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Comparison of Damage Induced by Mercury Chloride and Ionizing Radiation in the Susceptible Rat Model

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Abstract

Mercury (Hg), one of the most diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states. Although the reports indicate that mercury induces a deleterious damage, little has been reported from the investigations of mercury effects in living things. The purpose of this study is to evaluate the effects of mercury chloride and ionizing radiation. Prepubertal male F-344 rats were administered mercury chloride in drinking water throughout the experimental period. Two weeks after whole body irradiation, organs were collected for measuring the induced injury. Serum levels of GOT, GPT, ALP, and LDH were checked in the experimental groups and the hematological analysis was accomplished in plasma. In conclusion, the target organ of mercury chloride seems to be urinary organs and the pattern of damage induced by mercury differs from that of the irradiated group.

1. Introduction

Ionizing radiation, a widely used therapeutic modality in oncology, not only eradicates neoplastic cells but also generates inevitable side effects on normal tissues [1]. A deleterious effect of radiation is the production of reactive oxygen species (ROS) which include superoxide anion (O_2^- , a free radical), hydroxyl radical ($\cdot OH$), and hydrogen peroxide (H_2O_2). These reactive species may contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein oxidation, and muscle injury) and to metabolic and morphologic changes (e.g., increased muscle proteolysis and changes in the central nervous system) in animals and humans [2].

Toxic metals (lead, cadmium, mercury and arsenic) are widely found in our environment [3]. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Mercury (Hg), one of the most diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states, each of which has unique characteristics of target organ specificity [4]. Although the reports indicate that mercury induce a deleterious damage, little has been reported from the investigations of mercury effects in living things. For a study of biological injury, serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are the sensitive markers of hepatocellular damage [5]. It is suggested that the measurement of these isoenzymes in serum and urine may help to elucidate the localization of tissue damage. Especially, an increased level of serum alkaline phosphatase (ALP) was an indicator of renal damage [6].

Cortisol (hydrocortisone, compound F, $11\beta,17,21$ -trihydroxy-4-pregnene-3,11,20-trione) is the most potent glucocorticoid produced by the adrenal cortex [7]. Like other adrenal steroids, cortisol is synthesized from cholesterol through a series of enzymatically mediated steps. The first and rate-limiting step in adrenal steroidogenesis, the conversion of cholesterol to pregnenolone, is stimulated by the pituitary adrenocorticotropic hormone (ACTH) which is, in turn, regulated by hypothalamic corticotropin releasing factor (CRF). Cortisol acts through specific intracellular receptors and has effects in numerous physiologic systems in response to physical and psychological stress, including immune function, glucose-counterregulation, vascular tone, substrate utilization and bone metabolism [8,9]. In the present study, radioimmunoassay of cortisol in serum and analysis of hematological components and enzymes related to tissue injury were carried out to evaluate the effect of mercury chloride with ionizing radiation .

2. Materials and Methods

Animals

Fifteen, 4-week-old, 85-90 g male Fisher 344 rats were purchased from Daehan Biolink (Chungbuk, Korea). Rats were acclimated for at least 3 days before the experiment was started. They were kept in cages containing chip bedding, three rats per cage. All rats were maintained under the following conditions; temperature (23°C) and lighting (12 hr light: 12 hr dark) and allowed free access to food and water.

Irradiation and Treatment

The fifteen rats were allocated randomly into three groups of five rats each. Irradiated groups were exposed to γ -radiation from a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy/hr [10]. Mercury chloride (HgCl_2) was administered 1 mg/kg in drinking water. All the rats were euthanized two weeks after irradiation. Immediately after death, blood was collected from the heart. The blood was left to clot at room temperature for 1 hr, centrifuged and serum was immediately frozen at -70°C until analysis.

Measurement of Serum Enzymes Activity

Activity of glutamate-oxalate transaminase (GOT), glutamate-pyruvate transaminase (GPT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) was measured by using the automatic analyzer (Hitach, 747/200 type), which is based on the spectrophotometric quantification of NADPH loss using lactic dehydrogenase as a coenzyme [11].

Measurement of Cortisol

Cortisol concentrations in serum of the experimental groups were determined in a radioimmunoassay by using a Diagnostic Products (Diagnostic Systems Laboratories, USA) with a sensitivity of 8.28 nmol/liter [12]. The inter- and intra-assay coefficients of variation were < 8.3% and <10%, respectively.

