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Influence of cadmium and copper on tissue element levels of pregnant rats

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Abstract: In the current study, we examined the effects of Cd on Cd, Cu, Zn and Fe levels in placenta and maternal and fetal plasma and tissues, the placental weight, total fetal and maternal body weights, and fetal and maternal tissue weights during pregnancy. A total of 21 adult female rats were treated during gestation with drinking water containing one of the following: 70 mg/L of $CdCl_2$, a combination of 70 mg/L of $CdCl_2$ and 70 mg/L of $CuSO_4$, or no addition (control). Placenta Cu and Fe levels, fetal liver and kidney Cu levels, and fetal liver tissue weights were lower in the group administered Cd than in the control group. Also, Cd levels in the placenta, maternal and fetal liver, and maternal kidney were higher in the group treated with Cd than in controls. In the group administered both Cd and Cu, fetal body and tissue weights did not change, but Cd levels in the placenta, maternal and fetal liver, and maternal kidneys were higher than in controls. Zn and Fe levels in the maternal kidney and fetal liver were also lower in this group. Cd exposure during pregnancy resulted in Cd accumulation in maternal and fetal tissues during pregnancy and a decrease in the total weight of fetuses, and the combination of Cd and Cu caused some changes in the both maternal and fetal levels of Cu, Zn, and Fe, but it did not cause changes in the total fetal body weight or the weights of individual tissues.

Keywords: Pregnancy, cadmium, copper, fetus, rat

1 Introduction

Cadmium (Cd) is a toxic metal widely used in industrial processes and is a widespread environmental pollutant. It is toxic because it induces an early oxidative stress in cells and contributes to the development of serious pathological conditions because it persists

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in tissues for long periods of time [1] and is toxic to several organs [2]. The major sources of Cd pollution in ecological systems are mining, smelting, and industrial use. In addition, sources of human exposure include foods, cigarette smoke, and alcoholic beverages [3].

The fetal effects of Cd are less well understood and are less dramatic because the placenta is a target organ for Cd toxicity and serves as a partial barrier against transport to fetus, largely because trophoblasts synthesize metallothionein, a protein that complexes heavy metals like Cd [4].

Trace elements, however, have a key role in fetal development and growth [5, 6]. Copper (Cu), for example, is an essential mineral element of particular importance, especially early in life, for the development and maintenance of myelin [7]. Clinical manifestations of Cu deficiency are well characterized, and high Cu intake alters genetic control systems, leading to liver damage in infants [8]. The mechanisms of the pathogenic effects of Cu deficiency and excess, however, are not clear.

Several studies have reported strong positive correlations between zinc (Zn) and Cd concentrations in some tissues in humans, but the relationship between Cu and Cd is not well known [9–11]. The administration of Cd and lead (Pb) may induce changes in the metabolism of essential metals like Zn, iron (Fe) and Cu, altering the activity of metal-dependent enzymes [12].

Cd is known to affect the levels of trace elements in tissues such as, Zn, Cu, and Fe, which are important for the function of a variety of enzymes and are required for normal development and health. The liver and kidney are the main tissues in which Cd is accumulated. It is possible that exposure of pregnant women to high levels of Cd due to environmental exposure or cigarette smoking could also result in fetal exposure. Therefore, in the current studies, we examined the effect of high Cd exposure on Zn, Cu, and Fe levels in maternal and fetal liver, kidney, and plasma in rats. Additionally, we examined how Cd exposure affects fetal and placental numbers and weights and trace element levels in placentas. Finally, we investigated how Cu supplementation influences the effects of Cd.

2 Statistical methods and Experimental Procedures

2.1 Experimental Procedure

21 Wistar albino adult female rats (14−16 weeks, 212±11 g) were supplied by the Animal Care Unit of Pamukkale University Animal Research Center, Turkey. The rats were reared under the supervision of a veterinarian, kept in a well-ventilated, noiseless environment, and allowed free access to food and water. Animal care and all experimental procedures were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services).

