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Comparison of urinary creatine with other biomarkers for detection of cadmium-induced testicular damage

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In this study, biomarkers of testicular damage were compared. In particular, urinary creatine was evaluated as a non-invasive marker of damage. Male rats were exposed to various doses of cadmium chloride, an established testicular toxicant. Pathological damage, testes weights, urinary creatine and creatinine, serum LDH-C4 and serum testosterone were determined. Cadmium chloride caused dose-dependent damage to the testes undetectable at the lowest dose (0·75 mg kg^{-1}) but apparent at a dose of 1·125 mg kg^{-1}. Urinary creatine was significantly raised after doses of 1·125 mg kg^{-1} and above 24–48 hr after dosing, and at the highest dose within 24 hr after dosing. Testes weight and serum testosterone were significantly decreased, and LDH-C4 significantly increased, at the highest dose (3·0 mg kg^{-1}). Therefore urinary creatine was the most sensitive marker of acute cadmium-induced testicular damage and dysfunction.

Keywords: creatine; testicular damage; cadmium; biomarker.

Introduction

Testicular damage is an important toxicological effect of many types of chemicals. Its detection by non-invasive biomarkers is therefore of particular interest. We (and others) have previously shown that a number of testicular toxicants increase the amount of creatine in the urine of rats (Timbrell *et al.* 1995) while Traina *et al.* (1997) have shown this in mice. Thus cadmium (Nicholson *et al.* 1989, Gray *et al.* 1990), 2-methoxyethanol (Rawcliffe *et al.* 1989, Nahas *et al.* 1993), 2-methoxyacetic acid, di-*N*-pentylphthalate, 1,3-dinitrobenzene (Moore *et al.* 1992) and 2,3,5,6-tetramethyl *p*-phenylenediamine (Draper *et al.* 1994) all cause creatinuria and various types of acute testicular damage. Ischaemic damage to the testis will also lead to raised urinary creatine (Gray *et al.* 1990). Repeated exposure to 2-methoxyethanol also results in sustained elevation of urinary creatine (Butterworth *et al.* 1995). However, female rats treated with cadmium (Nicholson *et al.* 1989) or 2-methoxyethanol (Rawcliffe *et al.* 1989), and orchidectomized male rats dosed with cadmium (Gray *et al.* 1990) do not show significant creatinuria. Creatine is therefore a potential biomarker for testicular damage.

Testicular damage may be detected by other biomarkers, such as serum LDH-C4 and testosterone, and with testes weight. LDH-C4 is a germ cell-specific isozyme (Reader *et al.* 1991) whose presence in the serum is indicative of damage to the seminiferous tubules, while testosterone is produced by the Leydig cells under

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the influence of LH and gives an indication of the hormonal status of the individual and other effects on the Leydig cell.

Cadmium chloride has been previously shown to cause significant testicular damage in male rodents (Samarawickram 1979). This may be due to the effect of cadmium on the vasculature of the testis, i.e. constriction of the blood vessels, leading to ischaemic necrosis throughout the testis. The testes are especially sensitive and damage occurs after single doses which do not cause damage to other organs, with permanent and complete destruction of the seminiferous tubules (Samarawickrama 1979).

The aim of this study was to validate and further explore the use of urinary creatine as a marker of acute testicular damage by comparing its ability to detect cadmium-induced pathological damage with two serum markers, LDH-C4 and testosterone, and with testes weight and histopathology.

Materials and methods

Animals

Outbred male Sprague-Dawley rats (Glaxo Research and Development) were housed in individual metabolism cages and allowed to acclimatize for 2–3 days before the start of the study. Animals were allowed food and water **ad lib**.

Treatment

Dose ranges were designed with reference to the published literature to include doses causing both minimal and easily detectable toxic effects. Groups of animals, weighing 185–240 g, were given a single i.p. injection of $CdCl_2$ (Sigma Chemical Co., Poole, UK, 99%) in UHQ water (1 ml kg^{-1}) at doses of 0·75, 1·125, 1·5 or 3·0 mg kg^{-1} body weight. Control animals received the same volume of water i.p. Eight animals were used in each group except for dose groups 0·75 and 3·0 mg kg^{-1} where four animals per group were used.

Urine collection

Urine was collected in ice-cooled vessels for periods of 24 hours. The pH of urine samples was measured.

Experiment termination

Animals were sacrificed 48 h after dosing by exsanguination under anaesthesia, and blood collected from the abdominal aorta for the preparation of serum. The testes, seminal vesicles, liver and kidney were excised and processed for histology and/or biochemical analyses, as described below.

Histology

Testes were fixed in Bouin's fixative before transfer to 10·5 %buffered formalin. After processing and mounting in paraffin-wax blocks, sections were cut and stained with haemotoxylin and eosin. Sections were scored for damage in the following way: the severity of seminiferous epithelial damage and the proportion of affected tubules were each assigned a score out of 5, giving a combined score of 10 for each testis section.

