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COMPARATIVE EFFICACY OF CHELATORS TO REMOVE RENAL CADMIUM BURDEN IN ISOLATED PERFUSED RAT KIDNEYS

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Résumé

Trois agents chélateurs (l'acide diéthylène triamine penta-acétique, DTPA; l'acide méso-2,3-dimercaptosuccinique, DMSA; l'acide 2,3-dimercapto-1-propanesulfonique, DMPS) ont été comparés quant à leur efficacité à mobiliser du cadmium (Cd) accumulé dans le tissu rénal. Des reins prélevés chez des rats exposés durant 3 j au Cd (acétate de Cd , 0.75 mg/kg·j, i.p) ont été isolés et perfusés in vitro, à l'aide d'un système de reperfusion utilisant une solution de Krebs-Henseleit, pH 7.4, contenant 8 acides aminés et 6% d'albumine. Les concentrations de Cd dans le perfusat et l'urine ont été mesurées par spectrométrie d'absorption atomique. Six périodes de clearance, après une période d'équilibration de 20 min, ont été obtenues. Le DMSA et le DMPS ont mobilisé le Cd à partir du tissu rénal, comme l'ont montré les augmentations dose-dépendantes des concentrations de Cd dans l'urine et le perfusat. L'accumulation de Cd était nettement plus élevée dans le perfusat que dans l'urine, indiquant que l'effet des chélateurs se marquait surtout au niveau tubulaire basolatéral. Le DTPA n'induisait qu'une faible mobilisation de Cd dans l'urine et le perfusat, et son efficacité était clairement inférieure à celle des autres chélateurs. Comme prévu, la quantité de Cd présente dans le tissu rénal après perfusion par le DMSA ou le DMPS diminuait en fonction de l'efficacité des chélateurs, jusqu'à des valeurs inférieures de 46% au taux rénal de Cd avant perfusion. Le DMPS apparaissait induire une excretion urinaire de Cd plus importante que celle induite par le DMSA, une caractéristique qui pourrait être liée à une sécrétion tubulaire du chélateur, qui a été décrite antérieurement. Un intervalle de temps prolongé (1-2 semaines) entre le moment de l'administration du Cd et la perfusion du rein avec le DMPS induisait une augmentation de l'excrétion urinaire de Cd. Tous les chélateurs se sont montrés néphrotoxiques à concentrations élevées.

Mots-clés: rein isolé perfusé; DTPA; DMSA; DMPS; Cd; néphrotoxicité; antidotes; chélateurs.

Abstract

The ability of 3 chelators (diethylene-triamine pentaacetatic acid: DTPA; meso-2,3-dimercaptosuccinic acid: DMSA; 2,3-dimercapto-1-propanesulfonic acid: DMPS) to mobilize cadmium (Cd) accumulated in renal tissue following ip administration of Cd during 3 days

was evaluated in vitro by the isolated perfused rat kidney technique, using a reperfusion system with a Krebs-Henseleit solution containing 8 amino acids and 6% albumin. Cd concentration in urine and perfusate was measured, by atomic absorption spectrophotometry, in samples collected during three 20-min clearance periods, after a 20 min equilibration time. DMSA and DMPS mobilized Cd from renal tissue, as shown by dose-dependent increases of Cd in urine and perfusate. A much larger amount of Cd appeared in the perfusate than in urine, indicating that the chelators were mostly effective by removing Cd from basolateral side of renal tubules. DTPA elicited a much smaller increase of Cd excretion both in urine and perfusate and was clearly less effective than the 2 other chelators. As expected, total amount of Cd in renal tissue after exposure to DMSA or DMPS decreased in relation with the efficacy of chelators to mobilize Cd, down to 46% of the initial values of tissue Cd content. DMPS appeared to induce a larger Cd urinary excretion as compared to DMSA, a characteristic which may be related to a secretory tubular transport of the chelate, as reported previously. A larger time-interval between Cd administration and perfusion of DMPS was associated with an enhanced removal of Cd from renal tissue. All chelators showed renal, concentration-dependent functional toxicity.