After a 1-week adaptation period, the female rats were mated with male rats. Vaginal smears were obtained from the animals to assess the presence of sperm. A sperm-positive vaginal smear was taken to indicate the first day of pregnancy. Rats confirmed as being

pregnant were treated during pregnancy (21 days) with drinking water containing 70 mg/L of $CdCl_2$ (Sigma; n=7), a combination of 70 mg/L of $CdCl_2$ and 70 mg/L of $CuSO_4$ (Cd+Cu; Sigma; n=7), or no added metal (control; n=7). This dose of Cd was selected based on previous investigations [13], and Cu dose was the daily requirement for rats. At the end of pregnancy (on the 21^{st} day), all animals were anaesthetized with ketamine (50 mg/kg; Parke Davis, Turkey) and xylazine (5 mg/kg; Alfasan, Turkey). Blood was collected in heparinized tubes by cardiac puncture, and all animals were killed by decapitation. Blood was centrifuged, and the separated plasma was collected and stored at −20°C. Fetal and placental weights and the number of fetuses were noted. Fetal and maternal liver and kidney tissues were removed, weighed, and stored at −20°C.

2.2 Analytical methods

Tissues were prepared as described by Brown *et al.* [14]. Briefly, tissues were weighed and place in tubes with 2 ml of nitric acid. The samples were heated at 100°C until their volume was reduced by half. Next, 2 ml perchloric acid was added, and the samples were again heated at 100°C until their volume was reduced by half. Finally, the samples were diluted to 5 ml with deionized water.

For the analyses of elements in blood and tissues, standard solutions were prepared from Cu, Zn, Fe, and Cd standards (Merck). Deionized distilled water was used as the blank. The concentrations of Zn, Cu, and Fe in rat and fetus tissues were measured with flame atomic absorption spectrophotometer (Perkin Elmer AAS-700 Ueberlingen, Germany). Tissue concentrations of Cd were determined by atomic absorption spectrophotometer with a HGA graphite furnace (Perkin Elmer AAS-700 Ueberlingen, Germany). Blank and standard solutions were used to calibrate the atomic absorption spectrophotometer [14]. Calculations of concentrations were based on tissue weight.

2.3 Statistical methods

Mean values of all data, standard deviations of the means, and analysis of the differences between groups were determined using the SPSS 10.0 statistical package program. The non-parametric Mann Whitney U-test was to compare differences between groups because the number of our animals used was under 30. P values of less than 0.05 were considered to indicate significant differences.

3 Results

We examined the trace element levels in the tissues of pregnant rats and their fetuses who received Cd or Cd+Cu given their drinking water. There was no difference was found in the numbers of fetuses, fetal kidney weights, placental weights, Zn and Fe levels in the fetal liver, Fe and Cd levels in the fetal kidney, Zn, Cu, and Fe levels in the maternal plasma, or the kidney Cu levels between rats fed Cd and control rats (Tables 1–4).

Significant differences were found in the levels of placental Zn ($P < 0.01$), Cu ($P < 0.01$), Cd ($P < 0.01$), and Fe levels ($P < 0.05$) between rats administered Cd and control rats (Table 1). We also found a significant difference in the placental Cd ($P < 0.01$), Zn ($P < 0.01$), Cu ($p < 0.05$), and Fe ($P < 0.05$) levels between the rats administered Cd+Cu and the control rats (Table 1).

Table 1 Comparisons of placental weights and element levels (mean±SD).

