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Neurotoxic effects of antineoplastic drugs: the lesson of pre-clinical studies

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TABLE OF CONTENTS

1. ABSTRACT

Several antineoplastic drugs induce severe toxic damage of the peripheral nervous system and chemotherapy-induced peripheral neurotoxicity (CIPN) can be dose limiting. Moreover, CIPN signs and symptoms can be permanent and severely impair the patients' quality of life even after drug withdrawal. Despite extensive investigation, the exact mechanisms of neurotoxic action at the basis of CIPN are not completely known and it is likely that they can be at least in part different from the mechanisms of antineoplastic action of the drugs. A possible instrument to investigate on this important issue is represented by the evaluation of the effect of compounds used to reduce the toxicity of antineoplastic drugs in pre-clinical and clinical settings. This review will be focused on the most clinically-relevant neurotoxic antineoplastic drugs and on the results obtained with several different classes of putative neuroprotectants.

2. INTRODUCTION

The use of antineoplastic drugs has markedly improved the prognosis of cancer patients. However, an emerging and clinically-relevant problem in the administration of several of these compounds is represented by their side effects (1-7). Given their mechanisms of action, most of the antineoplastic drugs are toxic not only on fast-replicating cancer but also on normal cells; however, a significant proportion of effective agents can also be neurotoxic. In these cases the dorsal root ganglia and the peripheral nerves are the most common sites of damage, since the central nervous system is protected by an effective blood-brain barrier. Despite the well-established clinical and experimental observation that several antineoplastic drugs induce peripheral neurotoxicity, the fine mechanisms of this side effect is unclear, particularly in view of the absence of cell replication in normal adult neurons which should protect them from anti-mitotic drugs.

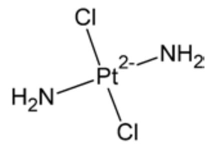

Figure 1. Cisplatin

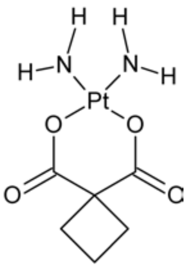

Figure 2. Carboplatin

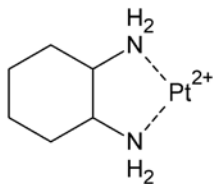

Figure 3. Oxaliplatin

In this review, we will describe the known mechanisms of action for the most commonly used antineoplastic drugs and we will discuss the role of these mechanisms in chemotherapy-induced peripheral neurotoxicity (CIPN) with the aim of speculating as to the pathophysiology of the peripheral nervous system damage and its possible modulation.

3. MECHANISMS OF ACTION OF THE NEUROTOXIC ANTINEOPLASTIC COMPOUNDS

3.1. Platinum-derived drugs

3.1.1. Cisplatin

Cisplatin, cis-diamminedichloroplatinum(II) (Figure 1), was discovered to have cytotoxic properties in the 1960s and, by the end of the 1970s, it had earned a place as the key ingredient in the systemic treatment of germ-cell cancers, although it is used also in several other malignancies. About 30 analogues were evaluated in clinical trials, but only carboplatin and oxaliplatin have achieved wide approval (8). The currently accepted paradigm regarding cisplatin's mechanism of action is that the drug exerts its cytotoxic properties through binding to nuclear DNA (cisplatin–DNA adducts could activate multiple signalling pathways including those involving p53, Bcl-2 family, caspases, cyclins, CDKs, pRb, PKC, MAPK and PI3K/Akt) and subsequent interference with normal transcription, and/or DNA replication mechanisms. If cisplatin–DNA adducts are not efficiently processed by cell machinery, cytotoxic processes eventually end up in cell death. However, before cisplatin enters the cell, it may bind to phospholipids and phosphatidylserine in the cell membrane. In addition, in the cytoplasm many potential platinum-binding sites are also available, including RNA and sulphur-containing biomolecules. There is a lot of evidence suggesting that the cytotoxic effects induced by the binding of cisplatin to non-DNA targets (especially proteins) may contribute to its biochemical mechanism of action.

3.1.2. Carboplatin

Carboplatin (Figure 2) was approved in the United Kingdom and Canada in 1985 and shortly thereafter in the United States (8). Although they share the same mechanism of action, compared to cisplatin carboplatin is better tolerated but it may have inferior efficacy in germ-cell tumours, head and neck cancer and bladder and oesophageal carcinoma, whereas both drugs seem to have comparable efficacy in advanced non-small cell lung cancer and extensive stage small cell lung cancer as well as suboptimally debulked ovarian cancer.

3.1.3. Oxaliplatin

Oxaliplatin (Figure 3) is the first platinum-based compound that has marked efficacy in colorectal cancer when given in combination with 5-fluorouracil and folinic acid. The mechanism of action of oxaliplatin is similar to that of other platinum derivatives that exert cytotoxic effects through the formation of DNA adducts, with the subsequent impairment of DNA replication and transcription and resultant cell death. Among the several putative targets, platinum-derived drugs active metabolites, quickly but in a diverse way, react with small proteins with sulphydryl groups, such as glutathione, cysteine and methionine, and then with high molecular weight proteins, such as albumin and gamma globulins through a covalent link (9). No platinum accumulation has been reported in plasma with oxaliplatin whereas, after cisplatin administration, both total and ultrafiltrable platinum progressively accumulate in plasma. This difference of interaction within both active metabolites and proteins may play a role in the lack of oxaliplatin nephrotoxicity and its more delayed and reversible neurotoxicity. Erythrocytes represent an important deep compartment, especially for oxaliplatin. In fact, the drug is trapped in erythrocytes through a covalent binding to globin. Hence, at the end of a 2-hr infusion, approximately 40% of the blood platinum is found in erythrocytes.

3.2. Antitubulin agents

The mechanism of action of tubulin-binding drugs has been extensively reviewed (10, 11). Soluble tubulin exists in the cell as a heterodimer of one molecule of alpha-tubulin and one molecule of beta-tubulin. There are currently six known isotypes of alpha-tubulin and seven of beta-tubulin in addition to a variety of known post-translational modifications. During polymerisation, the heterodimers link together to form protofilaments. Thirteen of these protofilaments organised in a hollow cylinder make up the backbone of the microtubule. Microtubules exist in an unstable equilibrium whereby free dimers are constantly incorporated into the polymerised structures and microtubule dimers are released into the soluble tubulin pool. This equilibrium is under the control of several factors, including microtubule-associated proteins (MAPs). Two patterns of microtubule dynamics—treadmilling and dynamic instability—that maintain this equilibrium have

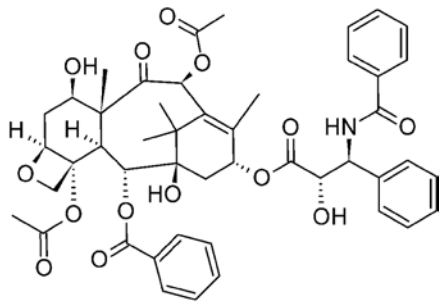

Figure 4. Paclitaxel

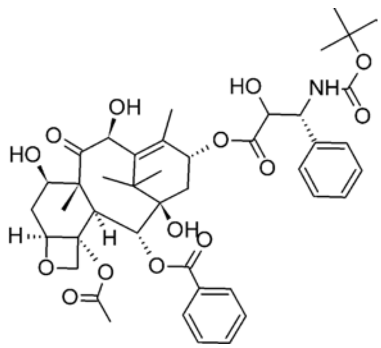

Figure 5. Docetaxel

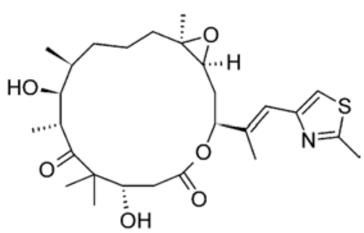

Figure 6. Epothilone

been reported. In treadmilling, tubulin units incorporated into the microtubule at the "plus" end are removed from the opposite "minus" end with no net change in microtubule length. Dynamic instability describes periods of relative stability interposed with rapid growth and a shortening of the microtubule. By binding to tubulin and interfering with heterodimerisation, drugs like taxanes, epothilones and vinca alkaloids disrupt microtubule dynamics without appreciably changing the microtubule mass. Microtubules are essential components of the cell cytoskeleton and their plastic nature gives them an important role in a number of cellular functions. They are critical for the movement of organelles during interphase and, during mitosis, form the mitotic spindle that transports daughter chromosomes to separate poles of the dividing cell. Drugs that interfere with microtubule function lead to the failure of alignment of the daughter chromosomes and their bipolar attachment to the mitotic spindle. The cell fails to pass through the checkpoints that exist to ensure that mitosis proceeds appropriately, leading to mitotic arrest at the metaphase/anaphase transition, followed by apoptosis.

