Effects of Combined Treatment of Rats with Cadmium and Ionizing Radiation on Nucleic Acids in the Kidneys, Liver and Haemopoietic Organs

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Abstract. The influence of Cd (1 mg/rat $CdCl_2$ i.p.) and/or gamma radiation (6 Gy) on RNA and DNA content and/or concentration in the intact kidney and hypertrophic kidney (on the 44th hour after UN) and in other slowly and quickly proliferating organs was studied. The period between administration of Cd and Ir in the group with combined treatment was 30 min; between treatment (administration of Cd, Ir and combination of both treatments – Cd+Ir) and UN it was 1, 7, 14 and 21 days.

The total extent of damage caused by the treatments in the investigated organs was following: intact kidney < liver < hypertrophic kidney and bone marrow < spleen < thymus. In the intact and hypertrophic kidney and liver, the administration of Cd caused more extensive changes in comparison with gamma irradiation, and the effects of combination of the treatments were similar to those of Cd alone. In the bone marrow, spleen and thymus, more profound changes were observed after Ir in comparison with Cd administration, and the effects of combined treatment were similar to the effects of Ir alone. The changes in the hypertrophic kidney after administration of Cd and/or Ir were more extensive than in the intact kidney, which suggests latent injury induction in the rat kidney by these noxa. The higher effectiveness of the treatments in the hypertrophic kidney than in the intact one was manifested mostly by the decrease in the RNA and DNA content, which was mainly due to inhibition of growth induced by UN and not by a real decrease in DNA and RNA contents caused by loss of damaged cells.

Ionizing radiation (Ir) causes a number of chemical and structural changes of nucleic acids (e.g. chemical changes of nitrogen bases, breaks of one or two polynucleotid strands, cross-link formation), which are accompanied with a decrease in the DNA and RNA content in radiosensitive organs, and/or lead to the formation of various cytological, histological, physiological and especially genetical injuries (Bedford and Phil, 1991; Hall, 1997; Cornforth, 1998; Ward, 1998, and others). In comparison with Ir, the effects of heavy metals including cadmium (Cd) have been much less investigated, although Cd load in populations living in industrial European countries has increased multiply during the 20th century (Drasch, 1983; Bernard et al., 1991). Very high doses of Cd are lethal; sublethal doses of Cd cause, similarly to Ir, a wide variety of species-related and dose-related changes leading to mutagenesis, cancerogenesis, teratogenesis and other toxic effects (Nordberg, 1993; Fedoročko et al., 1996; Fasanyaodewumi et al., 1998; Tang et al., 1998).

During life Cd accumulates especially in the kidneys and liver, which are its target organs (Dudley et al., 1985; Bernard et al., 1991; Friedman and Gesek, 1994; Theocharis et al., 1996). Single-strand breaks of DNA (Latinwo et al., 1997; Saplakoglu et al., 1997) and fragmentation of DNA together with histopathological changes characteristic for apoptosis, which appear mainly in coiled proximal tubules, were detected in the rat kidneys after different manners of Cd administration (repeated administration by a gastric tube or single i.p. administration) (Yan et al., 1997; Ishido et al., 1998, 1999).

Although there is a potential for simultaneous exposure to both hazardous factors, the interactions of Cd and Ir effects are not well known. It has been reported that Cd administration 24 h before X-irradiation increased radioresistance of mice to lethal doses of radiation, probably due to induction of metallothionein synthesis (Matsubara et al., 1987). In addition to the reduction of lethal effects of radiation, administration of Cd 24 h before gamma irradiation also decreased the radiation injury to haemopoietic stem cells and accelerated the recovery processes in the bone marrow and spleen of irradiated mice (Fedoročko et al., 1996; Macková et al., 1996).

The principal aim of the present study was to evaluate the effects of these factors acting alone or in combination in the rat kidneys, when the time between Cd administration and Ir was too short (30 min) for induction of metallothionein production. As some latent injury to the kidneys, which manifests itself during cell proliferation induced by unilateral nephrectomy (UN), can arise (Otsuka and Meistrich, 1990), we have investigated not only the normal (intact) kidney but also the kidney hypertrophing after UN. For comparison we

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Abbreviations: Cd – cadmium administration, Ir – ionizing irradiation, UN – unilateral nephrectomy.

have simultaneously analysed the effects of Cd and/or Ir even to other radioresistant and radiosensitive organs – liver and bone marrow, spleen and thymus, respectively.