	Controls (n=7)	Cd Group (n=7)	Cd+Cu Group (n=7)
Weight (g)	0.44 ± 0.10	0.42 ± 0.06	0.43 ± 0.05
Zn (μg/g tissue)	7.84 ± 0.93	$12.38 \pm 1.98 **$	7.37 ± 0.90††
Cu (μg/g tissue)	9.47 ± 2.90	$5.21 \pm 1.00 **$	7.32 ± 0.98†
Fe (μg/g tissue)	147.94 ± 49.39	$99.06 \pm 20.67*$	154.86 ± 39.35†
Cd (μg/g tissue)	0.03 ± 0.03	$0.13 \pm 0.08 **$	$0.14 \pm 0.02 **$

*P<0.05, **P<0.01 between controls and Cd or Cd+Cu groups.
†P<0.05, ††P<0.01 between Cd and Cd+Cu groups.

Table 2 Comparisons of number of fetuses and fetal tissue weights (mean±SD).

	No. of fetuses	Fetus weight (g)	Fetal liver weight (g)	Fetal kidney weight (g)
Controls (n=7)	10.00 ± 2.76	3.90 ± 0.42	0.54 ± 0.26	0.05 ± 0.01
Cd Group (n=7)	9.00 ± 2.00	$3.06 \pm 0.26 **$	$0.23 \pm 0.10*$	0.06 ± 0.01
Cd+Cu Group (n=7)	10.00 ± 0.95	3.35 ± 0.55	0.33 ± 0.06†	0.05 ± 0.01

*P<0.05, **P<0.01 between controls and Cd or Cd+Cu groups.
†P<0.05 between Cd and Cd+Cu groups.

Fetal liver weights were higher in the rats administered Cd+Cu than in the rats administered Cd ($P < 0.05$; Table 2), whereas the total weights of fetuses and the weight of fetal livers was lower in the rats administered Cd than controls ($P < 0.01$ and $P < 0.05$, respectively). The Cu levels in fetal livers and kidney tissues were lower in the rats administered Cd than controls ($P < 0.05$ and $P < 0.01$, respectively). In addition, the Zn, Fe, and Cd levels in fetal livers were significantly different between the rats administered Cd+Cu and controls ($P < 0.01$, $P < 0.05$, and $P < 0.05$, respectively). The Zn levels in fetal livers and the Zn and Cu levels in fetal kidneys were also significantly different between rats administered Cd and rats administered Cd+Cu ($P < 0.01$; Table 3).

Between the rats administered Cd and controls, there was a statistically significant difference in the Cd in the liver ($P < 0.01$) and the Cd and Zn ($P < 0.01$ and $P < 0.05$) levels in the kidney. Between the rats administered Cd+Cu and controls, there was also a significant difference in the kidney Cd ($P < 0.01$) and Fe levels ($P < 0.01$) and the liver Cd levels ($P < 0.05$). Furthermore, the levels Zn and Cd in the kidneys and Zn in the

liver were significantly different ($P < 0.01$) between the rats administered Cd and those administered Cd+Cu administered group (Table 4).

Table 3 Comparisons between element levels in fetal livers and kidneys (mean±SD).

		Controls (n=7)	Cd Group (n=7)	Cd+Cu Group (n=7)
Fetal Liver				
	Zn (μg/g tissue)	39.86 ± 22.13	27.71 ± 8.66	$6.13 \pm 4.54 * *, \dagger\dagger$
	Cu (μg/g tissue)	23.43 ± 10.44	$17.20 \pm 1.19*$	18.09 ± 2.94
	Fe (μg/g tissue)	266.93 ± 85.23	174.00 ± 66.81	$162.07 \pm 20.91*$
	Cd (μg/g tissue)	0.01 ± 0.01	$0.07 \pm 0.03 * *$	$0.05 \pm 0.02*$
Fetal Kidney				
	Zn (μg/g tissue)	25.24 ± 11.41	$47.67 \pm 6.93 * *$	$23.87 \pm 5.30\dagger\dagger$
	Cu (μg/g tissue)	54.26 ± 6.61	$13.36 \pm 3.37 * *$	$46.27 \pm 6.42\dagger\dagger$
	Fe (μg/g tissue)	304.29 ± 88.71	340.97 ± 52.05	336.35 ± 100.28
	Cd (μg/g tissue)	0.24 ± 0.09	0.33 ± 0.09	0.30 ± 0.07

*P<0.05, **P<0.01 between controls and Cd or Cd+Cu groups.
†P<0.05, ††P<0.01 between Cd and Cd+Cu groups.