Key words: IPRK; chelators; DTPA; DMSA; DMPS; Cd; nephrotoxicity; Cd antidotes.

Introduction

Exposure to cadmium (Cd) entails acute and/or chronic, dose-related toxicity which is expressed in a number of target organs (1). Acute toxicity induced by Cd results, depending on the exposure conditions, in gastrointestinal or pulmonary lesions. In cases of prolonged exposure, the metal accumulates mostly in the liver and kidneys: up to 80 % of the body burden is found in these two organs (2). Renal accumulation of Cd, related to its extremely

long biologic half-life (ca. 10-20 years) results in a nephropathy which has been described in occupationally exposed workers as well as in population groups living in a polluted environment (2). Cd is first accumulated in the liver, where it induces the synthesis of metallothionein (MT). The Cd-MT complex is then slowly released into the circulation and is taken up in the kidneys, where the metal induces new synthesis of MT and is stored for prolonged periods. Renal damage occurs above a threshold level of the toxic metal, when the capacity of MT synthesis by unbound Cd in tubular cells is exceeded (3).

In order to develop a possible therapeutic approach in cases of human Cd poisoning, the ability of various Cd chelators to mobilize and increase the excretion of the toxic metal has been investigated experimentally (4). The compounds used for this purpose included in particular the following: dimercaptopropanol (5,6,7); EDTA and DTPA (8); DMPS (9,10); DMSA (9,10); dithiocarbamates (5,11-15). The ability of chelating agents to reduce toxic metal cell content has also been tested in cell cultures previously exposed to Cd (16,17). While several studies indicated that chelators increased the mobilization and reduced the kidney levels of Cd, other investigations provided evidence that redistribution of Cd may occur following chelator administration, resulting in enhanced Cd levels in some tissues, notably the kidney, and thus suggesting potential deleterious effects of chelators for treating Cd poisoning (6,13). Such a redistribution appears to occur most notably when the time-interval between Cd and chelator administration is short (5,6).

The characterization of the specific effect of chelators on renal Cd content is made difficult by the metal redistribution mentioned above. The present study was therefore intended to investigate the comparative short-term effect of several chelators on the renal Cd burden in conditions where systemic redistribution could be precluded. For that purpose, we resorted to an isolated, perfused rat kidney (IPRK) preparation, which has been described in detail previously (18,19). Despite the functional limitations of such a preparation, it should allow to delineate the ability of chelators to mobilize Cd in kidneys from animals previously exposed to the metal, in the absence of any redistribution. This technique has been used, in relation with studies on Cd toxicity, to investigate the uptake of the metal in various experimental conditions (20,21), and to study the kinetics of chelators (22). We planned to evaluate the comparative efficacy of three chelators to mobilize Cd from kidneys of rats at short (3 days) or longer (1 or 2 weeks) times after Cd administration to the animals.

Methods

Male Wistar rats (Kleintierfarm Madörin, Füllinsdorf, BL, Switzerland) weighing between 350 and 550 g were used. The animals had free access to rat pellets and water, but were fasted overnight before perfusion. During the three days preceding the perfusion experiments, they received one i.p. injection per day of cadmium acetate (CdA), 0.75 mg/kg b.w. Twenty-four hours after the third injection, the right kidney was isolated and perfused.

Kidney preparation

After induction of anesthesia (Na pentobarbital, ca. 60 mg/kg i.p.), heparin (35 IU, Fluka AG, Buchs) was injected through the jugular vein, and the right ureter was cannulated with PE-50 polyethylene tubing with a short tapered tip. For the experiments with non-filtering kidneys, the right ureter was ligated during 20-30 min before removal of the kidney.

