Abstract. The clinical use of the efficient chemotherapeutic drug cisplatin is limited by its specific severe organ toxicities such as nephro-, oto-, and also peripheral neurotoxicity. Membrane transporters such as the copper transporter-1 (Ctr1), the copper transporter-2 (Ctr2), the P-type copper-transporting ATPases ATP7A and ATP7B, the organic cation transporter-2 (OCT2), and the multidrug extrusion transporter-1 (MATE1) mediate cellular transport of cisplatin. Since OCT2 is specifically expressed in the kidneys, its role as possible target of specific organ protection against undesired cisplatin toxicity is under investigation. We could show that OCT2 is also expressed in the cochlea in hair cells and in cells of the stria vascularis and also in dorsal root ganglia of mice. Moreover, we could show in a mouse model of cisplatin acute toxicities that the expression of OCT is critical for the development of ototoxicity, peripheral neurotoxicity and nephrotoxicity. Competition of cisplatin transport by the OCT2 substrate cimetidine was able to suppress ototoxicity, and reduce nephrotoxicity. Only few human tumors express OCT2, its expression being apparently down-regulated by epigenetic modifications, suggesting that a protective therapy by competition for the transport of cisplatin by OCT2 may be generally feasible without affecting its antitumor potency. There is already some evidence that patients bearing a mutation in OCT2 gene or co-medicated with cimetidine are protected against cisplatin nephrotoxicity. In conclusion, OCT2 seems to be an ideal target for the establishment of protective therapies aimed to specifically reduce cisplatin side-effects and increase the quality of life of the patients.

Introduction. Intracellular-acting drugs must cross biological membranes to reach their targets. In the case of lipophilic drugs, the passage through plasma membranes happens largely via passive diffusion at a rate related to their lipophilicity, while in the case of hydrophilic drugs this process is mediated by membrane transporters. Many membrane transporters have a specific tissue and cell distribution: in the morphologically- and functionally-polarized epithelial tissues, for example, transporters are even specifically expressed on the apical or basolateral cell membrane. In this way, a specific drug-transporter interaction can be exploited to target drugs to selected cells and tissues, but of course can also explain specific undesired adverse effects (1). While the pharmacological relevance of transport proteins is well-known because of their role in the development of resistance of tumor cells to chemotherapeutic agents (as for example in the case of P-glycoprotein (2)), their role in the development of specific drug adverse effects has been only in the recent years under critical investigation (1).

Cisplatin is a potent and efficient cytostatic drug, whose clinical use is limited by its severe acute and chronic nephro-, oto- and peripheral neurotoxicity (3). In this work I summarize our results presented at the the XIth International Symposium on Platin Coordination Compound in Cancer held in Verona (Italy) on the role of membrane transporters in mediating specific cisplatin uptake and toxicities and the thereby possible implications for protective therapies.

Cisplatin toxicities. Cisplatin treatment, even though effective against tumors, has severe side-effects such as nephrotoxicity, which is often dose-limiting, otoxicity, and peripheral neurotoxicity. Clinical signs of acute and/or chronic nephrotoxicity are a decrease in renal plasma flow (4) and in glomerular filtration rate (5), an increase in serum creatinine, and reduction of serum magnesium and potassium levels (6). Even though nephrotoxicity can be controlled by diuretics and pre-hydration of patients, the prevalence of cisplatin nephrotoxicity is still high, occurring in about one-third of patients undergoing cisplatin treatment (6). Studies in experimental animals showed that the kidney
accumulates more cisplatin than other organs (7), and that the proximal tubules are the renal structure specifically damaged by cisplatin (8). Otoxicity, an atypical side-effect for a chemotherapeutic drug, is caused by a damage first occurring at the cochlea base, where high-frequency sounds are processed, which, with increasing cumulative dose, proceeds towards the apex, affecting also hearing at lower frequencies (9). Cisplatin treatment causes hearing loss, possibly leading to deafness, which is still an unresolved clinical problem of dramatic importance in infants and younger children, where otoxicity can lead to a delayed language development which in turn might have devastating consequences for a young child’s social and educational development (10). Most patients treated with cisplatin develop a symptomatic and clinically-detectable sensory neuropathy, caused by its preferential uptake in the dorsal root ganglia (DRG), which produces a dose-related large fiber sensory neuropathy (11, 12).

