ. Prof. Dr. Peter KOSS,
alle Lenaugasse 10, 1082 Wien, Tel. (0222) 42 75 11, Telex 7-5400.

Für diesen Bericht behalten wir uns alle Rechte vor.