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In vitro and in vivo effects of low dose HTO contamination modulated by dose rate

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Abstract
The experiment performed *in vitro* intended to examine whether an adaptive response could be elicited on lymphocytes by low-level contamination of whole blood with tritiated water and if the modification of the dose rate has any influence on it. Lymphocytes pre-exposed to ^{3}HOH (0.2 - 6.6 MBq/ml) and subsequently irradiated with 1 Gy γ-rays showed micronuclei frequency significantly lower (40% - 45%) than the expected number (sum of the yields induced by ^{3}HOH and γ-rays separately). The degree of the radioresistance induced by HTO pre-treatments became higher with decreasing dose-rate for a rather similar total adapting dose.
In vivo, the aim of the study was to investigate if different dose rates are inducing modulation of the lipid peroxidation level and of the thymidine uptake in different tissues of animals contaminated by HTO ingestion. The total doses varied between 5 and 20 cGy and were delivered as chronic (100 days) or acute contamination (5 days). It was observed that only doses about 20 cGy caused a dose-rate dependent increase of the lipid peroxidation level in the tissues of small intestine, kidney and spleen.
Both chronic and acute contamination did produce reduced incorporation of thymidine in the cells of bone marrow. The most effective decrease of thymidine uptake was induced by the acute contamination in the lower dose domain (≡ 5 cGy). Our hypothesis is that in this dose domain the modification of thymidine uptake could be due to changes at the level of membrane transport.

Introduction

The interest about possible ^{3}H induced health detriments corresponding to low dose irradiation domain is justified by the use of nuclear power as well as by the ongoing research on fusion reactor technology. During the past decade a large number of radiobiological studies have become available for ^{3}H - most of them focusing on the RBE of tritium beta rays [1]. A point of interest concerning the tritium induced low-level effects came from the research on the adaptive response of mitogen stimulated human lymphocytes to low-LET radiation exposure [2].

The present paper reports the results of two different types of experiments: one is referring to *in vitro* induced effects on human lymphocytes while the other one is referring to *in vivo* effects observed on internally contaminated animals.

The experiment performed *in vitro* examined the question whether adaptive response can be elicited by low-level irradiation from tritiated water (HTO) and can be influenced by the modification of the dose rate. The response induced by the pre-treatment of the human lymphocytes with HTO of different specific ^{3}H activities and for different time intervals was subsequently provoked by acute gamma irradiation of the cells. The frequency of micronucleus induction, used as biological end-point, was evaluated after culturing the whole blood.

The aim of the *in vivo* study was to investigate if different dose rates are inducing any modulation in the lipid peroxidation level or in the thymidine intake level in some tissues of animals contaminated by HTO ingestion.

Materials and methods

Part of the work, i.e. the experiment performed in vitro, has used human blood samples, taken from healthy young male donors. HTO diluted with RPMI-1640 medium was added to whole blood samples to achieve final ^{3}H levels ranging from 0.2 to 6.6 MBq/ml of plasma, which correspond to irradiation dose rates from 0.05 to 1.7 cGy/h. The incubation times varied between 1-24 h in order to obtain similar total doses, with different dose rates. Exposure doses were evaluated by measuring the

^3H in the supernatant of the first centrifugation for washing the cells and by taking also into account the incubation time and a value of 0.8 for the correction factor for the water content of the cells [3].
The cells were subsequently irradiated with 1 Gy of γ-rays (0.476 Gy/min) from a Gammatron-3 equipment. Immediately after the challenging irradiation, microcultures were set up and 72h later cytokinesis-blocked (CB) binucleated lymphocytes [4] were scored for micronucleus frequency [5]. The results were expressed as the average number ± SD of micronucleated cells per thousand of CB-cells.
The experiment designed to evaluate in vivo effects of tritium low dose contamination was conducted on Dowley-Sprague rats, devised in 5 groups of 10 animals each; on the whole duration of the experiment (100 days) they received standard food and as drinking water, some groups received tap water (groups M, C and D), while the other groups received tritiated water (groups A and B), with specific activity in the range of 9.25 kBq/ml and 37 kBq/ml. Group M were used as nonirradiated controls. Animals from groups C and D were subjected to acute irradiation by single intraperitoneal injection with HTO (41.6 MBq/animal and respectively 166.5 MBq/animal), and sacrificed 5 days later.
The dose evaluation was made by a combined procedure, using an equation reported in the literature for tritium retention in rats [6] and also using experimental values for the final tritium specific activity, measured by us in the blood plasma of the animals.
At the end of the exposure. the animals have been sacrificed, collecting liver, kidney, spleen, small intestine and bone marrow samples.
The amount of lipid peroxides was estimated in liver, kidney, spleen and small intestine by the thiobarbituric acid (TBA) reaction [7]; the results have been expressed as quantity of malondialdehyde (MDA) per tissue mass (nmol MDA/g of tissue).
The bone marrow samples were collected in TC-199 culture medium and cell suspensions were prepared
with a cell density of 10^7 cells/ml. After repeated washings of cells, the thymidine intake test was performed by incubating the cells (normal or pre-treated with KCN) at 37°C for 15 min with ^{3}HTdR with specific activity of 0.4 μCi/ml. The uptake in whole cell material was evaluated by liquid scintillator measurements and it was expressed as dps/10^7 cells. In order to compare the modifications observed in our experiment for the different aspects under investigation, a normalisation of the data have been operated; there were calculated for each type of treatment ratios relative to the unirradiated control, denominated as "arbitrary units".
The statistical significance of the differences observed between irradiated and nonirradiated groups was assessed by Student's *t* test.