This has been suggested as the primary anti-neoplastic mechanism of action of tubulin-binding drugs. However, it has also been postulated that at least part of the anti-tumour effect of these agents is related to their effect on microtubules in interphase cells.

Of the several new chemotherapeutic agents of the antitubulin family introduced recently, the taxanes have had a profound impact in a wide variety of malignancies and are approved for clinical use by the Food and Drug Administration (FDA) board for the treatment of breast cancer, ovarian cancer, non-small-cell lung cancer and prostate cancer.

3.2.1. Paclitaxel

Paclitaxel (Figure 4) was first discovered in the early 1960s as part of a National Cancer Institute screening study to identify natural compounds with antineoplastic activity. Paclitaxel was identified as the crude extract from the bark of the North American Pacific yew tree, *Taxus brevifolia*, in the early 1970s and was found to exert significant cytotoxic effects in preclinical studies against many tumours through tubulin stabilizationand hyperpolymeration. However, clinical development was slowed until the early 1980s owing to the scarce supply of the Pacific yew tree bark and its poor solubility.

3.2.2. Docetaxel

Docetaxel (Figure 5) is a semisynthetic compound produced from 10-deacetylbaccatin-III, which is found in the needles of the European yew tree, *Taxus baccata*. Although slightly more water soluble than paclitaxel, docetaxel also requires a complex solvent system for its commercial formulation.

3.2.3. Epothilones

Two cytotoxic compounds (Epothilone A and B, Figure 6) derived from the myxobacterium *Sorangium cellulosum* have been found to interact with microtubule polymerisation at nanomolar concentrations (12) inducing arrest in the G2/M transition. However, in preclinical models, epothilones are more resistant to multi-drug resistant (MDR) mechanisms than taxanes. The epothilones competitively inhibit the binding of paclitaxel to mammalian brain tubulin, suggesting that the two types of compounds share a common binding site on tubulin, despite the lack of structural similarities.

3.2.4. Vinca alkaloids

The family of vinca alkaloids includes two natural agents, vincristine (Figure 7) and vinblastine, together with several semisynthetic drugs such as vindesine and vinorelbine. Vinca alkaloids also act by arresting cell mitosis after binding with intracellular tubulin but, in this case, the effect is the opposite of that described for taxanes and epothilones. In fact, the exposure to vinca alkaloids induces disassembly of the normal microtubular array. The first molecule of this family to be used as an anticancer agent was vincristine, a drug which is still in use because of its effectiveness, despite the availability of less neurotoxic derivates.

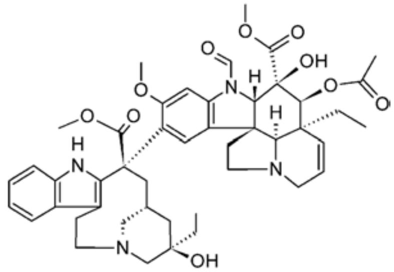

Figure 7. Vincristine

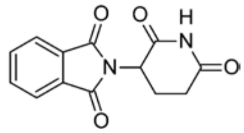

Figure 8. Thalidomide

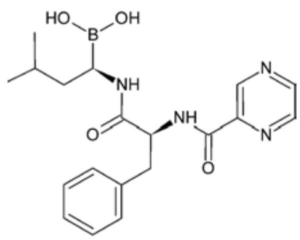

Figure 9. Bortezomib

3.3. Proteasome inhibitors

The inhibition of protein degradation through the ubiquitin–proteasome pathway is a unique approach to cancer treatment (13, 14). The proteasome carries out the regulated degradation of unnecessary or damaged cellular proteins; included in the array of proteins targeted by the proteasome are proteins that regulate cell-cycle progression and apoptosis. Proteasome inhibition adds another unique target to the range of cellular targets for chemotherapy (e.g. DNA, the cytoskeleton, and transcription and replication enzymes). Alone, this novel mechanism of action is lethal to many types of cancer cells, and preclinical activity has already been demonstrated in many tumour types, including solid tumours. The 26S proteasome (1,500 to 2,000 kD) consists of a core 20S catalytic complex (approximately 700 kD) and a 19S regulatory complex. It consists of two outer and two inner rings that are stacked to form a cylindrical structure with three compartments. Each outer ring has seven alpha-subunits (alpha 1 to alpha 7) whereas each inner ring contains seven beta-subunits (beta 1 to beta 7). The 20S proteasome complex has chymotryptic, tryptic, and peptidylglutamyl-like activities. The dipeptidyl boronic acid bortezomib (N-pyrazinecarbonyl-L-phenylalanine-L-leucine boronic acid, Figure 8), the first-in-class of this new family of antineoplastic agents, has demonstrated a unique cytotoxicity profile in the National Cancer Institute screen of 60 cell lines. Bortezomib inhibits the proteasome pathway rapidly and in a reversible manner by binding directly with the 20S proteasome complex and blocking its enzymatic activity (13).

3.4. Thalidomide

Thalidomide (Figure 9) was synthesized and first marketed in Germany as a “non-barbiturate hypnotic” with a notable prompt action, lack of hangover, and apparently favourable safety profile (15). It was banned from commercial use in 1963, after it had been discovered that it exerted teratogenic effects if taken between the 34th and 50th day of pregnancy. Over 12,000 affected children were born with skeletal abnormalities, an event that led to a major reform of drug approval procedures in the United States and elsewhere. Despite its tragic initial experience, thalidomide has become a subject of major interest because of its newly demonstrated clinical value in infectious disease and cancer, and because of its relatively low level of toxicity. Given the complexity of thalidomide metabolism and the potential contribution of its numerous metabolites, our current understanding of the mechanism of action is limited. However, thalidomide has attracted the attention of investigators because of its wide range of biological actions. At least two properties, anti-angiogenesis and immune modulation represent the leading hypotheses regarding its anti-tumour activity. In fact, these two effects may be closely related through the effects of thalidomide on cytokine secretion. Thalidomide inhibits angiogenesis in several experimental assay systems. It suppresses Tumour Necrosis Factor-alpha (TNF-alpha) and interferon gamma (IFN-gamma) secretion, both of which upregulate endothelial cell integrin expression, a process crucial for new vessel formation. It inhibits the secretion of basic fibroblast growth factor (bFGF), an angiogenic factor secreted by human tumours. Thalidomide also has a broad range of inhibitory and stimulatory effects on the immune system. It inhibits the migration of both immune and phagocytic cells in experimental systems. It reduces tumour-associated macrophage infiltration possibly through suppressing expression of endothelial cell adhesion molecules. In addition, two indirect anti-tumour effects of thalidomide have been recognized: the inhibition of secretion of IL-6, a cytokine secreted by the bone marrow stroma essential for the survival and proliferation of myeloma cells, and the stimulation of secretion of IL-12, a potent inhibitor of angiogenesis. Finally, thalidomide or its metabolites may have direct anti-tumour effects. In cell cultures, thalidomide suppresses the proliferation of human myeloma cells, but only at extremely high and probably pharmacologically not relevant concentrations. Thalidomide analogues have an at least 100-fold greater potency in directly inhibiting tumour cell growth, but thalidomide metabolites have not yet been clinically tested. Until its metabolism is better understood, the possibility of direct cytotoxic action cannot be ruled out.

4. CLINICAL ASPECTS OF CIPN

The target of drug-induced neurotoxicity is mostly dependent on the type of substance which may act predominantly on the nerve fibres (axon or myelin) or on the neuronal body (motoneurons or dorsal root ganglia primary sensory neurons). Accordingly, also the clinical features of drug-induced neuropathy are dependent on the type of agent involved, ranging from predominantly motor, to almost exclusively sensory or sensory-motor

Table 1. Summary of the main features of CIPN due the most commonly used antineoplastic drugs

Substance	Proposed target of neurotoxicity	Main clinical features	Pre-clinical models available
Platinum derived drugs			
Cisplatin	DRG neurons with secondary axonopathy	Sensory impairment (ataxia) Deep tendon reflexes reduction Rarely pain	Yes
Carboplatin	DRG neurons with secondary axonopathy	Sensory impairment (ataxia) Deep tendon reflexes reduction Rarely pain	Yes
Oxaliplatin	DRG neurons with secondary axonopathy	Sensory impairment (ataxia) Deep tendon reflexes reduction Rarely pain	Yes
	Voltage-gated sodium channels	Cold-related perioral and pharyngeal paresthesias	Yes
Antitubulin drugs			
Paclitaxel	Axons (DRG?)	Distal symmetrical polyneuropathy (sensory>motor) Occasionally pain	Yes
Docetaxel	Axons (DRG?)	Distal symmetrical polyneuropathy (sensory>motor) Occasionally pain	Yes
Epothilones	Axons	Distal symmetrical polyneuropathy (sensory>motor)	Yes
Vincristine	Axons	Distal symmetrical polyneuropathy (sensory and motor) Occasionally pain	Yes
		Autonomic neuropathy	No
Bortezomib	Unknown	Distal symmetrical polyneuropathy (sensory>motor) Severe pain	Yes
Thalidomide	Axons, rarely DRG neurons	Distal symmetrical polyneuropathy (sensory>>motor) Rarely pain	No

DRG = dorsal root ganglia

neuropathies, with or without any clinical evidence of autonomic impairment (7) (see a summary of the main clinical features of antineoplastic drugs in Table 1).