Table 4 Comparisons of element levels in maternal tissues (mean±SD)

		Controls (n=7)	Cd Group (n=7)	Cd+Cu Group (n=7)
Maternal Liver				
	Zn (μg/g tissue)	10.62 ± 4.17	13.20 ± 1.05	$10.53 \pm 1.50\dagger\dagger$
	Cu (μg/g tissue)	5.68 ± 1.63	4.72 ± 0.42	4.82 ± 0.62
	Fe (μg/g tissue)	95.43 ± 44.21	116.85 ± 34.99	115.62 ± 42.66
	Cd (μg/g tissue)	0.01 ± 0.01	$0.18 \pm 0.14 * *$	$0.16 \pm 0.03 * *$
Maternal Kidney				
	Zn (μg/g tissue)	12.65 ± 6.39	$16.21 \pm 1.52*$	$11.07 \pm 1.82\dagger\dagger$
	Cu (μg/g tissue)	10.53 ± 3.50	9.70 ± 2.03	11.20 ± 2.43
	Fe (μg/g tissue)	230.78 ± 234.35	102.53 ± 37.85	$71.71 \pm 15.64*$
	Cd (μg/g tissue)	0.08 ± 0.01	$0.26 \pm 0.10 * *$	$0.48 \pm 0.10 * *, \dagger\dagger$

*P<0.05, **P<0.01 between controls and Cd or Cd+Cu groups.
†P<0.05, ††P<0.01 between Cd and Cd+Cu groups.

There were significant differences in the Cd levels in the plasma between control rats and rats administered Cd or rats administered Cd+Cu as well as between rats administered Cd and those administered Cd+Cu (Fig. 1). With regards to the plasma Zn, Cu, and Fe levels, only the plasma Fe levels were significantly different between the rats administered Cd and controls ($P < 0.05$). Between rats administered Cd and those administered Cd+Cu, plasma Cu levels were significantly different ($P < 0.05$; Fig. 2).

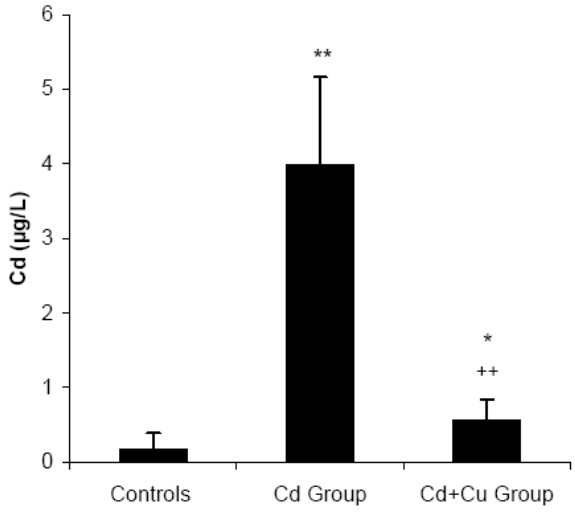

Fig. 1 Maternal Cd levels. *P<0.05, ** P<0.01 between controls and Cd or Cd+Cu groups. ++P<0.01 between Cd and Cd+Cu groups.