Results

Lymphocytes exposed in vitro to ^{3}HOH, to doses in the range of 1-10 cGy (intervals of 1-24 hours and specific activities of 0.2-6.6 MBq/ml) and subsequently irradiated with 1 Gy γ-rays (in the G_0 phase of their cellular cycle) showed micronuclei frequency significantly lower than the sum of the yield induced by ^{3}HOH and γ-rays separately. The frequency of micronuclei induced by ^{3}HOH alone remained similar to that of the non-irradiated control within the whole range of specific activities used in this experiment (Fig.1). Even doses of 10 cGy received by lymphocytes exposed for 24 hours to ^{3}H specific activity of 2 MBq/ml (0.5 cGy/h) were proved to be statistically ineffective for micronucleus induction The reduction of micronuclei induction appears to decrease with the increase in ^{3}H specific activity, for total conditioning doses between 1 - 2 cGy (Fig.1). We did observe individual variability in the responses of micronucleus induction either after HTO treatments, after 1 Gy γ-irradiation or concerning the amplitude of the adaptive reduction of micronucleus frequency. However, adaptive responses were positively observed for all 4 investigated donors .

The results obtained in the evaluation of the malondialdehyde content in tissues homogenates are summarised in Table I for both types (chronic or acute) of in vivo contamination with tritium.
In the case of liver and kidney, significant increase of lipoperoxides content was observed for contamination situations corresponding to total exposure doses of 20 cGy. The amplitude of the effect was higher at higher dose-rate. The spleen did respond the most sensitively to the investigated dose domain. A significant increase of the peroxide level was induced by very low dose rates (0.04 cGy/day), for total doses of 5 cGy; the same type of modification was observed for the other contamination situations and a dose-rate dependency was

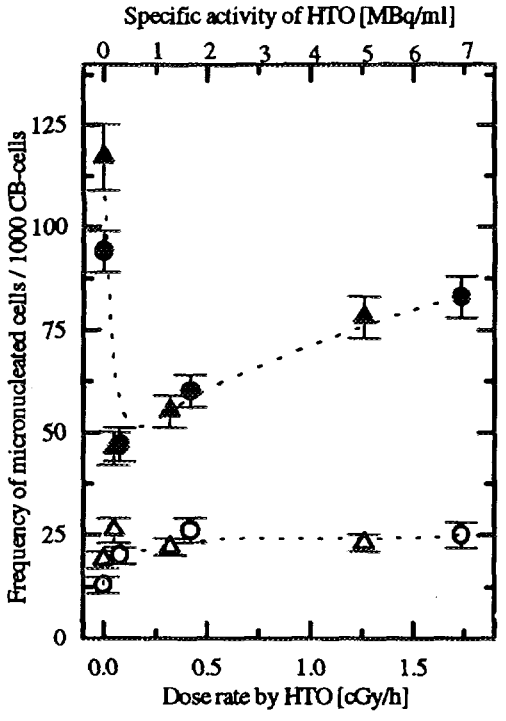

Fig. 1: Adaptive response: The relationship between the frequency of micronucleus induction and the dose-rate of the priming irradiation by HTO: o, Δ - HTO; o, Δ - HTO + 1 Gy γ - ray

noticed at doses of 20 cGy, the increase of the peroxide level reaching 300%. Similar sensitive response was observed in the case of the small intestine but the addition of a dose-rate dependency on the radioinduced effect was less pregnant

The results of the ^{3}HTdR incorporation assay, performed in vitro on bone marrow cells previously irradiated in vivo by HTO, are presented in Table II.

A significant decrease of TdR incorporation in KCN treated cells was shown for low total doses as 5 cGy; this decrease is larger at the higher dose rate. For larger dose, at 20 cGy, the decrease of the thymidine uptake is no more observed.

