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Full Length Research Paper

Toxicological Influence of ethanol and biochemical changes in rats exposed to cadmium

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The present study was performed to assess the function of the liver and kidney in rats exposed to 50 mg Cd/l (as cadmium chloride) and/or 10% (w/v) ethanol (EtOH) for 12 weeks. The activities of alanine aminotransferase (ALAT) and asparate aminotransferase (AspAT) in serum were measured as indicators of the liver function. As parameters of the kidney function, creatinine, total protein and urea concentrations in serum and urine, as well as urinary alkaline phosphatase (ALP) activity were determined, and creatinine clearance was calculated. Daily Cd intake ranged from 3.17 to 4.28 mg/kg body weight and from 2.41 to 3.17 mg/kg body weight in the Cd and Cd + EtOH groups, respectively. The daily intake of 10% EtOH ranged from 47.5 to 86.9 g/kg body weight in the EtOH and from 47.3 to 63.4 g/kg body weight in the Cd + EtOH-exposed rats. Cd and EtOH, independently of separate or combined application, changed liver and kidney function. Rats treated with Cd alone and those co-exposed to both substances showed qualitatively similar. Some functional (increased serum AspAT and urinary ALP, decreased urinary urea) and functional changes in the liver and kidney were more evident in the case of combined exposure, while others were more evident after single exposure. However, a decrease in creatinine clearance, noted only in the animals treated with Cd and EtOH, shows that functional changes indicating renal insufficiency are more serious in the co-exposed group. Due to lower Cd and EtOH intake (resulting from a stronger aversion to drinking water containing both substances) in the co-exposed rats, as compared to the Cd- and EtOH-treated groups, it is difficult to draw a definite conclusion from this study. The findings, however, seem to indicate that EtOH increases Cd nephrotoxicity in rats, and thus may suggest a higher risk of kidney damage in alcoholics exposed to Cd. Unfortunately, this study does not provide clear evidence if, and to what extent, EtOH influences Cd hepatotoxicity.

Keywords: hepatotoxicity- nephrotoxicity- cadmium- ethanol- rats

INTRODUCTION

It is well known that toxic effects of a xenobiotic can be modified by other substances (Skoczyńska and Smolik, 1994; Brus et al., 1999; Institoris et al., 1999; Gupta and Gill, 2000). As simultaneous exposure to two or more xenobiotics can take place in the environment and/or under occupational conditions, the investigation of interactions between toxic substances is an important problem in modern toxicology. The interaction between cadmium (Cd) and ethanol (EtOH) can be a good example. Exposure of certain human populations to Cd is often rather high (World Health Organization, 1992; Schrey et al., 2000) and EtOH consumption continues to

rise worldwide (Samson and Harris, 1992; Meyer et al., 2000); so those persons who are exposed to Cd may be simultaneously alcohol misusers (Maranelli et al., 1990; Schioeler, 1991).

Some publications provide data on Cd–EtOH interactions (Sharma et al., 1991, 1992; Brus et al., 1995) but many aspects are still not fully recognized. According to our earlier results short- and long-term EtOH administration affects Cd turnover in rats, and also modifies changes in the metabolism of some essential elements by this heavy metal (Moniuszko-Jakoniuk et al., 1999, 2001; Brzóska et al., 2000, 2002).

Liver and kidney are important organs of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites, and are especially vulnerable to damage. As the liver is an important target organ of EtOH (Bunout, 1999; Thurman et al., 1999), and the kidney of Cd toxicity (Kjellström, 1986; World Health Organization, 1992; Nordberg et al., 1994) we have also assessed liver and kidney function and histology in rats exposed cadmium and ethanol.

MATERIALS AND METHODS

Animals

For the experiments, 40 inbred 10-week-old male Wister rats of 250 g initial body weight were obtained were selected from inbred colony maintained in the Animal House of King Fahd center of medical researches. The animals were housed in stainless-steel cages under conventional conditions (temperature 22 ± 1°C; relative humidity 50 ± 10%, natural light–dark cycle) and had free access to drinking fluid and a standard rodent laboratory chow (The diet was prepared from: corn, wheat, barley, wheat bran, Soya-bruised grain, meat starch, skimmed powdered milk, phosphate, fodder-chalk, mineral and vitamin premix. Metabolizable energy of the LSM diet was 12.2 MJ/kg.) The Cd content of the diet was 0.211 mg/kg.