Cisplatin-induced neuropathy is sensory, predominantly characterized by symptoms of large myelinated fibre damage, such as numbness and tingling, paraesthesias of the upper and lower extremities, reduced vibration and position sense perception, reduced deep tendon reflexes, and incoordination with gait disturbance. Occasionally, Lhermitte's sign is reported by patients, suggesting the involvement also of the centripetal branch of the dorsal root ganglia neuron axons in the spinal cord. The first symptoms are often observed after a cumulative dose of 300–600 mg/m^2 of cisplatin. Risk factors for more severe neurotoxicity include diabetes mellitus, alcohol consumption or inherited neuropathies, all conditions which by themselves induce peripheral nerve damage. Advanced age has not been identified as an independent risk factor when there is no co-morbidity.

After completion of cisplatin chemotherapy, only a part of the patients has significant neurotoxic symptoms, whereas 3-4 months later the proportion is definitely higher. This phenomenon (called "coasting") is clinically very relevant, since it makes it difficult to assess the real severity of the dorsal root ganglia neuron damage during cisplatin administration. Due to the "coasting" phenomenon, cisplatin-induced neurological disorders should be carefully evaluated not only during treatment, but also 2-4 months after the end of its administration. Resolution or amelioration of symptoms occurs in most of the patients over the next 12 months (despite the fact that abnormal neurological examination is frequently permanent) and, in patients with mild signs of cisplatin-related neuropathy, re-treatment with platinum drugs is generally feasible after several months.

Conventional dosages of carboplatin have been associated with a lower risk of peripheral neuropathy (e.g. mild paraesthesias) than cisplatin. Although they are generally less severe, qualitatively, the symptoms of carboplatin peripheral neuropathy are exactly the same as those observed with cisplatin. Patients over 65 years of age or patients pre-treated with other neurotoxic agents may be at a slightly higher risk. When high dose regimens have been tested in order to achieve a better antineoplastic response, carboplatin peripheral neurotoxicity has become clinically relevant, with symptoms and signs identical to those observed after cisplatin administration.

The features of oxaliplatin neurotoxicity are rather different from those of cisplatin and carboplatin (16). In fact, besides chronic sensory neurotoxicity, in about 90% of patients, oxaliplatin treatment has been associated with acute neurosensory toxicity including dysaesthesia and paraesthesia. This particular type of neurosensory toxicity predominantly affects the fingers, toes, the pharyngolaryngeal tract, the perioral and oral regions, and it is generally induced or aggravated by exposure to cold. Such symptoms, which can be effectively treated with different antiepileptic agents, may occur within 30-60 minutes from the beginning or shortly after each course of oxaliplatin. Acute neurotoxicity is generally mild in severity, it disappears within a few hours or days and does not require oxaliplatin treatment withdrawal. Some patients may also develop muscle cramps or spasms. The acute neurotoxic effects of oxaliplatin result from the drug-related inhibition of voltage-gated sodium currents (17). It has been suggested that oxalate ions, which are released during oxaliplatin metabolism, might be responsible for the inhibitory effects on the voltage-gated sodium channels because of their calcium chelating activity. In addition to the acute neurotoxic symptoms caused by oxaliplatin, about 10–15% of patients treated with this agent develop moderate neuropathy, particularly after cumulative intravenous doses of 600–800 mg/m^2. The symptoms of chronic neuropathy include non-cold-related dysaesthesia, paraesthesias, superficial and deep sensory loss and, in some cases, sensory ataxia and functional impairment which persists between treatment cycles. Most of these symptoms usually disappear a few months after oxaliplatin withdrawal. Neurophysiological studies in platinum drug-treated patients evidence a reduction in the amplitude of the sensory potentials with minimal changes in the sensory nerve conduction velocity. Pathological examination of sural nerve biopsies has evidenced axonal degeneration without any evidence of primary demyelination.

Paclitaxel is more neurotoxic than docetaxel but, also for the latter drug, neurotoxicity may be dose-limiting (18), although at low doses antitubulin drugs demonstrated a protective effect in *in vitro* models of neurotoxicity (19). The clinical features of the peripheral neurotoxicity induced by taxanes are qualitatively identical, and they are mostly represented by distal, symmetrical hypoaesthesia in the upper and lower extremities with a length-dependent distribution. In most cases, all sensory modalities are affected and deep tendon reflex loss is an early feature of taxanes' peripheral neurotoxicity. Motor signs and symptoms may occur during treatment with paclitaxel or docetaxel, although only rarely is motor impairment a clinically-relevant feature. Very rarely distal neuropathic pain may ensue during taxane treatment, while myalgia is a frequent symptom. The signs of taxanes' peripheral neurotoxicity tend to be reversible but, in a minority of cases, persistence of sensory impairment is observed and incomplete recovery may occur. Nerve conduction studies in patients treated with taxanes evidence a reduction in sensory (and more rarely motor) potential amplitudes, with a mild reduction in sensory and motor conduction velocity. These findings suggest that axonal damage may be the main pathological change, but direct confirmation of this is still missing: in fact, so far, no conclusive pathological studies have been performed in humans, mainly because taxanes are generally used in combination schedules.

Given the common mechanism of action, it is not surprising that epothilones, similarly to taxanes, can induce dose-limiting peripheral neurotoxicity. However, the data available so far do not make it possible to have a clear picture of the clinical presentation of epothilone-induced nerve impairment. Using the schedules currently reported, it seems that the incidence of clinically-relevant peripheral neuropathy is rather high (experienced by up to two-thirds of the patients exposed to ixabepilone, known also as BMS-247550, a very effective epothilone B analogue), and patients present with sensory symptoms and signs (18). No conclusive data are available on the time course of epothilone-induced neuropathy or on its site of action, but it is likely that this side effect will significantly affect the clinical use of this very promising class of antineoplastic drugs.

Most of the patients treated with vincristine develop dose-dependent potentially treatment-limiting neurotoxicity with the clinical features of sensorimotor peripheral neuropathy (6). Sensory signs and symptoms are predominant in the majority of patients, with distal symmetrical hypoaesthesia and dysaesthesia involving all the sensory modalities (i.e. deep and superficial), sometimes with a painful component. Muscular cramps may occur, in association with reduced strength in the distal muscles in the most severely affected patients. Severe impairment of motor function leading to tetraplegia has occasionally been described in children or in patients with pre-existing hereditary neuropathies. Similarly, there have been occasional reports of isolated peripheral nerve functional impairment. Rarely, the "coasting" phenomenon has been described also with vincristine. Autonomic nervous system involvement is observed in about one third of the subjects exposed to vincristine presenting with orthostatic hypotension, urinary bladder dysfunction and erectile impotence. However, the most severe clinical occurrence is constipation due to a paralytic ileus or megacolon. The toxic signs induced by vincristine are reversible in most cases, but long-lasting impairment or incomplete recovery are frequent. Nerve conduction studies show decreased distal motor and sensory nerve action potentials with less prominent changes in nerve conduction velocity, while electromyography evidences denervation in distal muscles. These findings are consistent with the largely predominant axonal damage which has been described in sural nerve biopsy pathological examinations.