Creatine and creatinine extraction and determination

Creatine was extracted from the testes, seminal vesicles and liver by a modification of the method of Lee *et al.* (1988). Serum and tissue creatine and urinary creatine and creatinine were determined by the enzymatic method of Siedel *et al.* (1984), using a kit obtained from Boehringer as previously described (Gray *et al.* 1990).

LDH-C4 determination

Tissue extracts were prepared and serum and testicular LDH-C4 were assayed by the method of Reader *et al.* (1991), as previously described by Draper and Timbrell (1996).

Testosterone radioimmunoassay

Serum and testicular testosterone were measured using a commercially available single-antibody radioimmunoassay kit (Testosterone/dihydrotestosterone [^{3}H] assay system, Amersham International plc) as described previously (Draper and Timbrell 1996).

Statistics

Values were either compared with pre-dose values by paired Student's 't' test, or for multiple comparisons with a single control by using Dunnetts 't' test. Correlations between biomarkers were carried out using linear correlation analysis.

Results

Clinical effects of $CdCl_2$ administration

The lowest dose of $CdCl_2$ reduced weight gain 24 h after dosing, while doses above 0·75 mg kg^{-1} resulted in a significant weight loss compared with controls (figure 1). At 3·0 mg kg^{-1}, the animals were still losing weight 24–48 h after dosing. Food and water intake were significantly decreased at all but the lowest dose of $CdCl_2$ 24 h post-dose, and were still depressed 24–48 h post-dose (figures 2 and 3).

The effect of $CdCl_2$ on various organ weights

A significant effect on testes and liver weights was only seen at 3·0 mg kg^{-1} (table 1), but there was no significant effect on actual or relative seminal vesicle weights,

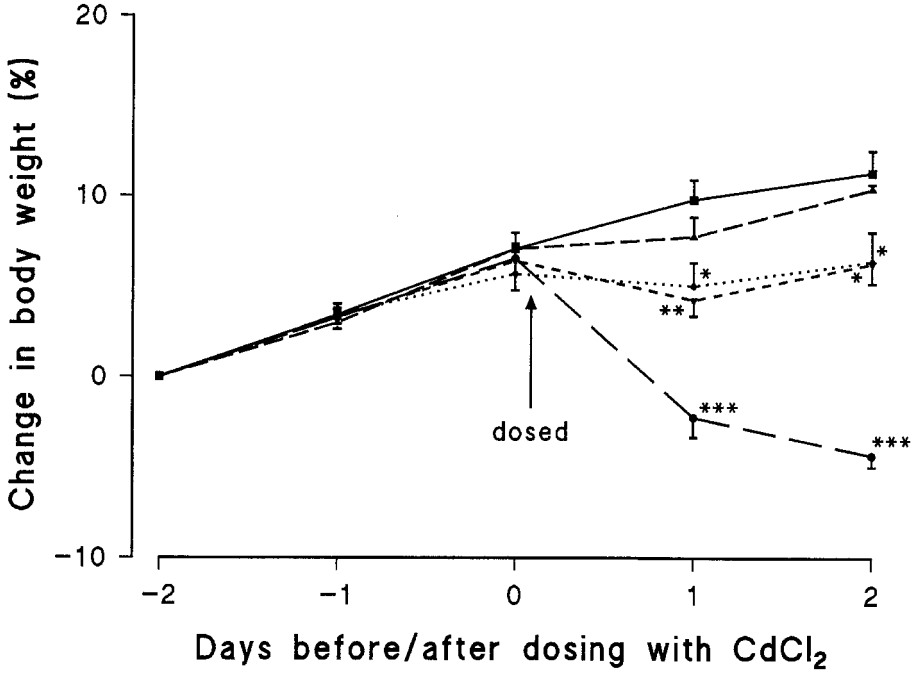

Figure 1. Effect of various doses of cadmium chloride on body weight in rats. (■) Control rats; (▲) rats dosed with 0·75 mg kg^{-1} $CdCl_2$; (▼) 1·125 mg kg^{-1} $CdCl_2$; (♦) 1·5 mg kg^{-1} $CdCl_2$; (●) 3·0 mg kg^{-1} $CdCl_2$. Significant difference from pre-dose value using paired test : * p<0·05; ** p<0·01; *** p<0·001. N =8 except for 0·75 and 3·0 mg kg^{-1} where N =4.