Chemicals

All reagents and chemicals were of analytical grade or higher purity. Trace-free nitric acid (Merck, Dormstadt, Germany) and Cd standard solution assigned for atomic absorption spectrometry (Sigma, St Louis, MO, USA) were used for Cd analysis.

Experimental design

The experiment was conducted for 12 weeks. The animals were randomly allocated to four experimental groups of 10 rats each: (1) a control group, which received redistilled water; (2) an EtOH group, which received 10% (w/v) EtOH; (3) a Cd group, which was exposed to CdCl2 at a concentration of 50 mg Cd/l; (4) a Cd + EtOH group, which received a redistilled water containing 50 mg Cd/l and 10% EtOH. Fluid consumption was measured daily during the whole experiment.

After 12 weeks of treatment all rats were placed separately in glass metabolic cages for 24-h urine collection. After overnight starvation, blood was taken by cardiac puncture, the liver and kidney were removed under ether anesthesia, washed thoroughly in ice-cold physiological saline [0.9% (w/v) NaCl], and weighed. Whole blood was centrifuged after clotting, and the serum was separated and stored frozen until further analysis.

Analytical procedures

Cd and EtOH concentrations. Cd concentration in the blood, liver and kidney was determined by atomic absorption spectrometry as described (Brzóska et al., 2000, 2002). Blood-EtOH concentration was analysed by head-space gas chromatography (Hewlett-Packard, model 5890, series II) according to the manufacturer's recommendations.

Alanine aminotransferase (ALAT) and asparate aminotransferase (AspAT) activities in serum. The activities of ALAT (EC 2.6.1.2.) and AspAT (EC 2.6.1.1.) were determined colorimetrically (SEMCO S/E-uv spectrometer) according to standard procedures using commercially available diagnostic laboratory tests.

Biochemical indicators of renal function

Total protein in serum and urine was determined according to Lowry et al. (1951). Concentrations of creatinine and urea in serum and urine, as well as urinary alkaline phosphatase (ALP, EC 3.1.3.1) activity, were assessed spectrophotometrically (SEMCO S/E-uv spectrometer) using diagnostic laboratory tests (POCh). Creatinine clearance was calculated.

Statistical analysis

Statistical analysis of results was performed using the Mann–Whitney non-parametric U-test. The level of significance was $P < 0.05$. In order to discern the possible interactions between Cd and EtOH, two-way analysis of variance (ANOVA/ MANOVA) was used. F values having $P < 0.05$ were considered significant. A linear Pearson correlation was performed for testing relationships between certain parameters. All statistical calculations were done with the STATISTICA 5.0 computer program.

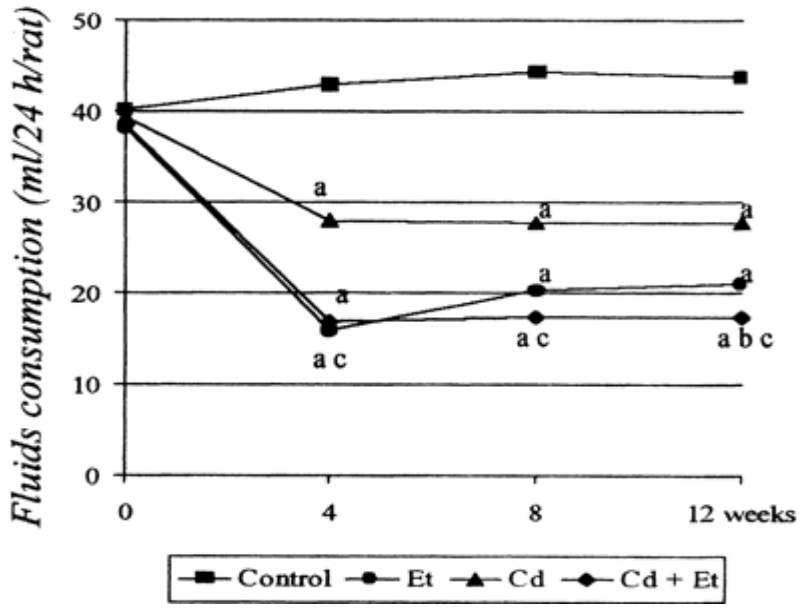

Figure 1. Effect of Cd, EtOH (Et), and their co-administration on fluid consumption. Each point represents the mean value of 10 rats. The animals were exposed to 10% EtOH or 50 mg Cd/l separately (EtOH and Cd groups) and to their combination (Cd + EtOH group) for 12 weeks. a,b,c Values were significantly different ($P < 0.05$; Mann–Whitney U-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