Isolated kidney perfusion was carried out as described previously (18,19). Briefly, the right renal artery was cannulated via the superior mesenteric artery, and perfusion was started immediately. The isolated right kidney was then transferred to the perfusion setup, using a recirculating system (using 70 ml of perfusion fluid at 37 °C). Perfusion fluid was a Krebs-Henseleit bicarbonate (KHB) buffer containing (mM): NaCl 116, KCl 3.5, $NaHCO_3$ 25, $MgSO_4 \cdot 7H_2O$ 2.44, KH_2PO_4 1.53, $CaCl_2 \cdot 2H_2O$ 1.5. Bovine serum albumin (Fraction V, Boehringer Mannheim, Germany) was added at a concentration of 6%. The albumin was previously dialyzed for 18-24 h against protein-free KHB. The medium contained also glucose (6.6 mM) and eight amino-acids (Sigma, St-Louis, USA): methionine 0.5, alanine 2, glycine 2, serine 2, arginine 1, proline 2, isoleucine 1, aspartic acid 3, isoleucine 1 mM. Before the experiments, the perfusate was filtered through a 0.22 μm filter, was gassed with O_2/CO_2 (95:5) for 20 min, and pH was adjusted to 7.4. The perfusate was prepared fresh for each experiment. Perfusions were carried out at 37 °C.

Experimental protocol

Isolated kidney perfusion was carried out in kidneys of either normal or Cd-exposed rats. In the latter group, Cd was administered (0.75 mg/kg, i.p) during 3 days prior to the perfusion.

The Cd-mobilizing effect of three chelators was studied: calcium trisodium salt of diethylenetriaminepentaacetic acid (DTPA); *meso*-2,3-dimercaptosuccinic acid (DMSA); 2,3-dimercaptopropane sulfonic acid, Na^+ salt (DMPS). DTPA was prepared according to Cantilena et al (8). The following concentrations of chelators were used: DTPA: 7.1 and 14.2 mM (3.6 and 7.2 g/L, respectively); DMPS: 2.4, 11.9 and 23.8 mM (0.5, 2.5 and 5 g/L, respectively); DMSA: 5.5 and 27.4 mM (1.0 and 5.0 g/L respectively). All chemicals were from Sigma.

Kidneys from Cd exposed rats were perfused with various concentrations of the chelators (cf Table 3), 24 hours after the last Cd injection. In additional groups of rats, the effect of DMPS (11.9 or 23.8 mM) was evaluated after 1 or 2 weeks following the last Cd injection.

Perfusions lasted 80 min. Renal function was measured during 6 consecutive 10-min clearance periods, beginning 20 min after renal artery cannulation. Samples (1 ml) of perfusate were collected at the mid-point of each period for clearance estimates. Urine samples were collected during 10 min, and volume was estimated gravimetrically.

The total amount of Cd was measured in left kidneys, and in right kidneys after perfusion. Tissues were digested in 2 ml HNO_3 65% (Suprapure, Merck) at 110°C during 3 h.

Analyses

Inulin clearance measurements were used to evaluate GFR. The concentration of inulin (Polyfructosan, Laevosan) in urine and perfusate was measured by spectrocolorimetry, after acid digestion (23). Na and K were estimated by flame photometry (Beckman, Electrolyte 2). Glucose was measured by the glucose-oxydase method. Cadmium in acid-digested tissues and fluids was estimated by atomic absorption spectrometry, using electrothermal activation, as reported previously (24)

Calculations

Cd content in renal tissue was calculated from the wet weights and the concentration in the ashing solutions.Total Cd urinary excretion was calculated from urine concentrations and urine volume. Total amount of Cd in the perfusate was calculated from the concentration at the end of the experiment, and the volume of perfusate.

Statistics

All results are expressed as mean ± standard error of the mean. The statistical significance of differences was evaluated by the Mann-Whitney, non-parametric test. $P<0.05$ was considered as the level of significance.