Rather surprisingly, although thalidomide sensory neurotoxicity was first recognized in the early 1960s (20), the features of this dose-limiting side effect of the drug are still not completely understood and several important issues have yet to be resolved. With regard to this, the absence of reliable animal models of thalidomide neurotoxicity poses a problem in attempting to understand the mechanism and site of the neurotoxic action of this drug. In fact, although several clinical studies have investigated thalidomide-induced sensory neurotoxicity, a consensus has not yet been reached even on some key aspects such as the site of action, the dose-dependency, the incidence, the correlation between clinical and neurophysiological results and the course of the neuropathy once it has ensued. The reasons for the discrepancies observed in the literature might be due, at least in part, to the evaluation of small series of patients, frequently exposed to a limited dose range of thalidomide and examined with different methods. In fact, a recent study (21) has demonstrated that thalidomide-induced peripheral neurotoxicity is dose-dependent only when relatively high doses are administered for the treatment of myeloma, while this dose effect is not evident when thalidomide is administered at lower doses (i.e. for the treatment of dermatological or rheumatologic diseases). The clinical features in the majority of patients are those of a length-dependent sensory neuropathy, mainly involving tactile and thermal modalities, with a reduction in or the disappearance of deep tendon reflexes and distal dysaesthesia. Dorsal root ganglia neuron damage may occur at least in some cases, as demonstrated by clinical and neuroradiological findings. Very occasionally, mild distal motor impairment has been reported. Thalidomide neurotoxicity is assessed during treatment in humans by means of serial sensory nerve examinations. The crucial event, which may be the cause of treatment withdrawal, is represented by a marked (i.e. > 50%) reduction in the amplitude of sensory potentials, while nerve conduction velocities change only slightly. Motor nerve conduction changes may also occasionally occur, but they are generally of no clinical relevance. It is noteworthy that clinical signs of thalidomide sensory neurotoxicity may occur also in the presence of "normal" (i.e. without a severe reduction in the amplitude of the nerve sensory potentials) neurophysiological results, particularly in the low-dose range.

Although it is in no way clear how and where bortezomib administration can affect the peripheral nervous

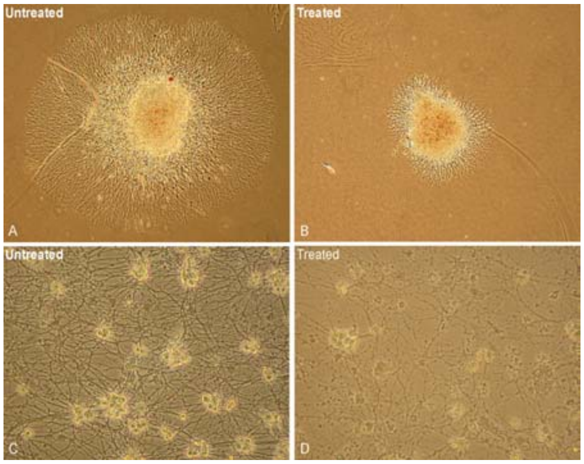

Figure 10. Examples of dorsal root ganglia explants (top) and isolated sensory neurons (bottom).

system, at least one-third of the patients under treatment have had evidence of clinically-relevant sensory peripheral neuropathy (22). Treatment withdrawal resulted in clinical improvement, but the low number of patients studied makes it impossible to draw any firm conclusion about this at the moment. Further studies are essential in order to clarify the very important issue of the clinical relevance of proteasome inhibitors' peripheral neurotoxicity and to define the mechanisms of this toxic effect, since it may be potentially a dose-limiting side effect in the future clinical use of these substances.

5. PRE-CLINICAL STUDIES

Several pre-clinical models have been implemented to investigate the pathogenesis of CIPN and most of them have also been used to test neuroprotective strategies. In the following sections the main features of these models will be reviewed.

5.1. *In vitro* models

Although the animal models have obvious advantages, they are expensive and time- consuming. Moreover, the interpretation of the results of *in vivo* experiments is often difficult at the molecular level due to the great number of variables present in the model. As a consequence, reliable pre-clinical *in vitro* tests to assess the effect of these drugs on the peripheral nervous system are mandatory. For these reasons different *in vitro* models to screen the neurotoxicity of antineoplastic agents and to investigate their molecular and cellular mechanisms of action and of toxicity have been optimized. In the literature, the models most commonly employed are based on two different cell lines (i.e. the SH-SY5Y human neuroblastoma and the rat PC12 pheochromocytoma cell line) and on dorsal root ganglia explants, from which organotypic cultures and sensory neurons primary cultures are obtained.

The human neuroblastoma SH-SY5Y cells express genes associated with neuronal differentiation (neuron specific enolase, neurofilament proteins, cathecolamine synthesis) and may be considered as neuroblasts at various stages of neuronal differentiation (23). Retinoic acid (RA) differentiated SH-SY5Y cells are biochemically, ultrastructurally and electrophysiologically comparable to human sympathetic neurons (24). The evaluations of RA-induced neurite elongation (considered as a marker of differentiation) in the absence of neurotrophic factors makes SH-SY5Y a suitable model for screening antineoplastic drug neurotoxicity. The length of neurites in SH-SY5Y cell cultures treated with different concentrations of antineoplastic drugs (compared with the length of neurites of control cultures) allows a wide spectrum of concentrations of the drugs to be tested quickly (25). Moreover, this *in vitro* model can be used to investigate the cellular and molecular mechanisms implicated in the drugs' neurotoxicity (26-29).

On the other hand, PC12 rat pheochromocytoma cells in response to Nerve Growth Factor (NGF) stop the proliferation, extend long and branching neurites and become electrically excitable acquiring properties of sympathetic neurons (30).The cells produce, store and can release catecholamines (norepinephrine and dopamine but not epinephrine). In the PC12 model, neurotoxicity is assessed, as in SH-SY5Y cells, by quantitative morphological methods including the counting of the number of cells exhibiting neurites and the measuring of neurite length (31-33). NGF-induced PC12 neuronal phenotype differentiation is associated with the expression of different neuronal proteins such as growth associated protein 43 (GAP-43) and presynaptic membrane-associated proteins such as synpatophysin and synapsin, but PC12 do not form "true" synapses with each other (34). Disadvantages of this cell line are that PC12 cells are not of human origin and they cannot be easily used to study growth factor-dependent neuroprotection considering their NGF requirement for differentiation.

Embryonic (E15) rat dorsal root ganglia are used to establish organotypic cultures containing sensory neurons and satellite cells. Sensory neurons at this stage of embryonic development are post mitotic and neurite growth is induced by NGF supplementation (35). The measure of the longest neurite of each dorsal root ganglia treated with different concentrations of antineoplastic drugs (compared with the longest neurite of control dorsal root ganglia, Figure 10) (36) allows the rapid testing of the same wide spectrum of concentrations of drugs tested in the human neuroblastoma SH-SY5Y cell line. Embryonic rat dorsal root ganglia represent a suitable model for assessing the neurotoxicity of antineoplastic drugs also because *in vivo* dorsal root ganglia are the main target of several neurotoxic drugs. Moreover, the concentrations of the drugs used to elicit changes in this *in vitro* model are, in general, comparable to those achievable *in vivo*. Embryonic (E15) rat dorsal root ganglia are also used to establish sensory neuron primary cultures in order to investigate the cellular and molecular mechanisms involved in the neurotoxicity of the drugs tested on the organotypic cultures. Furthermore, embryonic rat dorsal root ganglia cultures allow the effect of antineoplastic drugs on myelination to be studied (37).

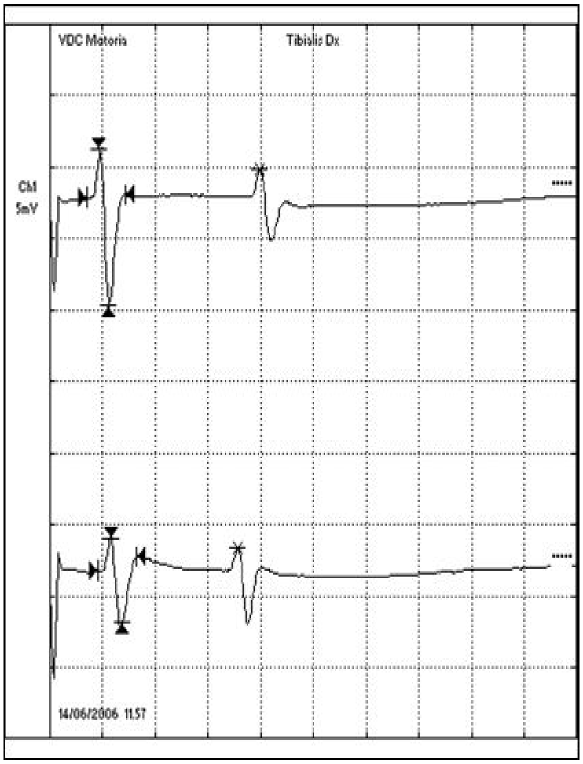

Figure 11. Example of rat sciatic nerve neurophysiological examination.