Table 1. Effect of $CdCl_2$ on testes and liver weights in the rat

Dose of $CdCl_2$ (mg kg^{-1})	Actual testes wt. (g)	Relative testes wt. (g 100 g^{-1} body wt)	Actual liver wt. (g)	Relative liver wt. (g 100 g^{-1} body wt)
0	2·39 ± 0·07	0·91 ± 0·02	11·95 ± 0·29	4·54 ± 0·10
0·75	2·53 ± 0·03	0·94 ± 0·01	13·00 ± 0·39	4·81 ± 0·07
1·125	2·38 ± 0·05	0·96 ± 0·01	10·97 ± 0·28	4·41 ± 0·09
1·5	2·30 ± 0·04	0·91 ± 0·02	11·06 ± 0·60	4·36 ± 0·12
3·0	1·78 ± 0·08**	0·87 ± 0·05	9·64 ± 0·35**	4·26 ± 0·09

Data are mean ± SEM. Significant difference from control (Dunnett's test): **$p<0·01$.
$N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

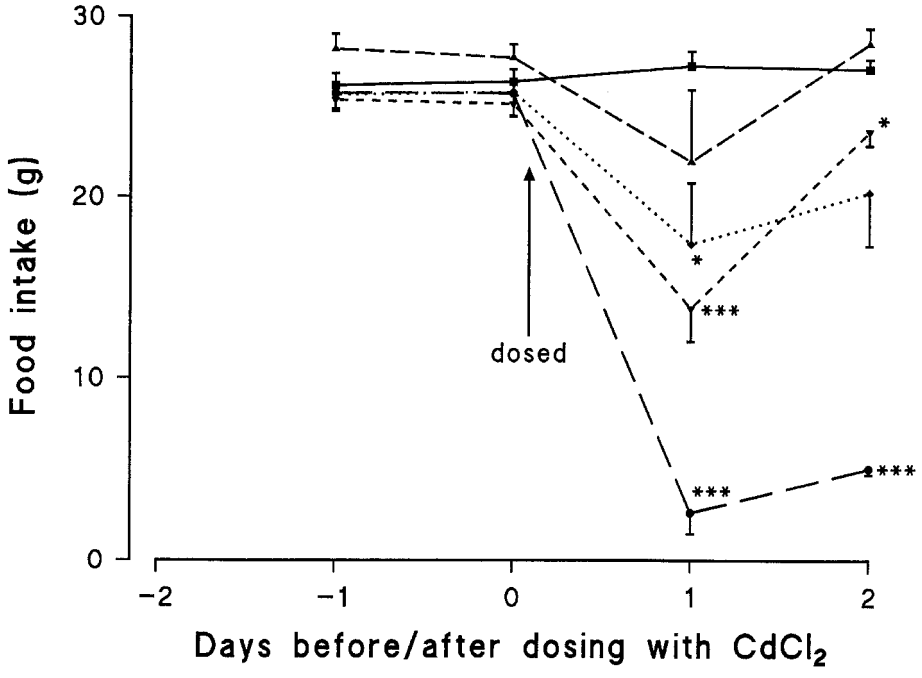

Figure 2. Effect of various doses of cadmium chloride on food intake in rats. (■) Control rats; (▲) rats dosed with 0·75 mg kg^{-1} $CdCl_2$; (▼) 1·125 mg kg^{-1} $CdCl_2$; (♦) 1·5mg kg^{-1} $CdCl_2$; (●) 3·0 mg kg^{-1} $CdCl_2$. Significant difference from pre-dose value using paired test: * $p<0·05$; *** $p<0·001$. $N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

although they were decreased to about half the control value at the highest dose (data not shown).

Effect of $CdCl_2$ on the histopathology of the testis

There was no observable effect of cadmium on the testis at a dose of 0·75 mg kg^{-1}, and minimal damage at 1·125 mg kg^{-1}. Damage was characterized by vacuolization and cellular necrosis in the seminiferous tubules, especially in Stages VII–IX and

