administration in diuresis experiments) by the determination of GR activity and the concentrations of GSH and GSSG in renal tissue (Table 1). Despite using the effective dose and different time schedules in diuresis experiments, the riboflavin effect on Tl induced proteinuria has never been observed (not shown).

Although there was no decrease of GSH concentration in renal tissue in Tl-treated rats [3], we tested the effect of N-acetylcysteine (ac-cys) and buthionine sulfoximine (BSO) on Tl-induced proteinuria. At 12 h after ac-cys (corresponding to the time of Tl administration in diuresis experiments), GSH concentration was significantly enhanced in renal tissue (see the table in Figure 1). Repeated administrations guarantee continuously high GSH levels for >36 h after the administration of Tl. Nevertheless, there was no detectable influence on Tl-induced proteinuria (Figure 1).

BSO was effective in decreasing both GSH and GSSG concentrations in renal cortex and medulla (see table in Figure 1) which was followed by significantly decreased proteinuria in comparison with Tl-treated rats (Figure 1). Previously, it has been shown that this protective effect is caused by accelerated urinary Tl excretion [3].

From our results, it can be concluded (i) that the hypothesis of Tl interaction with riboflavin has to be rejected because Tl did not decrease GR activity and riboflavin administration did not influence Tl-induced nephrotoxicity; and (ii) Tl affinity for GSH has to be rejected because Tl did not decrease GSH concentration in renal tissue and enhanced GSH concentration did not ameliorate Tl nephrotoxicity. Decreased GSH concentration did ameliorate Tl nephrotoxicity by accelerated Tl excretion.

The molecular mechanism which works in Tl toxicity remains to be clarified.

References


Unexpected electrophysiological effects of D-19575, a new cytostatic drug

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Objective

As a consequence of renal and urotoxic side effects of the oxazaphosphorines cyclophosphamide and ifosfamide (IFO) [1] D-19575 (β-D-glucosylisophosphoramide-mustard) with a presumed transmembrane transport like that of glucose was developed [2,3]. In D-19575 the active metabolite of IFO ifosfamide-mustard (IFM) is coupled to glucose.

Active glucose transport is maintained by means of SGLT1, SGLT2 and perhaps SAAT1 (pSGLT2, SGLT3). Glucose reabsorption in the renal proximal tubule is a Na+-coupled process depending on the membrane voltage (V′m). LLC-PK1 cells are a well suited model for the renal proximal tubule and their electrophysiological properties have been studied in detail [4].

Methods

We used the slow whole-cell patch–clamp technique to examine the acute effects (1 mmol/l each) of D-19575, ifosfamide-mustard (IFM), and β-D-glucose (5 mmol/l) (Figure 1) on the membrane voltage (V′m) and the membrane conductance (Gm) of differentiated renal proximal tubular cells, LLC-PK1 [4]. Xenopus laevis oocytes were injected with in vitro synthesized cRNA encoding for flounder renal organic anion transport (iROAT) as described [5], and oocytes were examined by means of a two-electrode voltage clamp device.

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showed no significant effect on $V_m$ and $G_m$ ($n=12–16$) (Figures 2 and 3).

Differentiated LLC-PK1 cells have been shown to express SGLT1 as well as SAAT1 [6–8]. Therefore, a depolarization accompanied by an increase of $G_m$ was expected if D-19575 was transported by these systems.

Because LLC-PK1 cells are lacking organic anion transport [9], we assayed $X. laevis$ oocytes expressing fROAT [5] for possible D-19575-induced currents. D-19575 as well as $\alpha$-D-glucose (1 mmol/l each) elicited barely detectable currents at a clamp potential of $-60$ mV, whereas in the same oocytes, the $p$-aminohippurate (PAH)-induced current (0.1 mmol/l) was $-10.3 \pm 5.7$ nA ($n=6$).

**Conclusion**

In summary, we show in renal proximal tubular LLC-PK1 cells an effect on $V_m$ and $G_m$ by the cytostatically active metabolite IFM indicating interference with cell metabolism. We were able to demonstrate the electrical equivalents of $\text{Na}^+$-coupled glucose transport in LLC-PK1 cells. We could not observe effects by D-19575 on either membrane voltage or ion currents indicating that D-19575 is not transported like glucose, which is in contrast to previous findings [10]. Because D-19575 shows no direct effect on the membrane voltage or ion currents in comparison to IFM we speculate that D-19575 is not directly cytotoxic, which is in contrast to previous findings [2]. From the physiological point of view we doubt the usefulness of cytostatic drugs transported like glucose.

**Results**

Glucose revealed a significant reversible and fast depolarization of $V_m$ by $11 \pm 2$ mV and an increase in $G_m$ of $11 \pm 5\%$ ($n=26–38$). IFM showed a significant and slow depolarization of $V_m$ by $9 \pm 1$ mV and a decrease of $G_m$ by $19 \pm 8\%$ ($n=13–14$). In contrast D-19575

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**Fig. 1.** Chemical structures of $\alpha$-D-glucose (A), ifosfamide-mustard (B), and D-19575 ($\beta$-D-glucosyl-isophosphoramidemustard) (C).

**Fig. 2.** Summary of effects of glucose (5 mmol/l), ifosfamide-mustard (1 mmol/l) and D-19575 (1 mmol/l) on the membrane voltage in LLC-PK1 cells. Empty bars represent pre- and post-control. * indicate statistical significance between controls and effects. Shown are mean values $\pm$ SEM, $n$ refers to the number of experiments.
Glycosaminoglycan prevents hyperglycemia-induced renal TGF-β1 gene expression

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Patients with diabetes mellitus account for nearly half of all patients on haemodialysis. Progressive expansion of the mesangial matrix and thickening of the glomerular and tubular basement membranes are hallmarks of human and experimental diabetic nephropathy. These lesions may lead to glomerular fibrosis, a central feature in the development of diabetic nephropathy. We have previously reported that chronic therapy with a low-anticoagulant, heparin-derived glycosaminoglycan preparation (GAG/mH) may prevent diabetic nephropathy, as assessed by albuminuria and histo-