Cisplatin transporters. Several different transporters seem to be involved in the cellular transport of cisplatin: the copper transporter-1 (Ctr1) (13), the copper transporter-2 (Ctr2) (14), the P-type copper-transporting ATPases ATP7A and ATP7B (15), the organic cation transporter-2 (OCT2) (16, 17), and the multidrug and toxin extrusion transporter 1 (MATE1) (18, 19). While Ctr1, Ctr2, ATP7A and ATP7B are ubiquitously expressed, OCTs and MATE1 are highly expressed in secretory organs, such as the liver and the kidneys, and for this reason, are of special interest as mediators of specific organ toxicities. Focussing on OCTs, these transporters have a species- and subtype-specific organ expression: human OCT2 (hOCT2) is specifically expressed in the basolateral membrane of renal proximal tubule cells, while human hepatocytes express mainly hOCT1 in their sinusoidal membrane (20). For the right interpretation of translational studies, it is important to mention that in rodents, both OCT1 and OCT2 show a high renal expression in the basolateral membrane of proximal tubule cells, with higher OCT2 expression in male animals (21, 22).

We furnished the first direct in vitro evidences for the transport of cisplatin by hOCT2 showing that it inhibits the uptake of the fluorescent organic cation 4-((dimethylamino)styril)-N-methylpyridinium (ASP+) by hOCT2 stably transfected in human embryonic kidney (HEK) cells. The expression of hOCT2 was also linked to a higher cisplatin accumulation compared to wild-type HEK cells (16). These findings were confirmed by other groups studying cisplatin uptake by rat OCT2 (23) and by hOCT2 (24). We could also showed that cisplatin competed with ASP+ basolateral transport in freshly-isolated human proximal tubules (where hOCT2 is expressed), whereas it did not influence ASP+ transport in human hepatocytes (where hOCT1 is expressed) (16). Moreover, competition of hOCT2 transport by the OCT substrate cimetidine effectively suppressed cisplatin cellular toxicity in vitro (16). The introduction of mice, where the genes for OCT1 and OCT2 were genetically deleted (OCT1 and 2 knock-out -ko- mice) (25) allowed us (17) and others (26) to study the importance of these transporters for the development of acute cisplatin toxicities in vivo. Compared with wild-type mice, OCT1 and 2 ko mice developed a much milder nephrotoxicity under acute treatment with cisplatin (17). Interestingly, these mice were also protected against cisplatin induced otoxicity (17). Because of this finding, we investigated the expression of OCT2 in the mouse cochlea and were able to show its expression in hair cells and in the cells of the stria vascularis, structures sensitive to cisplatin damage. Recently, performing laser-ablation mass spectrometry experiments in wildtype mice acutely treated with cisplatin, we were able to show a high accumulation of cisplatin in the kidney cortex and also in the stria vascularis of the cochlea, areas where OCT are specifically expressed (27). Interestingly, treatment of the wildtype animals with cisplatin together with cimetidine, a competitor for the transport by OCT, eliminated or lowered the ototoxic and nephrotoxic effects of cisplatin, respectively (17). The reason for incomplete protection from cisplatin nephrotoxicity by cimetidine may reside in the vectorial nature of cisplatin transport in renal proximal tubules: cimetidine inhibits with higher affinity the apical expressed MATE1 (28), probably an efflux transporter for cisplatin, in this way probably reducing its renoprotective effect. A study in rats showed that such a protective strategy did not interfere with the antitumoral activity of cisplatin (29). Interestingly, OCT2 has been demonstrated to be expressed in DRG within the nervous system (30). Genetic or pharmacological knockout of OCT2 protected mice from hypersensitivity to cold or mechanical-induced allodynia induced by oxaliplatin treatment (30). Even though the idea of a specific protective therapy aimed to reduce cisplatin toxicities by competing its uptake by OCT is very attractive, it is important not to compromise its uptake in tumor cells, the target of cisplatin therapy. Investigating the transporter expression in several different tumor derived cell lines, we found a strong expression of Ctr1 but no or only a very low expression of OCT, at least at the mRNA level (17). However, colorectal tumor samples expressed hOCT2 (31), while a reduced expression of OCT1, OCT2, and OCT3 due to epigenetic modifications such as DNA methylation was observed both in animal models and in prostate and liver tumor patients (32-34). There are few studies in patients suggesting a protective effect of cimetidine against and an important role for hOCT2 for the development of cisplatin nephrotoxicity. One study suggested already 1987 an efficient nephroprotection by cimetidine in patients treated with cisplatin, without compromising the success of tumor
therapy (35). Another study showed that patients bearing a mutation in hOCT2, which decreases transport function, were protected against cisplatin nephrotoxicity (26). A recent study showed that both co-medication with cimetidine and genetic polymorphism of hOCT2 protected patients against cisplatin nephrotoxicity (36).

Collectively, these results indicate the critical importance of OCT2 in the handling of cisplatin in the kidney, in the inner ear and possibly also in DRG and provide a rationale for the development of new targeted approaches to mitigate these debilitating side effects of cisplatin chemotherapy.

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References