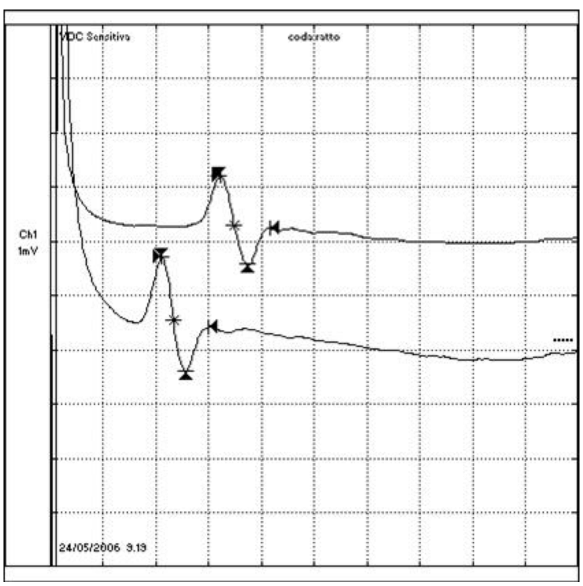

Figure 12. Example of rat tail nerve neurophysiological examination.

5.2. *In vivo* models

Several animal models of CIPN have been developed in recent years. Using these models, behavioural, neurophysiological, analytical, molecular biology and pathological methods have been developed to assess the effect of most of the currently available neurotoxic antineoplastic drugs. Among the most commonly used behavioural methods, pain perception testing has frequently been used to detect hypoaesthesia, hyperpathia and allodinia, although occasionally coordination, motility and strength have also been evaluated. The main advantage of these methods is that they allow repeated determinations during the experiments and make it possible to follow-up the animals for a long period of time. However, they are time-consuming and reproducibility is largely dependent on the experience of the examiners and adequate animal training. Some of these problems can be overcome by the neurophysiological examination of peripheral nerves. The sciatic nerve (Figure 11) can be used to assess the involvement of a large, rather proximal, mixed sensory and motor nerve while the caudal nerve (Figure 12) is easily accessible for repeated determinations of conduction velocity throughout the experiments. The implementation of new and more sensitive analytical methods allows the drug distribution to be also investigated, thus giving useful information regarding the site of action (and, therefore, of the putative targets) of the neurotoxic compounds. As already mentioned, while they are reliably used in *in vitro* models, the results of molecular biology studies are often difficult to interpret in animal models, since it can be difficult to separate the effects obtained in the different cell types. However, none of these *in vitro* techniques can substitute the pathological examination of parts of the peripheral nervous system and the use of morphological and morphometric methods. Regarding the possibility of using pathological methods to address the issue of the molecular changes induced by antineoplastic drugs, immunolocalization studies have already been used, particularly in neuropathic pain models, with interesting results. Very recently, skin biopsies have also been used with the aim of investigating the most distal part of sensory nerves which is supposed to be the site of the earliest changes in axonopathies and of applying a method already available also in a clinical setting.

6. PATHOGENESIS OF CIPN AND NEUROPROTECTION

The molecular and cellular basis of the toxic effects of antineoplastic drugs will be reviewed starting from the results obtained with the use of several putative neuroprotectants and discussing their possible mechanisms of action (see Table 2 for a summary of the main results). The issue of pharmacological neuroprotection has very recently been extensively reviewed (e.g. see 38), although the incredibly rapid rate of accumulation of new data in this field makes a complete update almost impossible. For the purpose of clarity, putative neuroprotectants will be divided into major classes according to their known (or, in some cases, hypothesized) mechanism of action.

6.1. Antioxidants

The generation of free radicals and, in general, oxidative stress is one of the mechanisms most commonly hypothesized in cell injury and death. This assumption is true also for CIPN although the direct and conclusive demonstration of this mechanism of toxicity has never been reported for most anticancer drugs. Nevertheless, several

Table 2. summary of the results obtained in pharmacological neuroprotection during antineoplastic drugs administration

Substance	Reported positive pre-clinical results	Reported positive clinical results	Other protective effects
Antioxidants			
Reduced gluthathione	Cisplatin	Cisplatin, oxaliplatin	
Alpha-lipoic acid		Docetaxel, cisplatin, xaliplatin	
N-Acetylcysteine	Cisplatin		Cisplatin ototoxicity
Vitamin E		Paclitaxel, cisplatin	Cisplatin ototoxicity
Growth factors and related compounds			
Nerve Growth Factor	Paclitaxel, cisplatin		Cisplatin ototoxicity
Brain-Derived Neurotrophic factor			Cisplatin ototoxicity
Neurotrophin-3	Cisplatin		
Leukemia Inhibitory Factor	Paclitaxel		
Vascular Endothelial Growth Factor-1	Paclitaxel, cisplatin, thalidomide		
Erythropoietin	Paclitaxel, cisplatin		Cisplatin ototoxicity
Org 2766	Cisplatin		
Acetyl-L-carnitine	Paclitaxel, cisplatin, oxaliplatin, vincristine		
Glutamine		Paclitaxel, oxaliplatin	Reduced central pain
Detoxicants			
Amifostine		Cisplatin (conflicting)	Cisplatin oto- and nephrotoxicity
Diethyldithiocarbamate		Cisplatin (conflicting)	
BNP7787	Paclitaxel, cisplatin		
Ions and channel modulators			
Carbamazepine		Oxaliplatin	
Nimodipine	Cisplatin		
Other compounds			
Venlafaxine	Oxaliplatin	Paclitaxel, oxaliplatin	Reduced central pain
GCP II inhibitors	Paclitaxel, cisplatin		
EGb761	Cisplatin		Cisplatin ototoxicity
Calpain inhibitors	Paclitaxel		
Xaliproden	Paclitaxel, cisplatin, vincristine	Cisplatin	

antioxidants have been tested as neuroprotectants in different experimental models and, in view of the tolerability and safety of most of these drugs, clinical trials have also been attempted.

Glutathione (GSH) is a naturally occurring tripeptide (glutamyl-cysteneil-glycine) with a high affinity for heavy metals and it is one of the most effective physiological radical scavangers. Platinum administration\ depletes the amount of reduced glutathione and increases the oxidized form, thus reducing the antioxidant capacity of the glutathione pool. Platinum deposits in humans undergoing treatment with cisplatin have been shown to decrease after coadministration of glutathione. The mechanism of neurotoxicity induced by platinum-based antineoplastic drugs has been shown in preclinical studies to involve heavy metal accumulation in the peripheral nervous system and reduction of this accumulation, associated with antioxidant activity, has been postulated as a key mechanism of glutathione neuroprotection. In fact, preclinical and clinical experiences have provided evidence that GSH is effective for the prevention of cisplatin-induced neurotoxicity without reducing the clinical activity of cisplatin, a major concern since high intracellular levels of glutathione have been associated with cancer cell resistance to treatment (39-43).

Alpha-lipoic acid, an essential cofactor for mitochondrial enzymes, is a cyclic disulfide acting as an endogenous antioxidant and as a potent free radical scavenger (44-46). Indirectly, alpha-lipoic acid is involved in the recycling of other antioxidants such as glutathione, vitamin C, and vitamin E. Two case series have reported that alpha-lipoic acid may be beneficial in the treatment of neuropathy caused by a combination of docetaxel an cisplatin or oxaliplatin alone. Moreover, Rybak *et al.* (47) have demonstrated that alpha-lipoic acid is able to reduce the cisplatin-induced ototoxicity acting as scavenger of reactive oxygen species (ROS) and as chelator of platinum, preserving the antioxidant system in the cochlea. Comparable results have been obtained by Husain *et al.* (48) studying alpha-lipoic acid neuroprotection against carboplatin ototoxicity.

N-Acetylcysteine (NAC) is an antioxidant thiol that is able to induce *de novo* synthesis of glutathione (49). The proposed mechanism of neuroprotection of N-acetylcysteine is related to its ability to decrease plasma levels of homocysteine while increasing serum glutathione. The known mechanism of protection of NAC from platinum ototoxicity (50, 51) may be also correlated with its binding to the platinum, resulting in an inactive complex (52). Experimental models of neuropathy have frequently implicated hyperhomocysteinemia, although this effect has not yet been associated with CIPN. It has been suggested (53) that reactive oxygen species generated by platinum compounds play an important role in platinum-induced neuronal apoptotic cell death via activation of the p53 signaling pathway. Preincubation of nerves from a mouse dorsal root ganglion neuron-neuroblastoma hybrid cell line (N18D3) with N-acetylcysteine was reported to attenuate the accumulation of p53 protein in response to platinum, resulting in a block of platinum-induced apoptosis and in a neuroprotective effect.