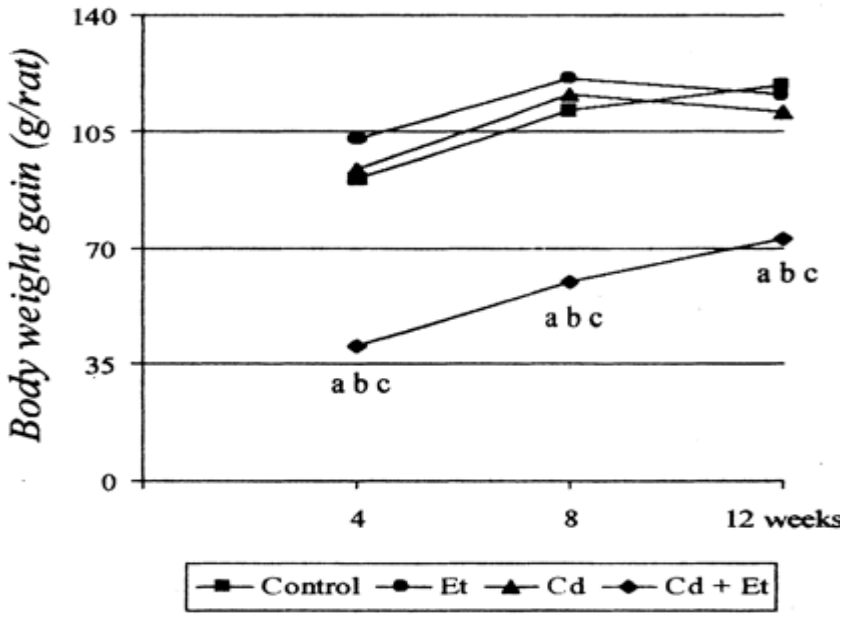

Figure 2. Effect of Cd, EtOH (Et) and their co-administration on body weight gain. a,b,cValues were significantly different ($P < 0.05$; Mann–Whitney U-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

RESULTS

Fluid consumption and Cd and EtOH intakes

Cd or EtOH administered alone depressed the drinking fluid consumption, which was further reduced by their co-administration (Figure 1). This effect was observed during the whole experiment. In the Cd, EtOH and Cd + EtOH groups, the mean consumption of drinking fluid was reduced by 37, 52 and 60%, respectively ($P < 0.001$ vs

Table 1. Effects of Cd, EtOH and their co-administration on liver and kidney weight Cd concentration in the whole blood, liver and kidney.

Group	Liver weight (g)	Relative liver weight (g/100 g body weight)	Kidney weight (g)	Relative kidney weight (g/100 g body weight)
Control	8.7154 ± 0.1969	2.067 ± 0.054	0.9865 ± 0.0261	0.233 ± 0.003
EtOH	8.7551 ± 0.1840	2.068 ± 0.038	0.9912 ± 0.0254	0.234 ± 0.005
Cd	7.9980 ± 0.217[a,b]	1.974 ± 0.042	0.9350 ± 0.0188[b]	0.231 ± 0.006
Cd + EtOH	7.1976 ± 0.2297[a,b,c]	2.035 ± 0.059	0.8688 ± 0.0278	

The animals were exposed to 10% EtOH and/or 50 mg Cd/l for 12 weeks. Values are means ± SEM of 10 animals.
[a,b,c]Values are significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to the control, EtOH and Cd groups, respectively.

