Abstract. To gain a better insight into the neurotoxicity of platinum drugs, it is important to increase our knowledge over the phenomena allowing their entry into dorsal root ganglia neurons. A deeper understanding of platinum-drug transport mechanisms in neurons would represent not only a step forward in the pathogenetic interpretation of their neurotoxicity, but would also disclose possible treatment options to prevent this severe side-effect achievable through modulation of transporter activity. Copper transporters and organic cation transporters have been identified as putative targets for the pharmacological modulation of neuronal cell accumulation of platinum drugs and damage, and this possibility has been demonstrated by animal studies. The modulation of drug transporter activity is a promising strategy to limit the neurotoxicity of platinum drugs, provided that a) complete characterization of drug transporters is obtained and b) selective neuronal activity is targeted without reducing anticancer drug efficacy.

Platinum drugs exert peripheral neurotoxicity by affecting the dorsal root ganglia (DRG) neurons (1-4), although the complete mechanism involved is not yet completely known (5). However, before reaching and possibly interacting with intracellular targets, platinum drugs need to cross the plasma membrane of DRG neurons, and this phenomenon is only in part due to passive diffusion (6). Active platinum drug transport mechanisms have been widely studied in cancer cells since they are relevant in explaining the sensitivity as well as resistance to chemotherapy (7, 8), but little is known regarding these mechanisms in neurons (9).

A deeper knowledge of cellular transport mechanisms in DRG neurons would be potentially very important, not only to increase our understanding of the pathogenesis of platinum drug-induced peripheral neurotoxicity, but also in order to try to modulate transporter activity to reduce toxicity of such drugs to normal cells, as already effectively demonstrated in experimental settings in vitro and in vivo (10-12). In the context of peripheral neurotoxicity, the final aim could be to limit the accumulation of toxic platinum drugs in neurons, but not in cancer cells, in order to maintain their anticancer activity.

Cisplatin and oxaliplatin, the two most severely neurotoxic drugs among platinum compounds, are reported to be substrates of transporters located on the neuronal plasma membrane (5). This observation is potentially relevant, since these drugs are unable to cross the blood brain barrier, but have very easy access to the unprotected neuronal bodies of the sensory neurons in the DRG, where platinum accumulates and reaches a very high concentration (13). As mentioned, the mechanism of neurotoxicity of platinum compounds is still not clear. In cancer cells, interstrand and intrastrand cross-link formation in DNA is a well-known, and highly relevant effects of platinum drug treatment (14) and platinum DNA adducts have also been reported in DRG neurons in experimental models (15). Moreover, platinum DNA interaction has also been demonstrated at the mitochondrial level in neurons (16, 17), suggesting that oxidative stress may be a relevant event in the pathogenesis of neurotoxicity from platinum drugs (18).

Among the different mechanisms determining resistance to platinum drugs, reduced intracellular accumulation by decreased drug uptake or increased drug efflux has been reported (19).

The Characteristics of the Principal Drug Transporters Involved in Platinum Drugs-induced Peripheral Neurotoxicity

Despite preliminary evidence suggesting that several other transporters might be relevant (6) two families of drug transporters seem to be particularly important in the passage...
of platinum drugs to and from the surrounding environment into DRG neurons: copper transporters and organic cation transporters.

The copper transporter family. This family of drug transporters includes molecules involved in both drug influx and efflux (20). Copper-transporting ATPases (Cu-ATPases) known as ATP7A and ATP7B are particularly interesting in their relationship with transport of platinum drugs. Physiologically, they are necessary for dietary copper uptake and homeostasis, allowing normal development and function of the central nervous system. ATP7A and ATP7B have structural and mechanistic similarities to other members of the P-type ATPase family, although the latter are unable to bind copper (21, 22). Besides their physiological role related to copper metabolism, ATP7A and ATP7B are also established regulators of the efflux of cisplatin and, possibly, of other platinum drugs. These transporters have been extensively investigated in cancer cells since it is possible that changes in their expression or activity might contribute to resistance to anticancer treatment, although conclusive data are available only for ATP7A (23).

Human copper transporter-1 (CTR1) is a 190-amino acid protein with three transmembrane-spanning domains and exists in the plasma membrane as a homo-trimer. It is expressed in several tissues and its presence is necessary for development and survival, as evidenced by the fact that Ctr1-knockout mice are unable to survive (24, 25). CTR1 is the main high-affinity copper influx transporter related to the transport of cisplatin and recent studies have demonstrated the localization and distribution of Ctr1 in the DRG in normal rats and in rats treated with platinum drugs (26). The localization of CTR1 within the DRG is not uniform, since CTR1 immunoreactivity is limited to the sub-population of larger DRG neurons. This observation is potentially of great interest since a) it suggests a specific distribution of at least this transporter and b) these large DRG neurons are those representing the most likely target for platinum drugs within the DRG. Neuronal CTR1 immunoreactivity in DRG tissue was associated both with plasma membranes and with granular vesicular structures of the cell bodies of large neurons, with much lighter diffuse cytoplasmic staining of other neuronal cell bodies, without staining of nuclei or nerve fibres. No overlap between smaller-sized ATP7A-immunoreactive neurons and larger-sized CTR1-immunoreactive neurons was reported (26). A specific and remarkable contribution of CTR1 in platinum drug uptake has been suggested in vivo (27) and this has subsequently been confirmed by an in vitro functional study (28).