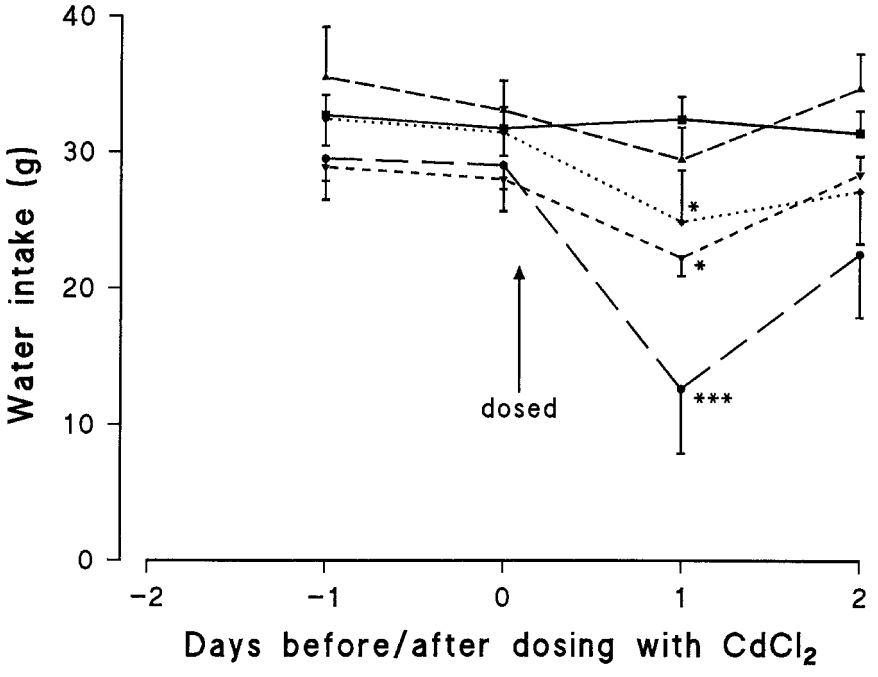

Figure 3. Effect of various doses of cadmium chloride on water intake in rats. (■) Control rats; (▲) rats dosed with 0·75 mg kg^{-1} $CdCl_2$; (▼) 1·125 mg kg^{-1} $CdCl_2$; (♦) 1·5 mg kg^{-1} $CdCl_2$; (●)3·0 mg kg^{-1} $CdCl_2$. Significant difference from pre-dose value using paired test: * $p<0·05$; *** $p<0·001$. $N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

XII–XIV. Sections from half the animals in the 1·5 mg kg^{-1} group showed little or no damage, while the others showed extensive damage, including tubular disorganization, widespread vacuolization, multinucleate bodies, some sloughing of cells into the lumen and, in some, interstitial hyperplasia and macrophage invasion. At the highest dose, all animals were affected, the damage being similar to that seen in the affected animals at 1·5 mg kg^{-1} but more extensive.

There was a dose-related increase in the mean pathology score (table 2). Severity was judged according to the degree of vacuolization, cellular necrosis and tubular disorganization apparent in the seminiferous tubules, and the degree of interstitial hyperplasia and macrophage invasion.

Creatine and creatinine levels in urine, serum and tissues after $CdCl_2$ administration

$CdCl_2$ caused a slight increase in urinary creatine 24 h after dosing, but this was only significant at 3·0 mg kg^{-1} (figure 4). At 24–48 h, there was a larger increase, significant both at 1·125 and 3·0 mg kg^{-1}. Although urinary creatine was elevated at 1·5 mg kg^{-1}, it was not significant due to great variation within this group. There was a dose-related decrease in urinary creatinine, which was significant at 3·0 mg kg^{-1} 24 h after dosing, and significant at all doses except 1·5 mg kg^{-1} at 24–48 h after

Table 2. Pathology score of testis sections from $CdCl_2$-treated rats

Dose of $CdCl_2$ (mg kg^{-1})	Average combined score
0	1·2
0·75	1·2
1·125	2·1
1·5	5·0
3·0	9·0

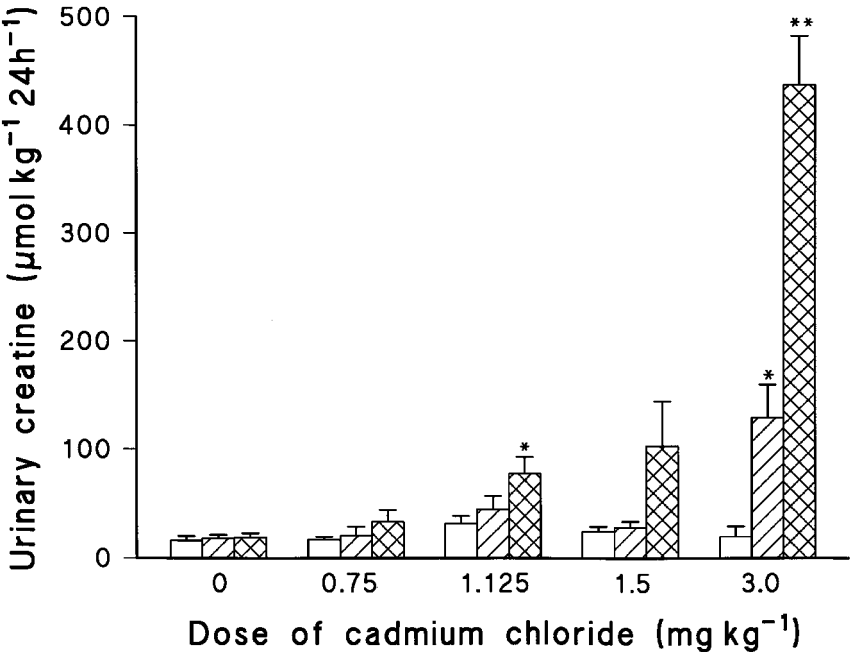

Figure 4. Effect of various doses of cadmium chloride on urinary creatine excretion by rats. (□)24 h pre-dose urine sample; (▨) 0–24 h post-dose urine sample; (▩) 24–48 h post-dose urine sample. Significant difference from pre-dose value using paired test: * $p<0{\cdot}05$; ** $p<0{\cdot}01$. $N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

dosing (figure 5). The urinary creatine:creatinine ratio showed the same dose-related effects as seen with creatine excretion, but was also significantly raised at 1·5 mg kg^{-1} $CdCl_2$ 24 h post-dose (figure 6).