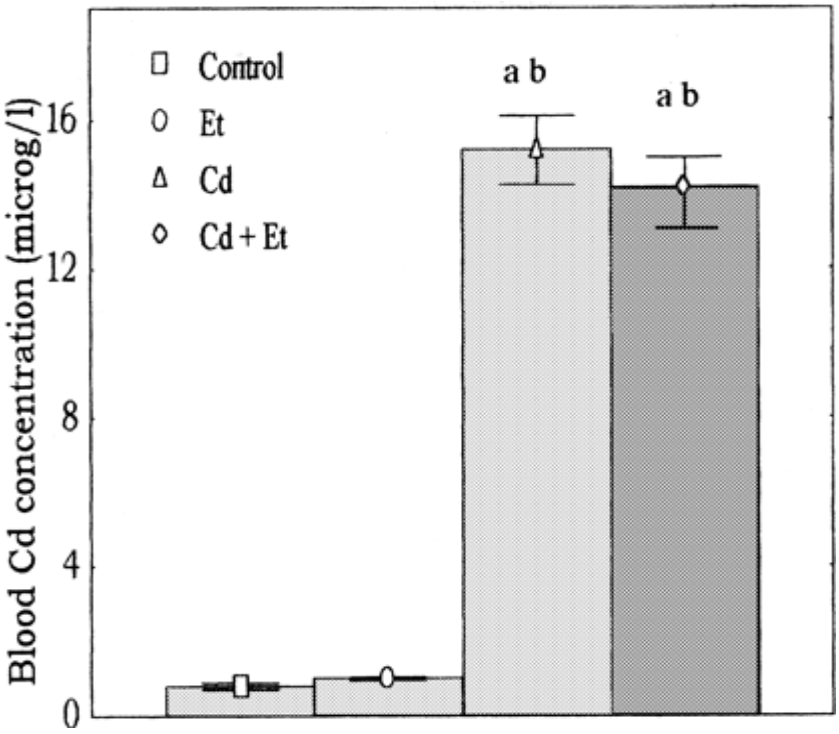

Figure 3. Cd concentration in whole blood. Each point represents the mean value ± SEM for 10 rats. a,bValues were significantly different (P < 0.05; Mann–Whitney U-test) compared to the control and EtOH groups, respectively.

control). The daily Cd intake ranged from 3.17 to 4.28 mg/kg body weight in the Cd and from 2.41 to 3.17 mg/kg body weight in the Cd + EtOH groups, while the EtOH intake from 47.5 to 86.9 g/kg body weight (EtOH group) and from 47.3 to 63.4 g/kg body weight (Cd + EtOH group). The average Cd and EtOH intake in the Cd + EtOH groups were lower by 38 (P < 0.001) and 18% (P < 0.01), respectively, compared to their separate dosages.

Body weight gain, liver and kidney weight

The body weight gain of rats exposed to Cd or EtOH alone was similar to that of controls (Figure 2), while combined administration of the two substances resulted in a significant retardation already during the first 4 weeks (Figure 2). The final body weight of the co-exposed rats was lower by 39, 34 and 37% versus control, Cd and EtOH groups (P < 0.001), respectively. Two-way analysis of variance has shown that both Cd (F = 25.7, P = 0.000) and EtOH (F = 15.9, P = 0.000) had independent effects on the decrease in body weight gain and an interaction between the two substances (F = 12.2, P = 0.001) was also noted.

Cd and EtOH alone had no effect on kidney weight (Table 1), but the liver weight was reduced by 8% (P < 0.05) following Cd administration (Table 1). The co-exposure to Cd and EtOH decreased the absolute weight