Results

IPRK function. In order to validate the IPRK set-up used in the present work, we measured the main functional properties of the present preparation, using 10 kidneys from normal rats for 3 clearance periods of 20 min each. Results are presented in Table 1. GFR values remained stable during the 60 min of clearance studies, while FE_{Na} tended to increase until the last period, but remained between approximately 6 to 10% of filtered load. Glucose reabsorption was extensive at the three time periods.

Effect of Cd on renal function. In order to evaluate the effect of Cd pretreatement on IPRK function, the main functional properties of 10 kidneys from rats pretreated or not with Cd were compared during 3 clearance periods of 20 min each. As shown in Table 2, renal functional parameters were little affected by 3 days of Cd administration, with the only exception of FE_K.

Effects of Cd chelators on Cd mobilization. The Cd chelating properties of the various agents used in the present study are summarized in Tables 3 to 5. Cd content of urine and perfusate was increased, as compared to controls, with all chelators. These effects were first estimated by measuring the total amount of Cd remaining in the renal tissue after perfusion, which was compared to the initial tissue concentration as measured in the left, unperfused kidney. The effect of various chelators is indicated in Table 3. It appears that DMPS and DMSA induced a clear-cut, dose related effect on Cd tissue content, while DTPA remained ineffective (Table 3). Table 4 summarizes the changes in urinary and perfusate Cd content during perfusion with chelators. The results are expressed as percentages of initial Cd concentrations in renal tissue. It appears that both perfusate and urinary Cd content increased in a dose-related manner with DMPS or DMSA perfusion, but not with DTPA. The highest mobilizing effect (urine + perfusate) of the chelators reached 15.7% (DMPS) and 18.5% (DMSA) of the initial amount of Cd in the renal tissue. The urinary contribution (Table 4, 4th column) decreased with increasing concentrations of chelators. The concentration-related effects of DMPS and DMSA on Cd mobilization into urine or perfusate are represented in Fig. 1. The effects of the larger dose of the three chelators on urinary cumulative Cd excretion are presented in Fig. 2, showing the higher efficacy of DMPS.

Effects of DMPS at longer time intervals after Cd administration. The efficacy of DMPS at two doses to mobilize renal Cd was evaluated after 1 or 2 weeks following the last Cd injection. As shown in Table 5, the total amount of Cd released into urine and perfusate was higher following a delayed administration of DMPS, as compared to an early (24 h) administration. This increase, which occurred in the perfusate but not in urine, reached statistical significance at lower concentration of DMPS, at 1 and 2 week intervals. Average Cd content in renal tissue was higher after 1 and 2 weeks than 24 h following Cd injection.

Effects of chelators on overall renal function. As shown in Table 6, GFR decreased with increasing concentrations of DMPS. Similar changes occurred with the two other chelators, GFR decreasing from 0.42 (controls) to 0.26 ml/min with Ca-Na_3 DTPA (14.2 mM), and to 0.12 ml/min with DMSA (27.4 mM). Corresponding increases of fractional Na reabsorption were observed.

Non-filtering kidneys. The effects of DMPS (11.9 mM) upon Cd levels in perfusate were evaluated in 5 non-filtering kidneys (ureter ligated 30 min before, and during perfusion). The amount of Cd recovered at the end of the experiment in the perfusate of non-filtering kidneys (2.7 ± 0.7%), expressed as percentage of the initial amount of Cd in renal tissue, did not differ significantly from that measured in filtering kidneys (3.3 ± 0.8%).

Discussion

The present study aimed at evaluating the efficacy of three chelators on mobilizing Cd accumulated in the kidneys of rats previously exposed to the toxic metal. In order to prevent a possible redistribution of metal from other organs, and to avoid systemic effects resulting from exposure to chelators, we resorted to an isolated perfused rat kidney preparation. The system used was similar to that pioneered by Bowman (19) and described by Maack (18). The functional properties of our preparation, as reported in Table 1, appeared appropriate to carry out the present study. Thus, GFR, Na^+, K^+ and glucose excretion were in the range of those measured previously by other authors using IPRK preparations (18-21). The arterial perfusion pressure was kept in the low range (75-80 mm Hg) in order to maintain a fractional reabsorption of Na^+ between 90-95%. Functional parameters showed a relative stability during the three 20-min experimental periods, and were little affected by previous administration of Cd (Table 2).