At the lowest dose, $CdCl_2$ caused a slight decrease in serum creatine, but an increase at all other doses, which was significant at 3·0 mg kg^{-1} (table 3). There was also a significant decrease in testicular creatine concentration at 3·0 mg kg^{-1} (table 3). The concentration of creatine in the liver and the total creatine content were decreased at all doses, but these effects did not seem to be dose-dependent (table 3). Cadmium chloride had no significant effect on seminal vesicle creatine concentration (table 3).

Table 3. The effect of $CdCl_2$ on serum and tissue creatine levels

Dose of $CdCl_2$ (mg kg^{-1})	Serum creatine (μmoles ml^{-1})	Testicular creatine		Liver creatine		Seminal vesicle creatine	
		(nmoles mg^{-1} tissue)	(μmoles $tissue^{-1}$)	(nmoles mg^{-1} tissue)	(μmoles $tissue^{-1}$)	(nmoles mg^{-1} tissue)	(μmoles $tissue^{-1}$)
0	0·19 ± 0·01	19·93 ± 1·16	47·25 ± 2·31	0·81 ± 0·14	9·62 ± 1·73	14·00±0·14	4·75±0·89
0·75	0·17 ± 0·01	18·90 ± 0·48	47·92 ± 1·34	0·32 ± 0·09**	4·18 ± 1·09	11·32±1·41	4·36±0·76
1·125	0·23 ± 0·01	20·80 ± 0·59	49·57 ± 1·81	0·29 ± 0·03**	3·22 ± 0·27	14·08±0·88	4·53±0·62
1·5	0·23 ± 0·02	15·00 ± 2·56	34·98 ± 8·66	0·35 ± 0·04**	3·77 ± 0·32	13·93±0·99	5·07±0·67
3·0	0·28 ± 0·01**	1·14 ± 0·33**	2·04 ± 0·62**	0·40 ± 0·02	3·89 ± 0·22	11·25±1·76	2·23±0·70

Data are mean ± SEM.
Significant difference from control (Dunnett's test): *$p<0·05$; **$p<0·01$. $N=8$ except 0·75 and 3·0 mg kg^{-1} where $N=4$.

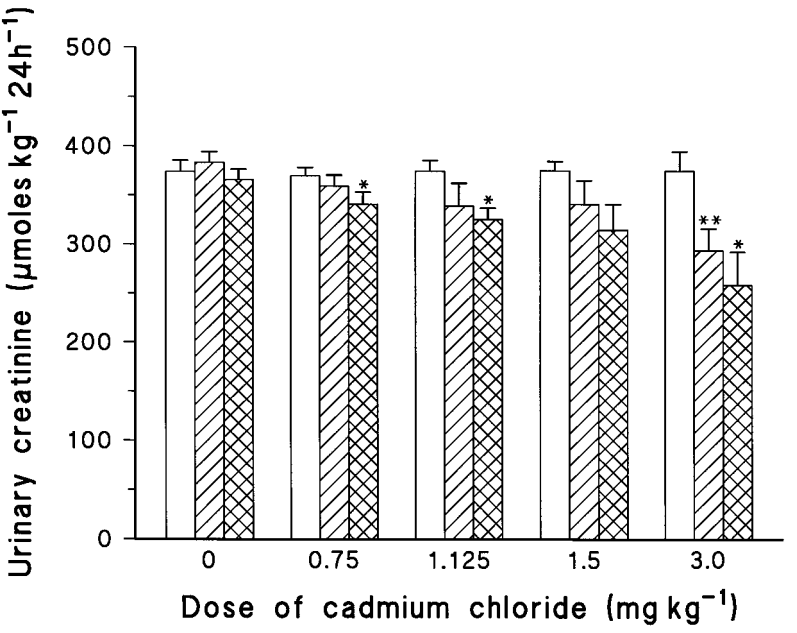

Figure 5. Effect of various doses of cadmium chloride on urinary creatinine excretion by rats. □ 24 h pre-dose urine sample; ▨ 0–24 h post-dose urine sample; ▩ 24–48 h post-dose urine sample. Significant difference from pre-dose value using paired test: * $p<0{\cdot}05$; ** $p<0{\cdot}01$. $N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

Serum and testicular LDH-C4 and testosterone levels

A significant increase in serum LDH-C4 and a significant decrease in testicular LDH-C4 occurred only at the highest dose. There was a dose-related decrease in serum testosterone that was significant at 3·0 mg kg^{-1}, but no change in testicular testosterone (table 4).