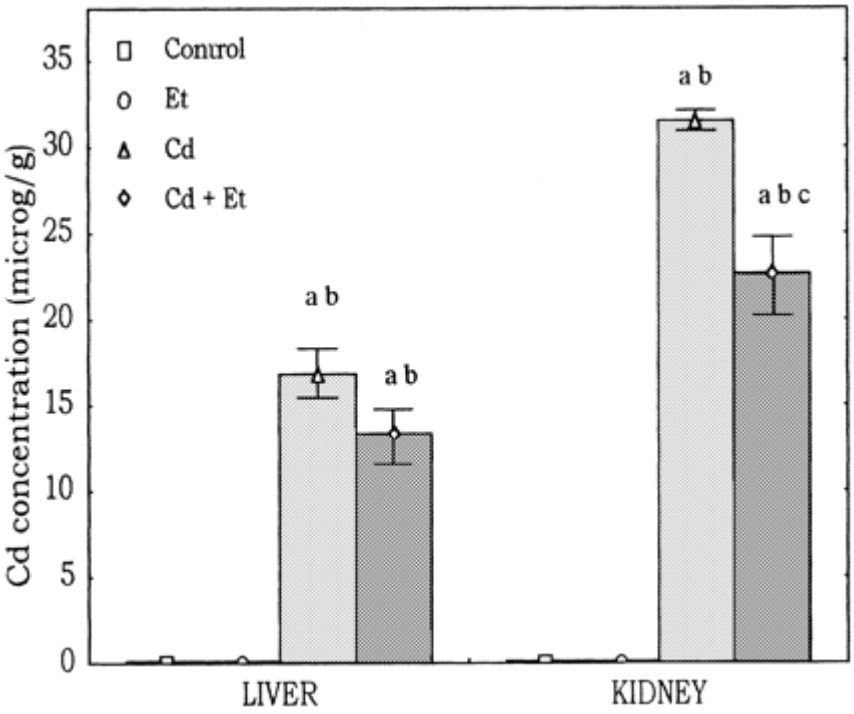

Figure 4. Cd concentration in liver and kidney. Each point represents the mean value ± SEM for 10 rats. a,b,cValues were significantly different (P < 0.05; Mann–Whitney U-test) compared to the control, EtOH and Cd + EtOH groups, respectively.

of both organs by 17 and 13% (P < 0.01), but the relative liver and kidney weights did not change (Table 1). The decrease in liver (F = 30.0, P = 0.000), and also in kidney weight (F = 25.7, P = 0.000) was mainly dependent on Cd administration, and an interactive effect between Cd and EtOH was also observed (F = 9.2, P = 0.004).

The administration of EtOH alone had no influence on Cd concentration in the blood (Figure 3), liver or kidney (Figure 4). Cd concentration in the blood and liver of rats simultaneously treated with Cd and EtOH was in the range of values noted in the group exposed to Cd alone, while the kidney concentration was lower by 28% (P < 0.01) in the combined group compared to Cd alone.

Blood-EtOH concentration

The concentration of EtOH in the blood of rats which were not treated with EtOH (the control and Cd groups) was within the low physiological range (Figure 5). In the animals drinking 10% EtOH alone, its concentration was significantly higher (P < 0.001), but the joint presence of Cd suppressed this increase (Figure 5).

ALAT and AspAT activities in serum

In the serum of rats exposed to Cd, EtOH and to their combination, increased activity of ALAT and AspAT was measured versus control (P < 0.001), but no differences — except in one case — were observed between the enzyme activities of the treated groups (Figure 6).

The changes in serum AspAT activity were independent of Cd (F = 39.2, P = 0.000) and EtOH (F = 17.8, P = 0.000), but an interaction between the two substances (F = 5.9, P = 0.020) was observed. On the other hand, serum activity of ALAT was mainly influenced by Cd (F = 14.8, P = 0.001), and an interactive effect of the substances (F = 7.2, P = 0.011) was also noted.

Biochemical indicators of renal function

Both Cd and EtOH exposure affected some biochemical markers of kidney function. As shown in Table 2, the intensity of these changes was dependent on whether Cd and EtOH were administered separately or in combination. The creatinine clearance was unaffected by Cd or EtOH alone, but their co-administration decreased it by 29% (P < 0.05) versus control and by 25% (P < 0.05) versus the Cd-treated group. The total protein concentration in urine was not influenced by either treatment alone. However, urinary protein excretion in the co-exposed rats was higher (P < 0.05) than in those receiving EtOH or Cd separately (by 11 and 14%, respectively). An increase in serum urea (by 23%, P <