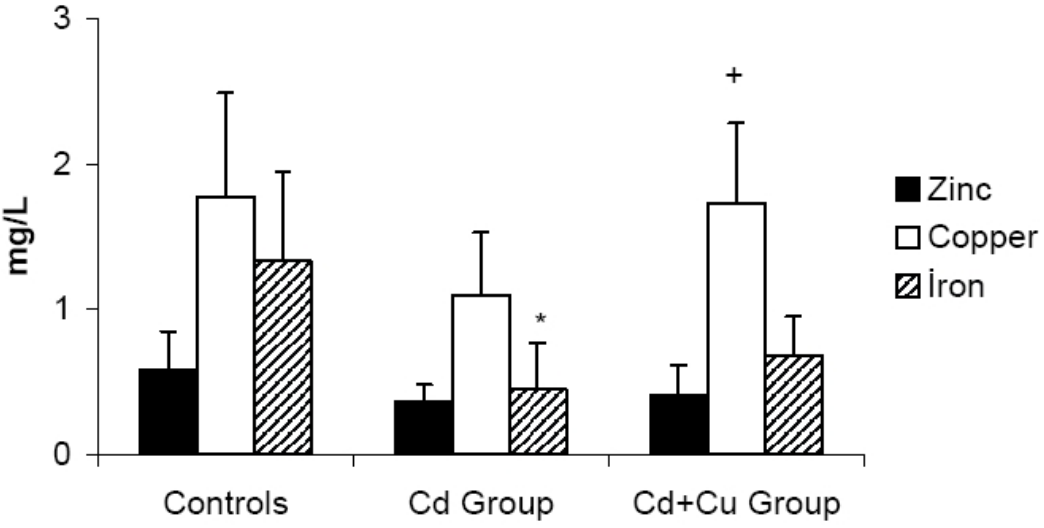

Fig. 2 Plasma Cu, Zn, and Fe levels. *P<0.05 between controls and Cd or Cd+Cu groups. +P<0.05 between Cd and Cd+Cu groups.

4 Discussion

Pregnancy causes many physiological and biochemical changes that may affect the maternal metabolism of trace elements. In rodents, cattle and humans, the placenta retains Cd and therefore acts as a partial barrier to protect the fetus from Cd exposure [15–17]. Here, we found higher levels of Cd in the placentas of rats administered Cd ($P < 0.01$). In addition, in these rats, the weights of fetuses and fetal liver weights were decreased but the numbers of fetuses did not change compared to controls. In addition, the Cd concentration in fetal kidneys did not change, whereas the concentration of Cd in the fetal liver increased compared to controls. The maternal Cd levels in plasma, liver, and kidney

were significantly higher in the Cd-administered group than to controls ($P < 0.01$). The reduction in the weight gain of pups exposed to Cd is thought to be due to the toxic effect of this metal. Specifically, Cd may lead to a growth delay by lowering the DNA synthesis or trace element bioavailability [18–20]. In our study, we observed that Cd accumulation in fetal tissues were higher than in maternal tissues. There are two possible reasons for the differences in Cd concentrations between fetal and maternal tissues: (i) the placenta may not serve as an effective barrier to Cd, and (ii) more Cd may be excreted by the maternal kidneys than by the fetal kidneys.

Accumulation of Cd, especially in the kidneys during pregnancy, is expected to produce serious toxic effects. Although the mechanisms by which Cd causes renal dysfunction have been extensively studied by many investigators [21, 22], the cellular mechanisms remain unclear. Clinically, Cd-induced nephropathy resembles acquired Fanconi's syndrome [21, 22]. Cd interacts with renal membranes and enzymes and disrupts energy production, calcium metabolism, glucose homeostasis, and ion transport [23].