Addition of chelators to the perfusate induced clear-cut changes in Cd handling by the perfused kidney. All compounds elicited an increase of Cd content either in the perfusate, the urine or both. These effects were concentration-dependent for DMPS and DMSA, whereas they were not for DTPA. Furthermore, the latter compound remained clearly much less effective than the two other chelators, as shown by the much smaller changes in perfusate and urine Cd content, and the absence of measurable decrease of Cd in perfused kidneys (Table 3). In contrast, both DMPS and DMSA elicited a large increase of Cd in perfusate, and a smaller rise in urine Cd content.

It is worth noting that Cd was mobilized from renal tissue by DMSA and DMPS to a much larger extent into the venous effluent than into urine, a discrepancy which even increased at larger chelator concentrations (Table 4). These findings would suggest that the effective chelating agents act mostly at the basolateral tubular side. Urinary Cd might result from Cd-chelator complexes ultrafiltered after chelation and recirculation, or from Cd directly mobilized at the luminal side by ultrafiltered free chelator compounds. Our findings favor the first hypothesis, most notably based on the observation that changes of Cd content in perfused kidneys with tied ureters did not differ from those of filtering kidneys. Since ureter ligation in IPRK has been shown to drastically reduce GFR to non measurable values (20), it appears that

reduction of renal tissue Cd induced by chelators results mostly from effects at the basolateral level.

It is well known that in chronic exposure renal Cd is mostly bound to intracellular metallothionein. Since metallothionein-bound Cd might be less available to chelating agents, we investigated the effects of increasing the time interval between Cd administration and exposure to a chelating agent. We assumed that essentially all renal Cd, at one or two weeks after the last Cd injection, would be bound to metallothionien. We used DMPS as a chelating agent, since it appeared the most effective in the previous experiments. As shown in Table 5, a longer delay increased rather than reduced the chelating effects of DMPS, as shown by the much larger Cd content in the venous effluent. We have no definitive explanation for these findings, though one could consider the hypothesis that the toxic effects of Cd in renal tubular cells were more widespread 1 or 2 weeks after Cd administration, resulting in an enhanced permeability of basolateral cell membranes to the chelator and an apparent increased chelating efficacy.

Though the overall chelating efficacy of DMPS and DMSA were similar in our experimental conditions, it appeared that DMPS enhanced the urinary excretion of Cd to at larger extent than DMSA when cumulated amounts of urinary excretion of Cd were considered (Fig. 2). Though, as discussed above, the urinary contribution to the mobilization of Cd remains small, DMPS might show distinct characteristics with respect to its tubular handling. Thus, Klotzbach et al (23) have shown, in IPRK studies, that net tubular secretion of DMPS occurs, indicating a carrier mediated transport process of the organic anion type, since the transport was inhibited by probenecid and para-aminohippurate. This secretory pathway may contribute to the efficacy of DMPS as a chelating agent for toxic metals accumulated in the kidney. Though our experiments were not designed at investigating the tubular transport characteristics of the chelators, they suggest that DMSA and DMPS differ in their ability to increase urinary Cd excretion, a property which may be related to the tubular kinetics of DMPS.

As mentioned previously, the fraction of mobilized Cd excreted into urine decreased with increasing doses of chelators. This observation may be related to the toxic effects of high concentrations of chelating compounds on renal function. Thus, as illustrated in Table 6,

marked decreases of GFR and other renal functions occurred at high concentrations of DMPS, and would be expected to reduce the excreted amount of chelated Cd. Similar conclusions applied to DMSA and DTPA as well.