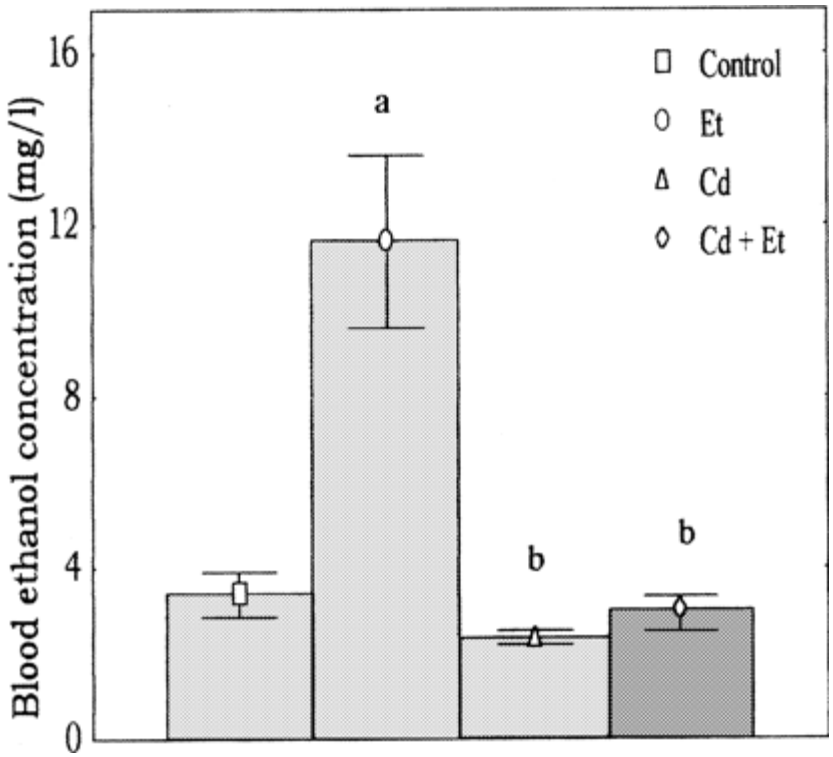

Figure 5. EtOH (Et) concentration in whole blood. Each point represents the mean value ± SEM for 10 rats. a,bValues were significantly different (P < 0.05; Mann–Whitney U-test) compared to the control and EtOH groups, respectively.

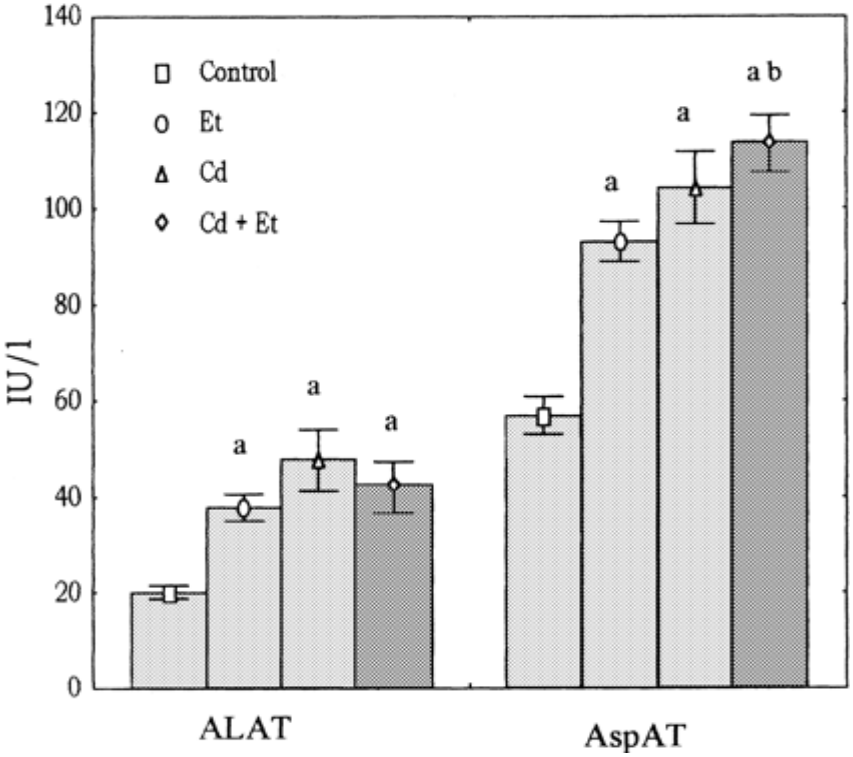

Figure 6. Effects of Cd, EtOH (Et) and their co-administration on serum ALAT and AspAT activities in serum. Each point represents the mean value ± SEM for 10 rats. a,bValues were significantly different (P < 0.05; Mann–Whitney U-test) compared to the control and EtOH groups, respectively.

Table 2. Effects of Cd, EtOH and their co-administration on biochemical indicators of renal function.