Correlation of parameters indicative of testicular damage after dosing with $CdCl_2$

All parameters indicative of testicular damage correlated with the 'pathology score'. There was also extensive correlation between parameters when compared with urinary creatine excretion at 24–48 h after treatment and with actual and relative testes weights. However, serum LDH-C4 and actual and relative testes weights did not correlate with serum testosterone (table 5).

Discussion

Cadmium chloride caused a marked, dose-dependent increase in testicular damage (table 2), characterized by necrosis, vacuolization and cellular disorganization of the seminiferous epithelium with some interstitial hyperplasia evident at the highest doses. Gray *et al.* (1990) have shown that surgical ligation of the pampiniform plexus produces similar effects. This supports the suggestion that cadmium-induced testicular necrosis is the result of ischaemia through some interference with the vascular supply (Aoki and Hoffer 1978).

Table 4. The effect of $CdCl_2$ on LDH-C4 and testosterone in the serum and testis of the rat

Dose of $CdCl_2$ (mg kg^{-1})	Serum LDH-C4 (U l^{-1})	Testicular LDH-C4 (U g^{-1} testis)	Serum testosterone (ng ml^{-1})	Testicular testosterone (μg g^{-1} testis)
0	34·36 ± 10·28	6·78 ± 0·37	701·8 ± 170·6	32·13 ± 6·98
0·75	16·28 ± 5·99	6·41 ± 0·46	462·9 ± 84·9	27·60 ± 5·72
1·125	15·71 ± 1·53	6·97 ± 0·39	506·9 ± 222·2	24·32 ± 8·63
1·5	38·83 ± 6·41	5·82 ± 0·76	331·9 ± 95·8	29·77 ± 6·43
3·0	82·00 ± 7·53**	2·11 ± 0·50**	3·6 ± 6·4*	38·47 ± 9·29

Data are mean ± SEM.
Significant difference from control (Dunnett's test): *$p<0·05$; **$p<0·01$.
$N=8$ except 0·75 and 3·0 mg kg^{-1} where $N=4$.

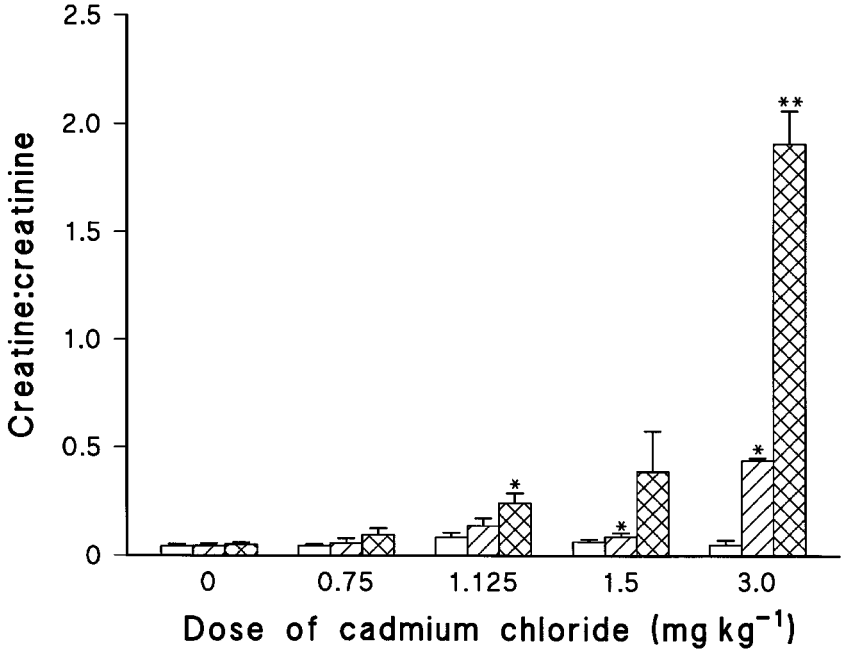

Figure 6. Effect of various doses of cadmium chloride on the urinary creatine:creatinine ratio in rats. □ 24 h pre-dose urine sample; ▨ 0–24 h post-dose urine sample; ▩ 24–48 h post-dose urine sample. Significant difference from pre-dose value using paired test: * $p<0·05$; ** $p<0·01$. $N=8$ except for 0·75 and 3·0 mg kg^{-1} where $N=4$.