In conclusion, our studies with the IPRK preparation point to a higher efficacy of DMPS and DMSA than of DTPA at mobilizing Cd from renal tissue. This observation is consonant with the known kinetic properties of these chelators, the distribution of DTPA being restricted to the extracellular space whereas the two other chelators are known to be able to cross the cell membrane. Cd mobilization from renal tubular cells proceeds mostly at the basolateral side, ultrafiltration and urinary excretion of the Cd-chelate occurring subsequently, and following systemic distribution in whole animals experiments. Specific transepithelial secretory transport of DMPS may further enhance the urinary excretion of the chelator and bound Cd. All chelators used in the present study showed functional renal toxicity at high concentrations.

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Table 1. Functional data of isolated kidneys from normal rats during 60 min perfusion.

	Time (min)		
	0-20	20-40	40-60
Renal artery pressure (mm Hg)	76.1 ± 1.5	75.7 ± 1.4	78.6 ± 1.4
Kidney perfusion rate (ml/min)	31.7 ± 0.6	35.7 ± 0.4	38.0 ± 0.4
GFR (ml/min)	0.43 ± 0.03	0.53 ± 0.03	0.49 ± 0.04
Urine flow (µl/min)	39.7 ± 4.2	66.2 ± 5.9	66.3 ± 6.0
Fractional excretion (%):			
Na^+	6.3 ± 0.8	8.8 ± 1.2	9.8 ± 1.2
K^+	55.0 ± 2.3	68.3 ± 3.1	63.2 ± 3.0
Fractional reabsorption glucose (%)	97.5 ± 0.1	97.1 ± 0.2	95.5 ± 0.4

(mean ± SEM; n=10 rats)

Table 2. Functional data of isolated perfused kidneys from untreated rats or rats injected with Cd (3 x 0.75 mg/kg Cd acetate)

	Control	Cadmium
Renal artery pressure (mm Hg)	76.8 ± 1.4	70.5 ± 0.8
Kidney perfusion rate (ml/min)	35.1 ± 0.6	33.4 ± 0.5
GFR (ml/min)	0.48 ± 0.03	0.42 ± 0.03
Urine flow (µl/min)	57.4 ± 7.6	53.2 ± 6.3
Fractional excretion (%):		
Na^+	8.3 ± 1.0	8.4 ± 1.3
K^+	62.2 ± 2.9	38.2 ± 2.1*
Fractional reabsorption glucose (%)	96.7 ± 0.3	95.8 ± 0.4

(mean ± SEM; n=10 rats in each group)
*: significantly different from controls ($p < 0.05$)

Table 3. Cd content of the right and the left (unperfused) kidneys.

	Cadmium content (μg/kidney)		Change (%)
	Left	Right	
Control	48.5 ± 5.0	47.8 ± 4.8	1.1 ± 2.9
DTPA (7.1 mM)	50.6 ± 8.8	46.0 ± 8.1	8.5 ± 3.7
DTPA (14.2 mM)	38.1 ± 4.2	42.2 ± 5.0	-10.5 ± 2.4
DMPS (2.4 mM)	59.0 ± 7.6	53.0 ± 7.2	10.1 ± 2.9
DMPS (11.9 mM)	50.4 ± 9.1	43.3 ± 8.3	13.3 ± 4.8
DMPS (23.8 mM)	48.4 ± 6.3	26.3 ± 4.2	45.9 ± 6.2
DMSA (5.5 mM)	38.9 ± 7.9	33.8 ± 7.0	9.4 ± 8.3
DMSA (27.4 mM)	36.2 ± 8.5	23.4 ± 6.1	34.8 ± 6.8

Cd (μg/kidney) measured in whole organs, after perfusion (24 h after the last Cd injection) of the right kidney (mean ± SEM; n= 6 rats in control, 5 rats in experimental groups).

Table 4. Effects of $CaNa_3$-DTPA, DMPS and DMSA on Cd content in urine and perfusate.