Group	Creatinine clearance (ml/min)	Total protein in serum (g/100 ml)	Urea in serum (mg/100 ml)	Total protein in urine (mg/mg creatinine)	Urea in urine (mg/24 h)	ALP in urine (IU/l)
Control	1.18 ± 0.06	3.07 ± 0.22	34.11 ± 0.76	3.02 ± 0.12	602.4 ± 23.7	1.64 ± 0.23
EtOH	1.07 ± 0.07	2.25 ± 0.14[a]	41.98 ± 1.71[a]	2.76 ± 0.09	409.6 ± 19.10[a]	4.26 ± 0.26[a]
Cd	1.11 ± 0.05	2.85 ± 0.16[b]	39.61 ± 0.87[a]	2.67 ± 0.11	385.9 ± 16.3[a]	7.43 ± 0.53[a,b]
Cd + EtOH	0.84 ± 0.11[a,c]	3.22 ± 0.10[b,c]	42.22 ± 2.72[a]	3.05 ± 0.10[b,c]	289.7 ± 9.5[a,b,c]	4.07 ± 0.19[a,c]
Main effect of						
EtOH	$F = 6.2$, $P = 0.018$	NS	$F = 9.4$, $P = 0.004$	NS	$F = 65.2$, $P = 0.000$	NS
Cd	NS	$F = 5.7$, $P = 0.022$	NS	NS	$F = 88.3$, $P = 0.000$	$F = 70.1$, $P = 0.000$
Interactive effect[d]	NS	$F = 14.0$, $P = 0.001$	NS	$F = 8.8$, $P = 0.005$	$F = 7.3$, $P = 0.011$	$F = 85.9$, $P = 0.000$

The rats were exposed to 10% EtOH and/or 50 mg Cd/l for 12 weeks. Values are means ± SEM of 10 animals.
[a,b,c]Values are significantly different ($P < 0.05$; Mann–Whitney *U*-test) compared to control, EtOH and Cd groups, respectively.
[d]Two-way analysis of variance (ANOVA/MANOVA). NS, non-significant.

0.01), and a decrease in serum total protein (by 27%, P < 0.05) accompanied by a decrease in urinary urea (by 32%, P < 0.001), and an increase in urinary ALP activity (2.6-fold, P < 0.001) were observed followed EtOH administration. Exposure to Cd alone decreased the urinary urea level (by 36%, P < 0.001), increased the urinary ALP activity (4.5-fold, P < 0.001) and the serum urea concentration (by 16%, P < 0.001), but had no effect on the total protein concentration in serum and urine. In co-exposed animals, serum protein concentration was unchanged, whereas serum urea was increased (by 24%, P < 0.05) vs controls. Furthermore, urinary excretion of urea was markedly reduced (2.1-fold, P < 0.001) while ALP activity was increased (2.4-fold, P < 0.001). In this group, the changes in urinary urea were more, while those in ALP were less pronounced than in the Cd and EtOH.

As revealed by two-way analysis of variance, depending on the parameter studied, the alterations in the indicators of kidney function were either significantly related to the intake of Cd or EtOH, or were a result of an interaction effect between the two substances (Table 2). An interactive effect was observed in serum total protein, in urinary urea, and in ALP activity. The changes of serum urea and creatinine clearance were mainly influenced by EtOH. In addition to the less or more pronounced interactive effect, total serum protein and urinary ALP were also influenced by Cd, while the urinary urea level was strongly independent from the effect of Cd and EtOH.

DISCUSSION

The present study was undertaken to evaluate the function of the liver and kidney in conditions of co-exposure to EtOH and Cd. Both substances are hepato- and nephrotoxic, but they affect these organs in different ways (Kjellström, 1986; World Health Organization, 1992; Nordberg et al., 1994; Epstein, 1997; Sakurama, 1998; Bunout, 1999; Thurman et al., 1999). Long-term EtOH consumption damages mainly the liver (Bunout, 1999; Thurman et al., 1999), whereas chronic exposure to Cd results, first of all, in tubular dysfunction (Kjellström, 1986; World Health Organization, 1992; Nordberg et al., 1994). Unfortunately, no data are available on the function and structure of both organs in conditions of co-exposure to Cd and EtOH.

We evaluated liver function by measuring plasma ALAT and AspAT activities. As parameters of kidney function, creatinine, total protein and urea concentrations in serum and urine as well as urinary ALP activity, were determined, and creatinine clearance was also calculated. The structure of both organs was assessed on the basis of histopathological analyses.