The organic cation transporters. Among the several wide-specificity transporters devoted to the excretion and distribution of endogenous organic cations, as well as of drugs and toxins, the organic cation transporters named OCT1 and OCT2 (full name SLC22A1, SLC22A2) and the cation and carnitine transporters OCTN1 (SLC22A4) and OCTN2 (SLC22A5) are those most likely involved in the influx of platinum drugs into DRG neurons (9). Moreover, in the central nervous system, OCTs participate in the regulation of extracellular concentrations of neurotransmitters in the brain and mediate the release of acetylcholine (29). Based on available literature data, OCT2 seems to be the most relevant in platinum drug-induced peripheral neurotoxicity, as well as in nephrotoxicity induced by cisplatin administration (10). In this respect, and as a remarkable proof-of-concept of the possible modulation of drug transporter activity as a strategy for minimizing drug toxicity, a very elegant study demonstrated that the co-administration of cisplatin and of the common drug cimetidine (an OCT2 transport competitor) can remarkably reduce experimental cisplatin-induced nephrotoxicity (10, 30). Another experimental study demonstrated that oxaliplatin is also transported via the same mechanism (31) and an involvement of OCT1 in platinum compound transport has also been suggested (32).

OCT2 is also implicated in the onset and severity of another key toxicity of cisplatin, namely ototoxicity, as demonstrated in an in vivo mouse model (10). In an attempt to better clarify the localization of OCT1, OCT2, OCTN1 and OCTN2 in rat DRG neurons, we demonstrated by real-time PCR the expression of their mRNAs using embryonic (E15) rat DRG of healthy animals. Interestingly, this expression was modulated by cisplatin treatment (9). Oct2 expression was confirmed by in situ hybridization experiments obtained ex vivo in normal rat DRGs, where virtually all the neurons were positively stained. On this basis, we treated rats using a well-characterized chronic animal model of cisplatin neurotoxicity and correlated the neurophysiological changes in the tail nerve conduction velocity and the pathological changes previously described in the DRG (13, 15, 33-35) with mRNA expression of Oct2. The results were intriguing, since a negative correlation, possibly reflecting an attempt by DRG neurons to limit cisplatin influx in order to prevent severe damage, was observed.

To test this hypothesis and to attempt a pharmacological neuroprotection strategy based on the study previously reported by Ciarimboli and collaborators (10) in their nephroprotection study, we tested the neuroprotective effect of cimetidine in neuronal cultures in vitro and subsequently treated rats chronically intoxicated with cisplatin rats with a pharmacologically-active dose of cimetidine. The results of this experimental approach were, however, only partially positive suggesting that a) the dose of cimetidine was not completely appropriated, or b) OCT2 is not the only nor the most relevant transporter involved in cisplatin neurotoxicity in DRG neurons.
Similar experiments, with positive results, were performed by other researchers focusing on the modulation of ATP7A and CTR1 through the administration of copper sulfate against cisplatin toxicity in the cells of the organ of Corti (36) or of cimetidine to prevent nephrotoxicity (37).

Very recently, a comprehensive in vitro and in vivo experimental paradigm confirmed at the protein level that OCT2 is expressed in DRG neurons and that cellular uptake of oxaliplatin is increased by 16- to 35-fold in cells overexpressing mouse or human OCT2. Interestingly, this increase in oxaliplatin uptake was associated with increased DNA platination and oxaliplatin-induced cytotoxicity. Furthermore, Oct2-knockout mice were protected from hypersensitivity to cold or mechanical-induced allodynia, signs typical of acute oxaliplatin-induced neurotoxicity. Additionally, overexpressing mouse or human OCT2. Interestingly, this increase in oxaliplatin uptake was associated with increased DNA platination and oxaliplatin-induced cytotoxicity. Furthermore, Oct2-knockout mice were protected from hypersensitivity to cold or mechanical-induced allodynia, signs typical of acute oxaliplatin-induced neurotoxicity (11).

Conclusion

The data regarding the distribution and activity of platinum drug-related transporters are still very limited and knowledge should be further improved. In particular, multiple expression of different transporters and their interplay should be carefully assessed. However, the preliminary data reported so far suggest that drug transporters play a critical role in the development of neurotoxicity from platinum drugs and that they might represent a valuable target for achieving effective neuroprotection, provided firm evidence that the accumulation of the anti-neoplastic drugs by cancer cells is not compromised by any treatment able to limit neuronal uptake.

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References