Statistical Analysis

Statistical analysis was performed by Student's *t* test for a simple comparison of each treatment group with the sham control group using Sigma Plot[®] software (Jandel Scientific, Germany). They are expressed as mean \pm SEM.

3. Results

The irradiated rats showed a significant loss of the weight of body, liver, spleen and testis compared with those in the control and mercury group ($p < 0.05$, Table 1). Especially, changes in the weight of kidney show a different pattern. The kidney weight significantly increased in the rats that were given mercury chloride. In general, the weights of body, liver, kidney, spleen and testis show a tendency of increase in the mercury chloride treated group compared with those in the control.

Results of the hematological analysis of plasma from the experimental group are represented in Table 2. Values of WBC, platelet, HCT and HB significantly reduced in the irradiated rats, while those of RBC and platelet were markedly elevated in the rats that were given mercury chloride. In case of serum level of WBC, both of the groups show a decrease compared to the control. MCV, MCH and MCHC were not different from those of the control.

The levels of SGOT and SGPT in the irradiated rats increased, while the value of SGOT significantly reduced in the mercury chloride treated group (Figure 1). Serum ALP was in a similar level to the irradiated group. The values of ALP, however, increased a little in the rats that were given mercury chloride. The level of LDH markedly reduced in both of the groups.

The ratio of serum concentration of cortisol in the experimental groups increased in the irradiated ($p < 0.02$) and the mercury chloride-treated ($p < 0.05$) groups (Figure 2).

4. Discussions

This study has shown that the effect of mercury chloride in drinking water on the whole body irradiated rats. The loss of body and organ (liver, spleen and testis) weights in the irradiated rats was as obvious as expected. However, the weights of body and organ show a rising tendency compared to those of the control and the irradiated group. The kidney weight went upto the distinguished values. It is suggested that the target organ of mercury chloride is urinary organs. According to the hematological analysis, values of RBC and platelet in rats that were given mercury chloride increased

markedly compare to the control. These values, when compared to the irradiated rats, increased by 2.37 times and 4 times, respectively ($p < 0.05$). Other checks in hematological criteria show a similar pattern to that of the irradiated rats.

Serum levels of the GOT and GPT indicated the hepatocellular damage in the irradiated and mercury chloride-treated groups. ALP, an indicator of renal injury, increased in the rats that were given mercury chloride. Elevated levels of circulating cortisol in both of the groups may indicate the ACTH hypersecretion, adrenal dysfunction, and biological stress. Particularly, the ratio of circulating cortisol of the irradiated rats increased higher than mercury chloride treated group. It is indicated in this study that mercury chloride gave lesser damage than ionizing radiation. Taken together, mercury chloride affects the organs including liver, kidney, spleen, and testis like ionizing radiation. Especially, the main target organ of mercury chloride seems to be urinary organs since it induced changes of kidney weight and ALP levels.

5. References

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Table 1. The weight of body and organs in experimental groups†

	CON	IRR	HgCl ₂
Body wt	144.6 ± 0.73	123.8 ± 3.35*	151.6 ± 1.25
Liver wt	6.60 ± 0.23	6.07 ± 0.27*	6.83 ± 0.11
Kidney wt	0.64 ± 0.03	0.62 ± 0.02	0.77 ± 0.01*
Spleen wt	0.42 ± 0.003	0.25 ± 0.01*	0.43 ± 0.006
Testis wt	0.87 ± 0.02	0.58 ± 0.02*	0.89 ± 0.01

†, All values expressed as means ± SEM (n = 5 in each group).

*, $P < 0.05$ versus control group.

Abbreviations; CON, control group; IRR, irradiated group; HgCl₂, mercury chloride treated group.

Table2. The hematological results from plasma of the experimental groups†

	CON	IRR	HgCl ₂
RBC (x10⁶)	7.37 ± 0.18	4.44 ± 0.18**	10.53 ± 0.08**
WBC (x10³)	5.08 ± 0.42	0.58 ± 0.03**	3.29 ± 0.08*
Platelet (x10³)	861 ± 6.51	235.75 ± 50.86**	945.33 ± 20.49*
HCT	48.33 ± 1.66	30.50 ± 1.52*	44.66 ± 0.66
HB	13.83 ± 0.46	8.92 ± 0.51*	13.63 ± 0.40
MCV	65.66 ± 0.88	68.25 ± 0.98	61.00 ± 1.02
MCH	18.66 ± 0.33	20.00 ± 0.47	18.66 ± 0.33
MCHC	28.66 ± 0.33	29.50 ± 0.34	30.33 ± 0.66

†, All values expressed as means ± SEM (n = 5 in each group).