The level of Cd treatment used in this study corresponds to human (especially smokers) occupational exposure to this heavy metal, or environmental exposure in heavily contaminated areas (World Health Organization, 1992). The level of intoxication with EtOH may be tantamount to its misuse in man (Wis′niewska-Knypl and Wrońska-Nofer, 1994; Brzóska et al., 2002).

Since the relative liver and kidney weights did not change in the co-exposed rats, the decrease in their weights reflects a retardation in body weight gain, which

is a consequence of reduced food (Brzóska et al., 2002) and water intake, and of Cd–EtOH interaction. Other authors also reported the unfavourable effect of co-exposure to Cd and EtOH on body weight gain (Tandon and Tewari, 1987; Gupta and Gill, 2000).

As animals receiving Cd and EtOH simultaneously develop a stronger aversion to drinking than those intoxicated separately, so they ingest less Cd and EtOH. The difference of intake is noteworthy and has to be taken into account in interpretation of the present results.

Cd accumulation in the liver and kidney of rats exposed to this metal alone as well as in combination with EtOH resulted in serious changes in the function of these two organs. Similar or more advanced changes in liver and kidney function under Cd influence, have been reported by others (Aughey et al., 1984; Kjellström, 1986; Mitsumori et al., 1998). Aughey et al. (1984) noted early pathological changes in rat kidney already after 6 weeks of administration of 50 mg Cd/l in drinking water. After 12 weeks, they revealed signs of tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in small areas of the kidney function.

Physiological changes in kidney were observed when Cd concentration in this organ exceeded 10 µg/g and they became more pronounced as concentration increased. At a Cd level of about 30 µg/g, enzymatic changes were observed (Aughey et al., 1984). In our experiment, Cd concentration in kidney ranged from about 20 to 30 µg/g, depending on whether Cd was administered alone or in combination with EtOH. The results of this study and of other investigations (Aughey et al., 1984) show that the critical Cd concentration in the kidney function is lower than 200 µg/g. Such high Cd concentrations in the kidney function were measured in rats fed with diet containing 200 mg Cd/kg for 2–4 months (Mitsumori et al., 1998).

Increased serum transaminase activities were observed in our study following Cd and EtOH co-administration and similar changes have been reported by other authors (Tandon and Tewari, 1987; Thurman et al., 1999).

Morphological observations, together with functional tests, show that Cd and EtOH, administered separately and especially in combination, lead to liver and kidney injury, thus posing a serious risk for health. The changes observed in these organs of co-exposed rats can be a result of an independent effect of Cd and EtOH and also of their interaction. Since EtOH alone also had affected the liver and kidney, on the basis of this study it is difficult to make any definite assessment as to whether EtOH influenced Cd toxicity, and if so, to what extent. However, such an effect of EtOH is very likely, and can be linked to changes in Cd body burden. In this work, we measured the Cd concentrations only in the liver and kidney, but in a previous study a profound effect of EtOH on Cd turnover was reported in the same experimental model (Brzóska et al., 2002).

We have noted that in the Cd + EtOH group the whole Cd pool in the internal organs was at the same level as in those receiving Cd alone, in spite of its lower intake. In the absence of the modifying effect of EtOH, the concentrations and content of Cd in the co-exposed animals should be lower, compared to the Cd-only exposed ones. Thus, our results clearly show that EtOH influences Cd turnover (increases gastrointestinal absorption and retention of absorbed metal), making the organism more susceptible to its accumulation.

Due to the different intakes of Cd and EtOH during their co-administration, than after their separate dosages, we cannot correctly interpret the interactive effects of the two substances on the liver and kidney. Nevertheless, our findings allow us to conclude that EtOH increases Cd nephrotoxicity, although the present results give no clear evidence of enhanced Cd hepatotoxicity. However, it seems likely that, if the consumption of Cd and EtOH were the same in co-exposed and separately exposed animals, the disturbances in liver and kidney function would be more serious in the co-exposed ones.

On the basis of the present and previous studies, we hypothesize that subjects exposed simultaneously to Cd and EtOH are more vulnerable to Cd accumulation and thus its deleterious health effects, including kidney damage. Further studies are needed to explain Cd–EtOH interactions in conditions of long-term co-exposure and their consequences for health.

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