Vitamin E (alpha-tocopherol) is an antioxidant that exerts a protective function on biological membranes inhibiting peroxidation of polyunsaturated fatty acids. Use of vitamin E as a neuroprotective agent resulted from an observation by clinicians that patients with neuropathy undergoing antineoplastic chemotherapy had low serum concentrations of alpha-tocopherol (54).

Dorsal-root ganglia are among the most vulnerable neural structures in vitamin E deficiency neuropathies. This observation could explain why the peripheral neuropathy induced by cisplatin treatment is a sensory neuropathy that cannot be clinically or neurophysiologically distinguished from a vitamin E deficiency neuropathy. Clinical trials have provided evidence of neuroprotection with vitamin E supplementation during treatment with paclitaxel or cisplatin (55). The hypothesis proposed to explain these results is that the peculiar ability of cisplatin to concentrate in the dorsal root ganglia induces a depletion of vitamin E and renders the neuron bodies more susceptible to oxidative stress. Vitamin E produced unexpected adverse effects on the occurrence of second primary cancers and on cancer-free survival in a population of patients at high risk of developing second primary cancers (56). Despite the fact that there is some concern about the generalizing of the study results to individuals in the general population who are at low risk of a first primary cancer, these results suggest that caution should be exercised regarding the use of high-dose vitamin E supplements in cancer patients. Different groups have demonstrated that vitamin E has also a significant otoprotective action in animals treated with cisplatin (57-60). In particular cotreated guinea pigs have shown preservation of Preyer's reflex, reduction in auditory threshold elevation and preservation of outer hair cells. In the same study, the cotreatment induced the reduction of lipid peroxidation and the reduction of DNA fragmentation in the cochlea (58).

6.2. Growth factors

Neuron development, survival and, probably, response to injury in adult life are markedly influenced by the presence and activity of growth factors specifically interacting with cognate receptors expressed by neurons and glial cells. For this reason, the use of these trophic factors has been suggested for preventing or treating CIPN in several different settings and the intracellular cascade of events induced by receptor interaction has been thoroughly investigated.

In the earliest studies a relationship was suggested between neurotrophins (NT) and the toxic action of cisplatin. Three of the members of the NT family, i.e. NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), have already been evaluated in *in vitro* models of cisplatin neurotoxicity and the results obtained by different groups which used *in vivo* experimental paradigms have consistently demonstrated a partially protective effect of NGF (61-64), an agent which has a strong trophic effect on dorsal root ganglia neuron subpopulations during development and, in particular conditions, possibly also during adulthood. These results have recently been further supported by the finding that circulating NGF levels are markedly reduced in neuropathic cancer patients who have been treated with different neurotoxic combination chemotherapy schedules, in most cases based on cisplatin or on the second-generation platinum-derived drug carboplatin (65). However, although it is likely that a relationship exists between cisplatin-induced peripheral neurotoxicity and reduced peripheral tissue and plasmatic NGF availability, the fine mechanism of this interaction is not yet clear. A possible platinum-induced downregulation of the expression of a specific NGF receptor (trkA) in dorsal root ganglia neurons has been suggested (62) although we were unable to determine any significant change in the mRNA expression of trkA and $p75^{NGFR}$, the high and low-affinity NGF receptors, in the sciatic nerve or in the dorsal root ganglia (personal observation). These results, therefore, do not support the hypothesis that the cisplatin-DNA binding which actually occurs in dorsal root ganglia neurons and satellite cells (66) significantly affects NGF receptor synthesis. A possible explanation for the reduced circulating levels of NGF is that the effect of the cisplatin-DNA binding in peripheral NGF-producing tissues might be functionally more relevant than that observed in the dorsal root ganglia neurons, a hypothesis which is in agreement with the reduced NGF constitutive levels observed in the intestine, bladder and paws after cisplatin treatment in mice (62). An additional possibility is that cisplatin might interact with other systems (i.e. the endothelium) which are in direct contact with the circulatory stream. The hypothesis of reduced peripheral NGF synthesis with a normal receptor expression in the dorsal root ganglia neurons and peripheral nerve would also be in agreement with the observation of a protective effect when exogenous NGF is administered. This would obviously necessitate the presence of normal receptor availability on dorsal root ganglia neurons and peripheral nerves in order to allow an effective replacement effect. Although the possibility of the direct injection of exogenous NGF is hampered in humans by the local and systemic side effects of the administration of the high dose of this substance needed to achieve sufficient bioavailability, different approaches might be considered. The latter should include the use of NGF-modulating drugs (61), or the implementation also for NGF of the same gene therapy strategies which have already been successfully used in animal models (67) and which might allow the production of biologically-significant amounts of NGF by the transfected tissues.

Although no evidence of a close relationship has been ever observed between NT-3 levels or activity and CIPN the administration of this neurotrophic factor has also been studied, based on the wide expression of its cognate high affinity receptor trkC; it has been reported that it is able to reduce cisplatin neurotoxicity (68) (e.g.cisplatin ototoxicity (69)) in animal models.

In order to clarify the real role of neurotrophins in neuroprotection against antineoplastic drugs and the molecular mechanisms through which such molecules could act, a great number of studies have been performed on in *in vitro* explant studies. Data are in agreement and show that NGF acting on tubulin polymerization and stabilization is neuroprotective against drugs such as paclitaxel and vincristine (70-72). On the contrary NGF is ineffective against cisplatin neurotoxicity except in pre-

NGF or co-NGF experiments (73, 74) and its effectiveness is strictly correlated with the concentration of cisplatin used (72). However, regardless its ability to prevent cisplatin-induced neurite outgrowth inhibition, NGF does not prevent the reduction of nerve fiber and cell density induced by cisplatin, suggesting that NGF is not effective in preventing cell death. Consequently, NGF positive effect seems to be limited only to surviving neurons after cisplatin treatment.

BDNF has been shown to protect auditory neurons (75, 76) and auditory hair cell from cisplatin-induced damage. Gabaizadeh *et al.* (77) have suggested that BDNF protection could be correlated with a glutathione-dependent reduction of ROS (reactive oxygen species).

Zheng *et al.* (75) have demonstrated the same protective effect on auditory neurons studying NT-3 but, surprisingly, opposite results regarding cisplatin protection of this growth factor have been obtained on dorsal root ganglia (73).

Leukemia inhibitory factor (LIF) is a cytokine involved in a variety of functions including stem cell differentiation and the regeneration of neurons. LIF expression has been shown to be up-regulated in response to neural injury and its specific receptor complex is expressed in the peripheral nervous system. LIF has been shown to mediate a number of potential therapeutic effects in models of neurologic dysfunction, and there is also evidence suggesting that LIF is neuroprotective in animal models of peripheral neuropathies (78-80). Kilpatrick *et al.* (79) have demonstrated that systemically administered LIF can abrogate paclitaxel-induced axonal atrophy in rat, suggesting that LIF might influence cytoskeletal structure. The same study indicates that LIF is not able to prevent the paclitaxel-induced reduction of Calcitonin-Gene Related Protein (CGRP) and Substance P expression in dorsal root ganglia neurons. Moreover, experiments carried out by Ozturk *et al.* (80) in mice have shown that LIF is effective in reducing cisplatin-induced morphological and functional damage. Probably such action is correlated with the action of LIF on Schwann cells which, secreting neurotrophic factors, may influence the phosphorylation of neurofilaments in the cytoplasm of neurons of myelinated fibres of the sciatic nerve. Although animal studies have demonstrated some benefit, a randomized double-blind placebo controlled study showed no evidence of neuroprotection in humans (81).

A vascular pathogenesis is attractive (at least in theory) for CIPN because most chemotherapeutic agents (e.g. paclitaxel, thalidomide and cisplatin) that induce neuropathy also show antiangiogenic activity in addition to their antimitotic properties. This mechanism has been suggested in a recent study and the Authors report also a reduction in nerve perfusion, claiming that antiangiogenic chemotherapeutic agents cause neuropathy, at least in part, by a vascular mechanism. Following this hypothesis, a strategy of intramuscular Vascular Endothelial Growth Factor- 1 (VEGF-1) gene transfer in proximity to the sciatic nerve has been applied. With this strategy, attenuation or reversal of damage has been obtained in animal models of neuropathy induced by paclitaxel, thalidomide and cisplatin (82, 83). Kirchmair *et al.* (83) have also demonstrated that VEGF is able to inhibit paclitaxel-induced endothelial cell apoptosis in *in vitro* experiments. However, an alternative or complementary mechanism for the effect of VEGF, including a direct neurotrophic effect on Schwann or neuronal cells, cannot be ruled out on the basis of these results.

