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Short Communication

Radiation induced oxidative DNA base damage and its repair in liver chromatin DNA of rats upon whole body γ-irradiation*

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The amount of all bases, except for 5,6-dihydroxyuracil were significantly increased in rat DNA upon cobalt-60 γ-irradiation. Control values were recovered 12 h after irradiation. The extent of DNA damage and repair was different for particular bases.

Ionizing irradiation produces different kinds of DNA lesions, among them free radical modified DNA bases. Base damage appears to be an important class of lesions since some of them may possess mutagenic properties [1–5].

The biological relevance of these lesions may depend on the efficiency of their repair. In the present work, we examined the formation and repair of DNA base damages induced by γ-irradiation in rat hepatic chromatin.

Male Wistar rats weighing 200–220 g were used. The animals were exposed *in vivo* to γ-rays using a cobalt-60 γ-source. The irradiation dose applied, 10 Gy, was not lethal. The rats were killed immediately (within 2 min), 1, 12, and 24 hours after irradiation. The livers were removed, frozen in liquid nitrogen and stored at –80°C until isolation of nuclei according to Lilja *et al.* [6] and of chromatin by the modified procedure of Mee & Adelstain [7] as previously described [8]. The chromatin was characterized as described by Gajewski *et al.* [8].

Each sample contained 100 μg of DNA (as determined by UV measurement), and internal standards as described by Dizdaroglu [9]. The samples were lyophilized and hydrolyzed with 0.5 ml formic acid in evacuated and sealed tubes for 30 min at 140°C [9]. After hydrolysis and derivatization, samples were analyzed by gas chromatography/isotope-dilution mass spectrometry with selected ion-monitoring (SIM) as described by Dizdaroglu [9].

We analyzed endogenous amounts of modified bases (control samples) as well as the level of the same modifications in samples isolated after whole body irradiation, at different time intervals after the treatment. The following modified bases were identified and quantified: 5-hydroxyhydantoine (5OHHyd), 5-hydroxycytosine (5OHCyt), thymine glycol (Thy-Gly), 5-hydroxymethyluracil (5OHMeUra), 5,6-dihydroxyuracil (5,6-diOHUra), 4,6-diamino-5-formamidopyrimidine (FapyAde), 8-hydro-

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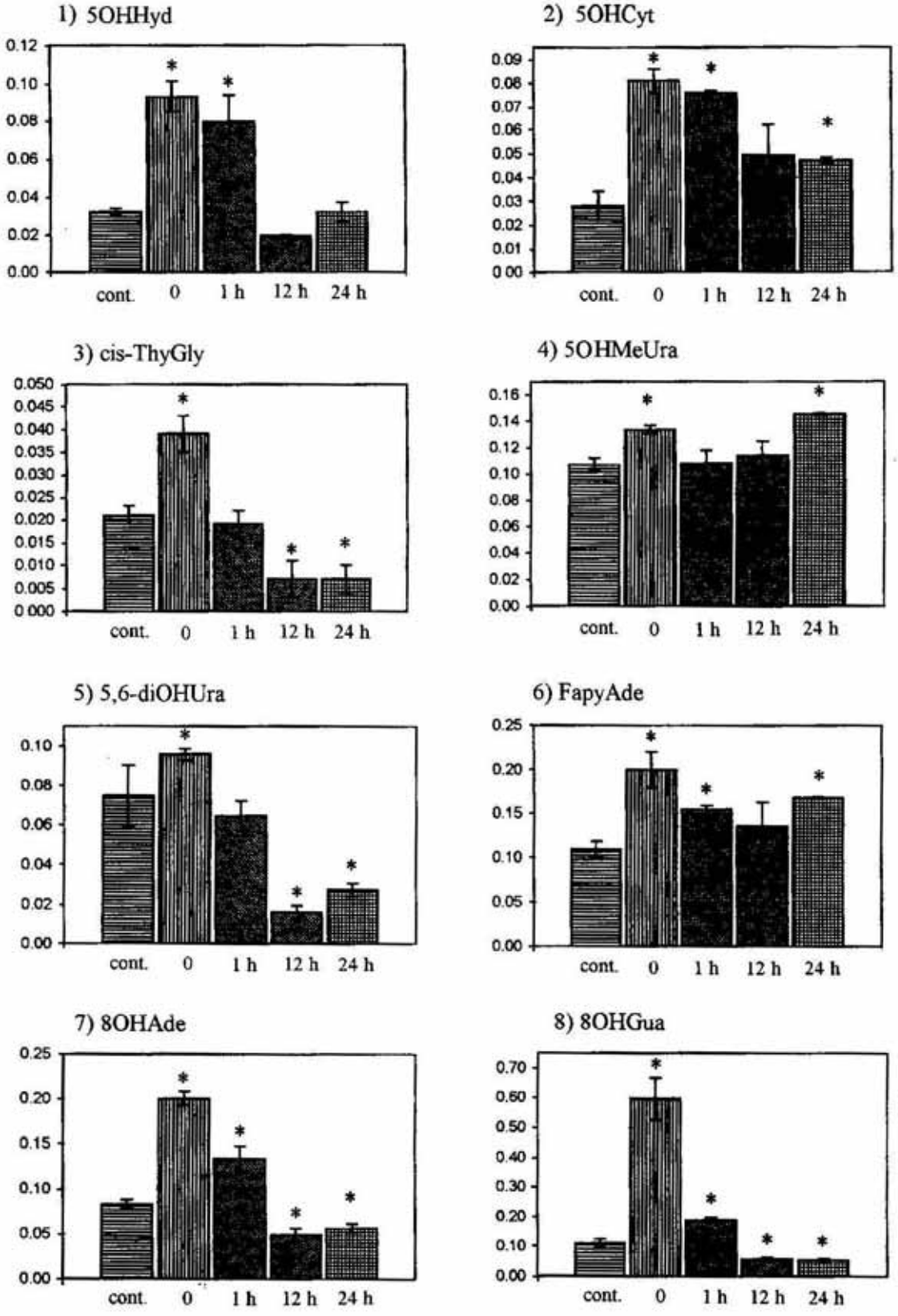