	U/L (%)	P/L (%)	U+P/L (%)	U/U+P (%)
Control	0.06 ± 0.01	0.57 ± 0.22	0.62 ± 0.22	16.21 ± 6.31
DTPA (7 mM)	0.26 ± 0.06**	0.75 ± 0.30	1.03 ± 0.38	31.17 ± 3.86
DTPA (14.2 mM)	0.22 ± 0.06*	0.61 ± 0.32	0.83 ± 0.32	38.22 ±12.09
DMPS (2.4 mM)	0.34 ± 0.19	0.60 ± 0.12	0.94 ± 0.30	28.08 ± 7.94
DMPS (11.9 mM)	0.67 ± 0.28**	3.30 ± 0.80*	3.97 ± 0.93*	17.72 ± 4.74
DMPS (23.8 mM)	2.84 ± 0.87**	12.88 ± 4.65**	15.72 ± 5.14**	18.47 ± 4.77
DMSA (5.5 mM)	0.25 ± 0.10**	0.61 ± 0.24	0.86 ± 0.34	29.44 ± 2.19
DMSA (27.4 mM)	0.86 ± 0.25**	17.60 ± 5.81**	18.47 ± 5.96**	5.76 ± 1.06

(mean ± SEM; n=6 rats in control, 6 rats in experimental groups).
U/L, P/L = urinary, resp. perfusate Cd content, as percentage of left kidney tissue (L) Cd content. U+P = sum of urinary and perfusate Cd content.
*,**: significantly different from control ($P<0.05$, $P<0.005$).

Table 5. Effect of delayed administration of DMPS on Cd content in urine and perfusate.

	Cd content (μg)					
	L. K.	R.K.	U/L(%)	P/L(%)	U+P/L(%)	U/U+P(%)
DMPS (11.9 mM):						
24 h.	50.4±9.1	44.9±9.6	0.7±0.3	3.3±0.8	3.9±0.9	17.7±4.7
1 week	54.4±5.8	45.0±2.1	1.7±0.5	11.6±3.6*	13.3±4.0*	14.6±5.3
2 weeks	69.4±5.2	64.7±8.1	1.2±0.3	16.4±3.8*	17.6±3.7*	8.4±2.7
DMPS (23.8 mM)						
24 h.	48.4±6.2	27.6±4.6	2.8±0.9	12.9±4.7	15.7±5.1	18.5±4.8
1 week	59.9±5.8	28.3±1.6	1.1±0.2	28.9±6.6	29.9±6.7	4.2±0.8
2 weeks	51.0±4.5	32.2±2.9	1.7±0.2	22.3±3.9	24.0±3.9	7.8±1.5

(mean ± SEM; n = 5 rats in each group)
All values expressed in %. Abbreviations as in Table 4.
*: significantly different from the 24 h. group ($p<0.05$).

Table 6. Functional data of isolated kidneys perfused at different DMPS concentrations.

DMPS:	0 (control)	2.4 mM	11.9 mM	23.8 mM
Renal artery pressure (mm Hg)	70.5±0.8	71.2±1.4	90.6±1.6	93.3±2.9
Kidney perfusion rate (ml/min)	33.4±0.5	38.6±1.0	29.0±0.6	26.6±0.6
GFR (ml/min)	0.42±0.03	0.31±0.04	0.30±0.03	0.20±0.02
Na^+ fractional excretion (%)	8.4±1.3	5.4±1.7	19.2±4.9	50.2±2.8
Fract. reabsorption glucose (%)	95.8±0.4	96.8±0.4	91.0±0.9	55.3±0.2

(mean ± SEM; n = 10 rats in control, 5 rats in experimental groups)

Legends to Figures.

Fig. 1. Cumulative amount of Cd (μg) excreted in urine (U) or perfusate (P) after 60 min of DMPS or DMSA perfusion in isolated rat kidneys, 24 hours after the last Cd injection. N=5 rats.

Fig. 2. Cumulative urinary excretion of Cd (ng) during 6 consecutive 10 min periods, in isolated rat kidneys, 24 hours after the last Cd injection. N=5 rats (6 controls). Data from the highest concentration of chelators.