Erythropoietin (EPO) is a cytokine originally used for its effect on erythropoiesis since it supports the survival, proliferation and differentiation of erythroid progenitor cells. However, in the past few years, it has become clear that EPO is a multifunctional trophic factor with potent neurotrophic activity on a variety of neural cells in the central and peripheral nervous system. EPO acts by binding with its receptors (EPOR) which are expressed in nerve axons, in Schwann cells and in dorsal root ganglia. EPOR over-expression after nerve injury, which is the basis for therapeutic use of exogenous EPO, have been demonstrated. Overall, experimental results have confirmed that EPO is an effective neuroprotectant that does not interfere with platinum-based tumour treatment. Moreover, the biological action of different doses of EPO on erythropoiesis and on the peripheral nervous system has also been demonstrated, supporting the development and use of EPO analogues lacking the trophic effect on erythropoiesis (84). Keswani *et al.* (85) have shown that Schwann cell-secreted EPO is able to protect dorsal root ganglia neurons against several toxic insults suggesting a central role for Schwann cells in the endogenous neuroprotective pathway. In the same experimental model, Keswani *et al.* (85) have demonstrated the protective effect of recombinant human EPO (rHuEPO) against acrylamide-induced distal axonal degeneration. Similarly Melli *et al.* (86) have shown that rHuEPO protects against paclitaxel-induced distal degeneration in mice, and Orhan *et al.* (87) have demonstrated that rHuEPO is efficient against cisplatin-induced neuropathy in rats. The protective effect of EPO and its carbamylated derivative have also been demonstrated by Bianchi *et al.* (88) in experimental cisplatin peripheral neurotoxicity. However, the mechanisms through which EPO exerts its neuroprotective action have yet to be clarified. Based on current knowledge, a direct protective effect of EPO and its derivatives can be suggested as acting on sensory neurons and/or peripheral nerves through direct binding to the EPOR which is widely expressed in the peripheral nervous system and is overexpressed after nerve injury. Independent studies have demonstrated that EPO is able to prevent neuronal apoptosis (89, 90). Melli *et al.* (86) have shown that the neuroprotective effect of rHuEPO against paclitaxel-induced neurotoxicity is correlated with its ability to prevent paclitaxel-induced accumulation of detyrosinated tubulin in sensory axons. *In vitro* experiments on dorsal root ganglia reveal that the rHuEPO modulation on the tyrosination state of microtubules is PI-3 kinase dependent. On the other hand, Orhan *et al.* (87), studying the neuroprotective effect of rHuEPO against cisplatin neurotoxicity, suggest that rHuEPO may also play a role in active myelination.

ORG 2766 (ACTH 4–9) is a hexapeptide melanocortin derived from adrenocorticotrophic hormone (ACTH) and melanocyte stimulating hormone (MSH) that has been shown to exert a trophic influence on nerve tissue without evoking corticosteroid secretion. Melanocortins are known to be present in degenerating nerves after injury and to promote neurite outgrowth even in the absence of NGF. Several studies have demonstrated ORG 2766 otoprotection in animals treated with cisplatin (91-93). Different hypotheses have been suggested to explain the action of ORG 2766 but further investigations are needed to elucidate the molecular mechanism of its otoprotection. One explanation could be that the co-administration of ORG 2766 and cisplatin reduces the amount of cisplatin reaching the cochlea through a direct interaction between the two compounds. On the other hand, the small dose of ORG 2766 utilized with respect to the dose of cisplatin has weakened this hypothesis. An alternative hypothesis suggests that ORG 2766 shares with cisplatin the same target in the cochlea. Different studies have demonstrated that cisplatin a) determines the degeneration and loss of outer hair cells (OHC) (94, 95), b) affects the vestibular neurons and spiral ganglion cells (69, 94, 95), c) induces structural and functional changes in the stria vascularis (94-96). Among these targets OHC seem to be the most plausible candidate and ORG 2766 could act on these cells in same way as in other cell types (97).

Acetyl-L-carnitine is a member of the family of carnitines, a group of natural compounds that have an essential role in intermediary metabolism. It has been suggested thatthey exert neuroprotective effects by various mechanisms including the regulation of acetyl-CoA, by controling the NGF level in the central nervous system of adult rats after total fimbria-fornix transection, by increasing the rate of transcription of the gene coding for the $p75^{NGFR}$ in the basal forebrain and cerebellum of aged rats, or by acetylation of tubulin and increasing NGF-induced histone acetylation (61). Acetyl-L-carnitine has shown promise as a neuroprotector in multiple animal models of chemotherapy-induced neuropathy including oxaliplatin, cisplatin, paclitaxel, and vincristine. A possible common mechanism of action is that the acetylation of important intracellular substrates (e.g. tubulin, histones) is enhanced after acetyl-L-carnitine administration (61, 98-100). Recent studies have reported symptomatic improvements and electrophysiological improvements in patients treated with acetyl-L-carnitine for paclitaxel or cisplatin induced neuropathy (101).

Glutamine is a non-essential gluconeogenic amino acid that is the main energy source for rapidly proliferating cells and the primary transporter of nitrogen between tissues. Glutamine up-regulates NGF mRNA in an animal model (102) and also in humans, and this event may play a role in the protection of patients undergoing chemotherapy with neurotoxic agents (103, 104). There may be additional benefits of glutamine such as acting centrally to mediate pain sensation by a complex mechanism involving glutamate downregulation. According to this theory, high systemic levels of glutamine could decrease the amount of glutamine transformed by astrocytes in glutamate thereby determining an attenuation of pain symptoms (105).

6.3. Detoxicants

One of the earliest attempts to prevent CIPN was based on the use of compounds able to protect different tissues from toxic agents (in some case originally developed for military purposes).

Amifostine is an organic thiophosphate cystamine analogue used as a radioprotectant. Recently, amifostine has been postulated as being cytoprotective in chemotherapy. Proposed mechanisms of cytoprotection include decreasing platinum-DNA adducts and DNA-DNA interstrand cross-links caused by alkylating agents as well as scavenging free radicals. The cytoprotection against cisplatin is thought to occur by a capping of the cisplatin adducts on DNA before cross-linking can be formed. In the literature, it has been reported that amifostine accumulates less efficiently into the brain and spinal cord but various studies (106, 107) have demonstrated that amifostine is able to provide protection from the toxic effect of cisplatin in peripheral nerves. On the other hand, several studies have shown that amifostine gives no protection (108) or only mild protection (109) against cisplatin-induced ototoxicity. Church *et al.* (110) suggested that high doses of amifostine are protective against cisplatin-induced peripheral ototoxicity but it is itself neurotoxic to the central auditory pathway, at least in the hamster model used. The selectivity for cytoprotection of nontumour tissue is thought to be secondary to the presence of functioning membrane bound alkaline phosphatase which dephosphorylates the thiophosphate to the active thiol metabolite WR-1065 before being taken up into the nontumour cell.

Diethyldithiocarbamate (DDTC) is the active metabolite of disulfiram and functions as a heavy-metal chelator. The neuroprotective mechanism is thought to be related to chelation and the removal of tissue-bound platinum, without affecting the antitumour bisguanosine-DNA adducts in patients undergoing cisplatin chemotherapy. Early human studies did not use neuropathy as a primary outcome, and later studies showed no benefit. Rather surprisingly, DDTC has been tested as a neuroprotectant though disulfiram is a well- known cause of peripheral neuropathy and DDTC itself is neurotoxic determining microglia activation (111).

BNP7787 (disodium 2,20-dithio-bis-ethane sulphonate; Tavocept) is undergoing development as a novel chemoprotector against common and serious cisplatin- and paclitaxel-induced toxicities (112, 113). BNP7787 is the disulphide form of mesna and, therefore, does not contain a free thiol group that would interfere with the antitumour effects of cisplatin.

6.4. Ions and channel modulators

Several attempts to modulate CIPN symptoms have been performed using compounds able to reduce pain perception, dysaesthesias and paraesthesias. Most of these compounds are antiepileptic drugs and the rationale for their use is stronger when a clear effect on the electrolytes of the antineoplastic drug has been demonstrated, as in the case, for instance, of oxaliplatin.

Direct electrolyte infusions (magnesium and calcium) are theoretically beneficial to patients taking oxaliplatin because of their proposed ability to stabilize the cell membrane.