In agreement with previous work by Gray *et al.* (1990) and Nicholson *et al.* (1989), this study showed a dose-dependent increase in urinary creatine excretion (figure 4). As $CdCl_2$ does not cause significant creatinuria in orchidectomized rats (Gray *et al.* 1990) and causes much smaller elevations in urinary creatine in female rats (Nicholson *et al.* 1989), the testes are the most obvious source. The effects of reduced food intake might contribute to this creatinuria but could not account for all of it, as the body-weight loss in the 48 h period after the hig

Table 5. Correlation of parameters indicative of testicular damage measured in rats treated with $CdCl_2$

Parameter 1	Parameter 2	*r* value	*p* value
Urinary creatine	Pathology score	0·81	<0·001
"	Actual testes weight	–0·79	<0·001
"	Relative testes weight	–0·47	0·007
"	Serum LDH-C4	0·73	<0·001
"	Serum testosterone	–0·52	0·003
Serum LDH-C4	Pathology score	0·67	<0·001
"	Actual testes weight	–0·59	<0·001
"	Relative testes weight	–0·43	0·017
"	Serum testosterone	–0·32	0·083
Serum testosterone	Pathology score	–0·51	0·003
"	Actual testes weight	0·32	0·078
"	Relative testes weight	0·03	0·868
Pathology score	Actual testes weight	–0·71	<0·001
"	Relative testes weight	–0·36	0·043

r values were determined using linear correlation analysis.

expected to contribute approximately 135 μmoles per rat of creatine (Draper unpublished observations) whereas the actual increase in creatine excretion during this period was about 530 μmoles per rat (figure 4). Thus, in the current study, the amount of creatine lost from the testes at 3·0 mg kg^{-1} $CdCl_2$ at 48 h (about 45 μmoles, table 3), added to the expected creatinuria due to body-weight loss following reduced food intake 48 h post-dose (about 135 μmoles per rat), accounts for about one third of the increase in urinary creatine seen (about 530 μmoles per rat, figure 4). At the lower dose of 1·125 mg kg^{-1} where there is no loss of body weight, only a decrease in weight gain (figure 1) there is no significant loss from the testes to account for the excess creatine in the urine. This suggests that $CdCl_2$ increases creatinuria partially by causing leakage from the testes, but may have some more subtle effects on creatine synthesis, secretion and metabolism.

The decrease in serum LDH-C4 at 0·75 and 1·125 mg kg^{-1} was unexpected (table 4), but might be due to interference from residual cadmium ions in the assay. This would also mask increases at higher doses, explaining why serum LDH-C4 was only significantly increased at the highest dose, although 1·125 mg kg^{-1} $CdCl_2$ caused obvious histopathological damage. Itoh (1984) showed a decrease in LDH-C4 activity in testicular extracts from mice at doses as low as 1·2 mg kg^{-1} $CdCl_2$. In our study, a slight decrease in testicular LDH-C4 was observed at 1·5 mg kg^{-1}, but was only significantly lowered at 3·0 mg kg^{-1} (table 4). Cadmium chloride caused a decrease in serum testosterone at all doses, significant only after 3·0 mg kg^{-1} (table 4). However, there was no effect on testicular testosterone.

The extensive correlation between the parameters indicative of testicular damage (table 5) suggests that all three biochemical markers were able to detect cadmium-induced damage. However, it seemed that urinary creatine showed a better relation to the degree of damage than the other two markers, and might have been able to detect this damage at lower doses. In addition, there was an observable, although statistically not significant, rise in urinary creatine excretion at 0·75 mg kg^{-1} $CdCl_2$ 24–48 h post-dose, with no obvious pathological damage. This rise did not seem to be due to reduced food intake and/or loss of body weight and may indicate subtle effects on the biochemical status of testicular cells. The Sertoli cells of t[illegible]

to synthesize creatine (Lee *et al.* 1994), which has been shown in isolated seminiferous tubules *in vitro* (Moore *et al.* 1989). Interference with this may lead to elevated levels. Isolated Sertoli cells *in vitro* will secrete creatine in response to exposure to sub-toxic concentrations of cadmium (Moore *et al.* 1997) and it has been suggested that the seminal vesicle will secrete creatine in response to androgen (Lee *et al.* 1991).

A dose-dependent decrease in urinary creatinine was also observed in our study. Urinary creatinine is routinely measured as an indicator of muscle status and this decrease may simply reflect the effects of reduced food intake or muscle wastage. However, a significant decrease occurred at doses where there was no loss of body weight (0·75 mg kg^{-1}) which may indicate more subtle effects on creatine uptake, metabolism and excretion in the testes. The urinary creatine:creatinine ratio may therefore be a useful marker along with urinary creatine.

Testes weights are a rough indicator of testicular damage and can only be measured after termination. In these studies, actual testes weights only showed a significant decrease at the highest dose of $CdCl_2$.

Serum LDH-C4 is known to be specific to the testes and is therefore ideal as a marker of testicular damage. Increases in serum LDH-C4 are then a clear indication of damage to testicular cells resulting in leakage into the general circulation. It will detect obvious damage, but may show no change if there are subtle detrimental effects on the biochemical status of those cells.

Serum testosterone indicates the hormonal status of the testes, and may be important in the detection of specific toxic effects on the Leydig cells or the hypothalamic-pituitary-testicular axis. However, results often show great variation, because of the cyclical nature of testosterone production. In this study, serum testosterone was significantly decreased only at the highest dose of $CdCl_2$.