* and ** indicate $p < 0.05$ and $p < 0.02$, respectively.

Abbreviations; CON, control group; IRR, irradiated group; HgCl₂, mercury chloride treated group; RBC, red blood cell count; WBC, white blood cell count; HCT, hematocrit; HB, hemoglobin; MCV, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration.

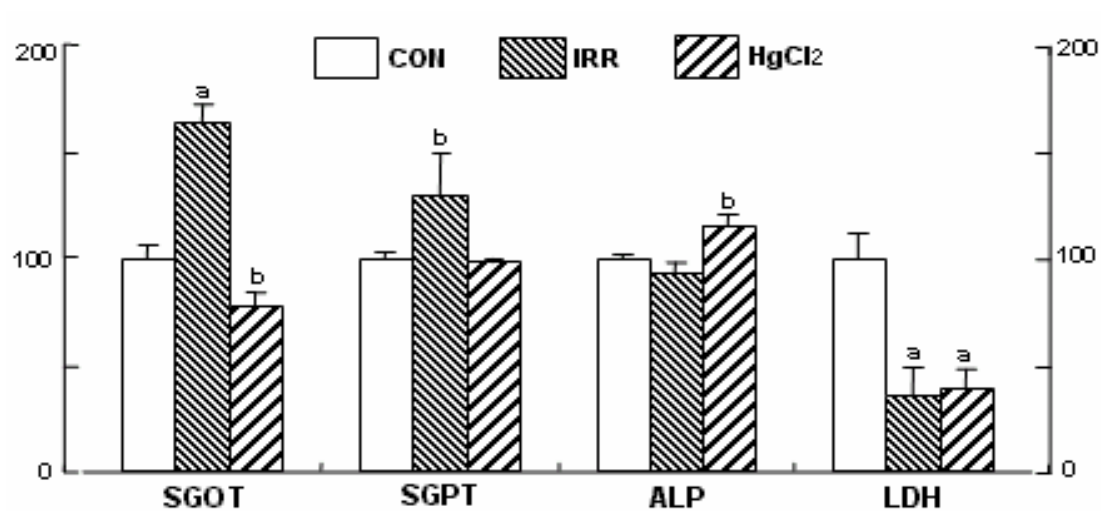


Figure 1. Ratio of serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) was measured by using the automatic analyzer (Hitach, 747/200 type) in experimental group. a, $p < 0.02$ and b, $p < 0.05$.

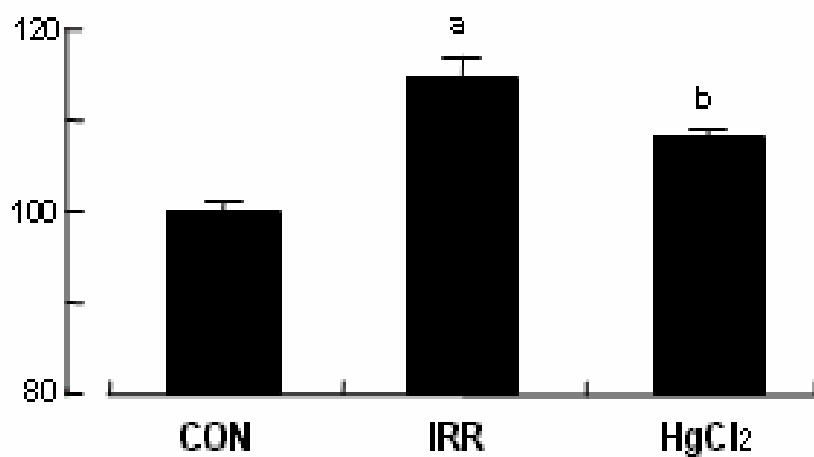


Figure 2. Ratio of circulating cortisol in serum of experimental group. The levels measured by commercial radioimmunoassay kits. a, $p < 0.02$ and b, $p < 0.05$.