Material and Methods

Animals

Male Wistar rats (SPF) weighing 280–300 g at the beginning of the experiment were used. The animals were kept under standard vivarium conditions (temperature 22–24°C and natural light rhythm), fed and watered *ad libitum.* They were housed in cages, with 5–6 animals in each. The experiment was conducted in autumn. It was preceded by another one with several variations of Cd and Ir doses and with results comparable with those of the present experiment.

Cadmium administration

Cadmium ($CdCl_2$, Lachema Brno, Czech Republic) was administered i.p. at the dose of 1 mg/rat alone (group Cd) or 30 min before irradiation (group Cd+Ir).

Irradiation

Animals (groups Ir and Cd+Ir) were irradiated with the dose of 6 Gy by gamma rays from a ^{60}Co source (Chisostat, Chirana, Czech Republic) at a dose rate of 0.235 $Gy.min^{-1}$.

To eliminate circadian variation, these treatments were performed at the same time between 7.30 – 9.00.

Unilateral nephrectomy

Rats of the three experimental groups (Cd, Ir, Cd+Ir) were subjected to UN 1, 7, 14 and 21 days after Cd administration and/or Ir, together with the non-treated control group (C). The surgical removal of the left kidney was performed under light ether anaesthesia after ligation of the ureter. The operation was performed between 12.00 – 14.00 and animals were sacrificed 44 h later. At that time, the first wave of DNA synthesis, which had been induced by the UN, was complete and the mitotic activity reached its maximum in the remaining contralateral kidney (Heine et al., 1971).

Nucleic acid determination

Quantitative changes in nucleic acids were determined by the method of Tsanev and Markov (1960). Tissue samples were homogenized in 5% trichloracetic acid and then deproteinized and purified by consecutive washing with methanol, chloroform, benzene and ether. The separation of RNA and DNA was carried out by hydrolysis in alkaline and acidic media, respectively. The concentration of DNA and RNA in the purified hydrolysates was determined by spectrophotometric measurements at two wavelenghts (DNA 268 and 284 nm, RNA 260 and 286 nm (Hitachi, Tokyo, Japan)) and expressed as mg of RNA or DNA per g wet tissue. The content of RNA or DNA was determined by calculation per total weight of an organ.

Statistical analysis

The experimental data were statistically evaluated by the Peritz' F-test (Harper, 1984) and are given as mean ± S.E.M.

Results

Changes of RNA and DNA were analysed in the "intact" kidney, excised in the course of the operation, and in the remaining contralateral hypertrophic kidney, liver, bone marrow, spleen and thymus, which were excised after sacrifice of Cd- and/or Ir-treated rats and control rats.

Kidneys

In the intact kidney, excised from Cd- and/or Ir-treated rats, we have not found any decrease in concentration and content of DNA and RNA in comparison with control values (Fig. 1). In the group with Cd administration (Cd, Cd+Ir), the RNA and DNA concentrations tended to increase until the 14th day, but this was not connected with the increase in the total content of DNA and RNA as the kidney weights slightly decreased at that time (Table 1). The combination of both treatments did not lead to summation of individual treatment effects in the intact kidney.

On the 44th h after UN, the concentration of RNA and DNA increased in the remaining contralateral hypertrophic kidney (Fig. 2) of control rats in comparison with the intact kidney (Fig. 1) of the same animals removed by nephrectomy in average by 22% and 61%, respectively, and the total content of RNA and DNA increased by 48% and 83%, respectively, while the weight of the hypertrophic kidney increased only by 26% in comparison with the intact kidney (Table 1).

The applied treatments caused more profound changes in the hypertrophic kidney than in the intact kidney of the same animals. After Cd administration or Ir alone, the concentration and content of RNA temporarily decreased on the 7th day. After combination of both treatments (Cd+Ir), the concentration and total content of RNA in the hypertrophic kidney also decreased on the 1st day and, in a lower extent, on the 21st day. DNA changes were more marked than RNA changes and they could also be seen after individual treatments (Cd, Ir) almost at each time interval of the investigation. First of all, in both groups of animals treated with Cd (Cd and Cd+Ir), the concentration and total content of DNA decreased on the 1st day; later, the concentration and total content of DNA also decreased after Ir alone. In rats of all three experimental groups, the most significant changes were found on the 7th day, when the DNA concentration decreased by 55–68% and the total content of DNA by 48–66% in comparison with controls. In the hypertrophic kidney, the combination of both treatments resulted in more significant changes on the 1st day and 21st day than individual treatments (Cd+Ir *vs.* Cd or Ir), but summation of the effects was only partial.