In the present study, Zn levels in both maternal and fetal livers were unchanged in the Cd-administered group, whereas the Zn levels in the placenta and the fetal and maternal kidneys was significantly higher than in controls. Interactions between Cd and Zn are known to occur during pregnancy. The livers of fetal and neonatal rats accumulate a high level of essential metals such as Zn (as metallothionein) during development [24]. To meet this requirement, Zn as well as Cu must be mobilized from the maternal tissues and transferred to the fetuses during gestation. The mobilization of these essential metals could result from hormonal changes during pregnancy. Significant decreases in hepatic Zn levels have been reported during pregnancy and lactation in rats injected with Cd [25]. In our study, we found that Zn levels decreased in the plasma of Cd-administered rats, although the decrease was not statistically significant. It is known that toxic levels of Cd can inhibit Zn absorption. It is important to recognize that some foods, such as cereals, contribute a small but significant amount of Cd to our daily diet. The extent to which, if any, these nontoxic levels of Cd affect Zn absorption in humans is not well known [26]. Zn accumulation in the kidneys of rats in this study may be related to metallothionein uptake. Metallothionein, a low-molecular weight protein with a high cysteine content and a high affinity for Zn and Cd, is suggested to play an important role in the concentration of these elements in the kidney [27].

We also found that placental Fe levels decreased but that Fe levels in fetal and maternal livers and kidneys were not significant different in the Cd-administered group. However, plasma Fe levels of Cd-administered rats were significantly lower than in controls ($P < 0.05$). Also, placental and fetus Cu levels were significantly lower in Cd-administered rats, whereas the maternal Cu levels in tissues was unchanged. Maternal plasma Cu levels were also lower, although the difference was not statistically significant. The administration of Cd during pregnancy is known to induce changes in the metabolism of Fe and Zn in pregnant rats and in their fetuses and newborns [20]. Moreover, iron deficiency has been shown to increase the absorption of Cd [28].

In this study, we examined the effects of Cu together with Cd because strong positive

correlations between Zn and Cd concentrations have been found in some human tissues [9–11], but the relationship between Cu and Cd has not been examined. We chose a nontoxic dose of Cu (10 mg/kg), which is approximately twice the daily requirement [29]. In our previous studies, we found that administration of this amount of Cu for one month to female rats did not cause any significant changes in the liver and kidney levels of Cu and Zn [30, 31]. Gee et al. also reported that parenteral Cu supplementation of the dam in late gestation had no effect on the liver Cu concentration of the foal at birth [32].

There were no changes in the levels of elements accumulated in the placenta except for an increase in Cd in the rats administered Cd+Cu compared to controls. Accumulation of Cd in fetal liver and maternal tissues was significantly higher than in controls. These results indicate that Cu does not reduce of the accumulation of Cd. There was also no change in the Cu levels of maternal and fetal tissues or in maternal plasma in the rats administered Cd+Cu. However, the Cu levels were lower, especially in fetal tissues in the rats administered Cd than in controls. Only the Zn levels in the maternal and fetal liver showed a decrease in the rats administered Cd+Cu compared to controls. This reduction in Zn levels in fetal livers was lower than in the Cd administered group. We suspect that this decrease in fetal liver Zn levels is due to the effect of Cu on Zn absorption [9].

No significant difference was seen in the numbers or weights of fetuses or the fetal organ weights in the Cd+Cu administered group compared to controls, but the fetal liver weights in this group were significantly higher than those in the Cd-administered group. Also, Fe levels in fetal livers and maternal kidneys in the Cd+Cu administered group were significantly lower than in controls. We suspect that this decrease is due to excessive Cd accumulation in the fetal liver and maternal kidney.

In our study, the maternal and fetal tissue element levels were affected by Cd exposure during pregnancy in different ways. In Cd+Cu administered group, both maternal and fetal tissue element concentrations were close to normal. Thus, it appears that the toxicity of Cd to tissues is lower when given together with Cu because especially weights of fetus and fetal organs did not show any difference compared to controls. A previous study also found that rats administered Cd show accumulation of this element in the heart and a decrease in both body mass growth and heart mass [33].

In conclusion, exposure to Cd during causes a decrease in fetal weights and accumulation of Cd in both mothers and fetuses. This, in turn, affects the maternal and fetal tissue levels of Cu, Zn, and Fe. In addition, administration of Cu together with Cd considerably reduces the negative effects of Cd on fetal body and tissue weights but did not affect the accumulation of Cd in maternal and fetal tissues.

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