Alternatively, on normal cells (non-treated with KCN), a modification in the level of the ^{3}HTdR incorporation is observed for all the dose range. This parameter is also sensitive to the dose rate.

The decrease of thymidine uptake in the cell could be due to the radiation induced modifications of membranes, of enzymes which catalyse the subsequent steps until thymidine enters into DNA or to the modification of the mechanism of DNA synthesis.

Table I. Effect of chronic and acute irradiation by HTO contamination on peroxide content of different tissues (nmol MDA/g tissue)

Exp. group Tissue	M (control)	A (4.2 cGy) 0.04 cGy/day	B (19.4 cGy) 0.19 cGy/day	C (5 cGy) ~1 cGy/day	D (20 cGy) ~ 4 cGy/day
Liver	8.01 ± 0.74	9.32 ± 0.86	15.69 ± 1.43 p_M=0.0004	10.30 ± 1.56	17.58 ± 1.12 p_M=0.00001
Kidney	16.52 ± 1.54	20.47 ± 3.15	22.19 ± 0.94 p_M=0.002	21.53 ± 2.39	31.94 ± 3.17 p_M=0.0009
Spleen	32.97 ± 1.59	50.58 ± 4.17 p_M=0.002	53.34 ± 4.48 p_M=0.001	52.94 ± 4.14 p_M=0.0007	108.95 ± 8.80 p_M=1*10^{-6}
Small intestine	20.43 ± 2.91	31.45 ± 3.86 p_M=0.04	26.72 ± 2.42	31.26 ± 1.99 p_M=0.001	35.10 ± 4.07 p_M=0.01

Table II. Effect of chronic and acute irradiation by HTO contamination on ^{3}HTdR uptake in bone marrow cells (the data expressed as "arbitrary units")

Exp. group	M (control)	A (4.2 cGy) 0.04 cGy/day	B (19.4 cGy) 0.19 cGy/day	C (5 cGy) ~1 cGy/day	D (20 cGy) ~4 cGy/day
KCN treated cells	1.00 ± 0.14	0.50 ± 0.10 p_M=0.018	0.91 ± 0.13	0.36 ± 0.12 p_M=0.007	0.92 ± 0.04
Normal cells	1.00 ± 0.07	0.73 ± 0.06 p_M=0.014	0.62 ± 0.05 p_M=0.001	0.53 ± 0.05 p_M=0.0003	0.75 ± 0.06 p_M=0.026

Discussion

The present results are sustaining the assumption that the micronucleus test, which represent a suitable indicator of the frequency of chromosomal aberration induced by ionising radiation, can be used also for the adaptive response assay, in good agreement with some previous reported results [8].Our experimental observations seem to demonstrate the possibility of inducing the adaptive response by small doses of β-irradiation delivered on G_0 lymphocytes, as long as the exposure occurred in the whole blood environment.

An important observation of our study is that the dose rate of the adapting irradiation, modulated by the level of tritium specific activity have some influence on the yield of micronuclei induction. The degree of the radioresistance induced by HTO pre-treatments became higher with decreasing dose-rate for a rather similar total adapting dose. However, the relative yield between observed and expected number of micronuclei decreased only until 40%-45%. This limit is already reached at dose rate of 0.3 cGy/h.

The interest of the peroxide level evaluation in the spleen and small intestine was suggested by their sensibility for the expression of radiological effects especially in the low dose domain [9]. Indeed our results showed a significant increase of the peroxide level for the lowest dose used (5cGy) only for the tissue homogenates of spleen and small intestine.. For doses of 20 cGy the effects were observed to occur for all the studied tissues and also to be dose-rate dependent: at higher dose rates the level of peroxides is significantly additionally increased.

Summing up, our study put into evidence only an increase of the lipoperoxide level due to HTO contamination., for the dose domain of 5 - 20 cGy and for dose rate range of 0.04 - 4 cGy/day. In most of the cases the effect is monotonically associated with the dose and the dose rate increase.

The test of DNA precursors incorporation in bone marrow was already previously used to characterise the biological response to low dose irradiation, by Feinendegen research group [10]. Our results showed that for chronic irradiation by HTO contamination in the low dose domain, the modification of the thymidine uptake could be due to changes at the level of membrane transport.
For the higher dose, the inhibition of the uptake could be due to a decreased activity of the thymidine-kinase, as it was suggested in the literature. Both phenomena are, most probably part of the same process of metabolic defence, triggered in the hit cells by low dose and low dose-rate irradiations. This mechanism might involve different steps, and their triggering could require different intensities of irradiation stress.

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