Both LDH-C4 and testosterone are measured in the blood. This is an invasive technique and is not ideal in routine clinical or industrial monitoring, or in animal studies where repeated sampling is required. Urinary markers are preferable because urine is easier to collect and provides larger volumes for analysis than blood or plasma. In this study, urinary creatine was raised in a dose-dependent manner at all doses of $CdCl_2$.

We found that urinary creatine was more sensitive than testes weight, serum LDH-C4 and serum testosterone at detecting acute toxicant-induced testicular damage. It may also be able to detect subtle toxicant-induced effects on the biochemical status of the testicular cells present prior to the appearance of pathological damage.

Acknowledgements

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References

Aoki, A. and Hoffer A. P. 1978, Reexamination of the lesions in rat testis caused by cadmium. *Biology of Reproduction*, **18,** 579–581.

Butterworth, M., Creasy, D. and Timbrell, J. A. 1995, The detection of subchronic testicular damage using urinary creatine: studies with 2-methoxyethanol. *Archives of Toxicology*, **69**, 209–211.

Draper, R. P., Waterfield, C. J., York, M. J. and Timbrell, J. A. 1994, Studies on the muscle toxicant 2,3,5,6-tetramethyl p-phenylenediamine: effects on various biomarkers including urinary creatine and taurine. *Archives of Toxicology*, **69**, 111–117.

Draper, R. P., Creasy, D. M. and Timbrell, J. A. 1996, Comparison of urinary creatine with other biomarkers for the detection of 2-methoxyethanol-induced testicular damage. *Biomarkers*, **1**, 190–195.

Gray, J., Nicholson, J. K., Creasy, D. M. and Timbrell, J. A. 1990, Studies on the relationship between acute testicular damage and urinary plasma creatine concentration. *Archives of Toxicology*, **64**, 443–450.

Itoh, R. C. 1984, Changes in lactate dehydrogenase isozyme-X activity induced by cadmium administration. *Toxicology Letters*, **20**, 173–176.

Lee, H. J., Gong, C., Wu, S. and Iyenagar, M. R. 1991, Accumulation of phosphocreatine and creatine in the cells and fluid of mouse seminal vesicles is regulated by testosterone. *Biology of Reproduction*, **44**, 540–545.

Lee, H. J., Ogawa, H., Fujioka, M. and Gerton, G. L. 1994, Guanidinoacetate methyltransferase in the mouse: extensive expression in Sertoli cells of testis and in microvilli of caput epididymis. *Biology of Reproduction*, **50**, 152–162.

Moore, N. P., Timbrell, J. A. and Gray, T. J. B. 1989, Isolated rat seminiferous tubules synthesize guanidoaceticacid and creatine from arginine. *Biochemical Society Transactions*, **17**, 520.

Moore, N. P., Creasy, D. M., Gray, T. J. B. and Timbrell, J. A. 1992, Urinary creatine profiles after administration of cell specific testicular toxicants to the rat. *Archives of Toxicology*, **66,** 435–442.

Nahas, K., Le Net, J-L, Provost, J. P. and Tomaszewski, K. E. 1993, An investigation of urinary creatine excretion as a potential marker for testicular damage. *Human and Experimental Toxicology*, **12**, 173–176.

Nicholson, J. K., Higham, D. P., Timbrell, J. A. and Sadler, P. J. 1989, Quantitative high resolution ^{1}H-NMR urinalysis studies on the biochemical effects of cadmium in the rat. *Molecular Pharmacology*, **36**, 398–404.

Rawcliffe, L., Creasy, D. M. and Timbrell, J. A. 1989, Urinary creatine as a possible marker for testicular damage: studies with the testicular toxic compound 2-methoxyethanol. *Reproductive Toxicology*, **3**, 269–274.

Reader, S. C. J., Shingles, C. and Stonard, M. D. 1991, Acute testicular toxicity of 1,3-dinitrobenzene and ethylene glycol monomethyl ether in the rat: evaluation of biochemical effect markers and hormonal responses. *Fundamental and Applied Toxicology*, **16**, 61–70.

Samarawickrama, G. P. 1979, Biological effects of cadmium in mammals. In *The chemistry, biochemistry and biology of cadmium*, M. Webb, ed. (Elsevier/North Holland Biomedical Press, Amsterdam), pp. 341–421.

Siedel, J., Mollering, H. and Ziegenhorn, J. 1984, Sensitive color reagent for the enzymatic determination of creatinine. *Journal of Clinical Chemistry*, **30**, 968–969.

Timbrell, J. A. 1995, Use of urinary taurine and creatine as biomarkers of organ dysfunction and metabolic perturbations. *Comparative Haematology International*, **5**, 112–119.

Traina, M. E., Fazzi, P., Urbani, E. and Mantovani, A. 1997, Testicular creatine and urinary creatine-creatinine profiles in mice after the administration of the reproductive toxicant methoxyacetic acid. *Biomarkers*, **2**, 103–110.