Fig. 1. Amounts of modified DNA bases (nmol of modified base/mg DNA) in whole chromatin and nuclear matrix DNA.

One nmol of a modified DNA base/mg of DNA = 32 modified bases/10^5 DNA bases. Stars above the bars indicate that the result is significantly different ($P < 0.05$ by Student's *t*-test) from the control value. Note the differences in scale for particular bases.

xy-adenine (8OHAde), and 8-hydroxyguanine (8OHGua). The results are illustrated in Fig. 1.

Damages of DNA bases induced by γ-irradiation are produced largely through oxidation of cellular DNA by free radicals which are generated as a result of radiolysis of cellular water [10–12]. Since the same type of free radicals is produced as a byproduct of oxidative metabolism in every aerobic organism, a background level of the modified bases can be detected in normal, non-irradiated cells. This level may simply represent a steady-state concentration reflecting the balance between formation and repair of these bases.

Mori *et al.* [13] were the first to report that all four bases in hepatic chromatin can be modified after whole body irradiation of mice. However, their investigations were focused on formation of these damages in chromatin. Therefore, in our study we examined both the formation and repair of DNA base damages induced by γ-irradiation in rat hepatic chromatin. Significant increases in the amounts of all the bases, except 5,6-diOHUra, over the control levels were observed in the samples isolated immediately after irradiation. The increases for particular bases differed from each other. The amount of most of the modified bases returned to the control value 12 hours after irradiation. However, the contents of 5OHCyt, 5OHMeUra and FapyAde in the chromatin were elevated over the control value even 24 h after irradiation. The decrease in base damages seems to point to removal of these damages from DNA by cellular repair. The observed differences between the removal of particular products could result from different kinetics of their repair.

Several enzymes that specifically recognize and remove modified bases analyzed in this work have been described [14–19]. However, little is known about repairability of individual modified bases in irradiated mammalian cells. To our knowledge so far the individual free radical-induced base damages have not been directly determined. However, results similar to those presented above were obtained by others using an indirect approach to determine base damage. By applying such an approach to analyze unspecific DNA base damage it was shown that, after irradiation of human white blood cells with a dose of 3 Gy, about 50% of the base damage was removed within 1.5 h [20]. Fornace [21] demonstrated that more than 50% of the base damage in human fibroblasts irradiated with 55 Gy, was removed after 1.5 h of repair incubation.

The level of some lesions (i.e. ThyGly, 5,6-diOHUra, 8OHAde, 8OHGua) dropped below the control value when they were analyzed 12 h and 24 h after irradiation. It is tempting to speculate that this phenomenon may be due to induction by γ-irradiation of the DNA repair enzymes which specifically remove modified bases. It is interesting that the most significant drop was observed for thymine glycol which is responsible for blocking replication and is likely to be lethal.

In conclusion, we have directly demonstrated, for the first time, the removal of free radical induced modifications of all four DNA bases generated by γ-irradiation in rat liver chromatin DNA after whole body irradiation.

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