Carbamazepine is a sodium-channel inhibitor prescribed in the treatment of epilepsy. A small case series reported a decrease in the severity of oxaliplatin induced peripheral neuropathy in patients on concurrent carbamazepine (114).

Nimodipine is a dihydropyridine calcium antagonist most commonly used for its quite selective cerebral vasodilatory effects. Animal studies suggest that nimodipine-induced limitation of intracellular calcium may provide neuroprotection to neural tissues (115) and against cisplatin neuropathy in a rat model system (116). On the other hand, a clinical trial of co-administration of nimodipine in a chronic oral dosing schedule with cisplatin based chemotherapy did not show a neuroprotective effect for nimodipine (117). This study does not exclude the possibility that nimodipine could be neuroprotective using a different dose/schedule and that nimodipine could effect the timing of the onset of recovery from cisplatin-induced neuropathy.

6.5. Other compounds

Several other compounds have been used in CIPN although the rationale for their use is rather elusive. Nevertheless, the results of these experimental attempts may be useful for generating new hypotheses regarding the pathogenesis of CIPN.

Venlafaxine has traditionally been used as an antidepressant, favoured for its selective reuptake of serotonin and norepinephrine and its low side effect profile. It does not bind to muscarinic-cholinergic, histaminic or α_1 –adrenergic receptors resulting in less severe effects than tricyclic antidepressants. Recent off-label use as a centrally acting pain medication has led investigators to consider its possible benefit to patients undergoing chemotherapy with agents known to cause painful neuropathy. In the case of oxaliplatin, the acute neuropathic reaction characterized by peripheral nerve hyperexcitability may be attenuated by venlafaxine. In fact, venlafaxine has been found to be neuroprotective not only against acute neurosensory symptoms secondary to oxaliplatin toxicity (118, 119) but also against chronic oxaliplatin-induced neuropathy (120, 121). Whether venlafaxine is able to block neuronal sodium channels has not yet been elucidated. On the other hand, a neuroprotective effect of venlafaxine against paclitaxel neurotoxicity has been reported (122), although the mechanism is unclear.

Excessive glutamate release is associated with neuronal damage as demonstrated by several studies in models of central nervous system damage. Glutamate Carboxypeptidase II (GCPII) is a metallopeptidase present in the central and peripheral nervous systems where it is responsible for cleaving the abundant dipeptide N-acetyl-aspartyl glutamate and liberating glutamate. Central and peripheral nervous system injuries are less severe in mice lacking the Folh1 gene encoding for GCP II (123), and the pharmacological inhibition of GCP II can both prevent and treat the peripheral nerve changes (124). In experimental cisplatin and paclitaxel neuropathy models GCP II inhibition induced a significant protection (personal observation).

Ginko biloba extract EGb761 could alleviate symptoms of cisplatin-induced peripheral neuropathy in mice, and primary sensory neurons from EGb761-treated animals retain their morphology and capacity to regenerate in culture (125). Multiple antioxidant actions of EGb761 are thought to be responsible for most of its protective effects in the central nervous system such as the scavenging of peroxyl radicals, superoxide anions and nitric oxide, and the inhibition of xanthine oxidase activity. Other explanations are also possible for the effectiveness of EGb761 in cisplatin neurotoxicity. For example, Ginkgo components may promote the expression of Glial cell line-Derived Neurotrophic Factor (GDNF) (126) and GDNF could then support the primary sensory neurons (although we found it had no effect in a study on GDNF as a neuroprotectant in cisplatin experimental neuropathy). Considering that cisplatin is expected to target peripheral neuroglial cells (characterized by an elevated mitotic capacity) another hypothesis suggests that EGb761 may rescue these cells. Nevertheless, *in vitro* studies performed by Ozturk *et al.* (125) did not show any positive effect of EGb761 on cisplatin-treated dorsal root ganglia migrating cells. Various studies have also demonstrated the efficacy of EGb761 as an otoprotectant against cisplatin-induced ototoxicity in rat (127) and in guinea pig. Huang *et al.* (127) have demonstrated that EGb761 is able to preserve hair cells from cisplatin-induced loss and they suggest that the EGb761 otoprotectant effect could be correlated with its ability to prevent lipid peroxidation, to increase GSH activity and to modulate SOD activity.

Calpains are ubiquitous cytosolic proteolytic enzymes involved in both physiological and pathological cellular functions. They are calcium-dependent enzymes belonging to the family of cysteine proteases. Limited activation of calpains results in the modification or activation of protein receptors, enzymes and cytoskeletal proteins. Pathological cellular insults lead to more generalized calpain activation resulting in cytoskeletal degradation and cell death. Calpain inhibition protects against neuronal loss and improves neurological function in several models of nervous system injury. Recent data (128) have demonstrated that paclitaxel can also activate calpains. Calpain activity in PC12 cells increased in a time and dose-dependent fashion in response to paclitaxel, and AK295 (a calpain inhibitor) reduced the severity of paclitaxel-induced CIPN.

Xaliproden (SR57746A) is a synthetic compound that exhibits *in vivo* and *in vitro* neurotrophic effects in several experimental studies, mostly performed in models

Table 3. Summary of the current knowledge of the main mechanisms of action and effects of the neurotoxic antineoplastic drugs on the peripheral nervous system

	Drugs						
Mechanism/effect	**Cisplatin Carboplatin**	**Oxaliplatin**	**Paclitaxel Docetaxel**	**Vincristine**	**Epothilone**	**Bortezomib**	**Thalidomide**
DNA adduct formation	+	+					
Oxidative stress	+/-	+/-					
Increased tubulin stability			+		+		
Reduced tubulin stability				+			
Reduced growth factor support	+	+	+				
Anti-angiogenic							+
Ion channel interference	+/-	+ (acute)					
Glutamate toxicity	+/-	+/-	+/-				
Protein breakdown modification						+	

+ = demonstrated; +/- = suggested

of central nervous system disorders. In some of these models it has been demonstrated that the neuroprotective effect of xaliproden is mediated by the activation of the mitogen activated protein kinase (MAPK) pathway. In particular, the xaliproden neuroprotective effect on mouse motoneurons determines the activation of MAPK ERK1/2 and of protein kinase C through the 5-hydroxytryptamine 1A receptor (129). *In vitro* xaliproden is able to potentiate the effect of NGF on neurite outgrowth of PC12 cells and is able to attenuate the reduction of neurite outgrowth induced by cytostatic drugs (vincristine, cisplatin, paclitaxel) in cocultures of rat dorsal root ganglia and Schwann cells (130). Coculture studies have shown that the neuroprotective effect of xaliproden against the reduction in the neurite length of dorsal root ganglia exposed to cytostatic drugs is partly due to the involvement of the NGF pathway. Ruigt *et al.* (130) assert that in this *in vitro* model, the xaliproden neuroprotective effect is not dependent on Schwann cell-secreted NGF as affirmed by Fournier *et al.* (131). According to Ruigt's hypothesis, xaliproden may be not considered merely as a weak NGF-mimetic and possible targets of xaliproden may be intracellular kinases and phosphatases. It is possible that by influence the equilibrium of the phosphorylated/dephopshorylated status in some cytoskeletal proteins such as actin, xaliproden could control the neurite integrity. A meeting report has recently suggested that xaliproden may be neuroprotective also in platinum-induced CIPN, but the full report of the study has not yet been published.

7. CONCLUSION

From the review of the currently available data it is evident that the knowledge of the fine mechanisms at the basis of the peripheral neurotoxicity of antineoplastic drugs is still rather limited. However, it seems quite clear that the assumption that the cyto- and neurotoxicity of these drugs are largely based on the same mechanisms is at least questionable (Table 3). However, this observation opens the theoretical possibility of minimizing the neurotoxic effects without reducing the anticancer cytotoxicity of these compounds, provided that clear evidence of their different mechanisms is obtained. At the moment this goal has not yet been achieved, but it is conceivable that in the next few years major advances in this kind of research will be made through the joint efforts of researchers in the fields of oncology and neuroscience.

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Abbreviations: CIPN: chemotherapy-induced peripheral neurotoxicity; MAPs: microtubule-associated proteins; MDR: multi-drug resistant; TNF-alpha: Tumour Necrosis Factor-alpha; IFN-gamma: interferon gamma; bFGF: basic fibroblast growth factor; NGF: Nerve Growth Factor; GSH: glutathione; ROS: reactive oxygen species; NAC: N-Acetylcysteine; BDNF: brain-derived neurotrophic factor; NT-3: neurotrophin-3; LIF: Leukemia inhibitory factor; CGRP: Calcitonin-Gene Related Protein; VEGF-1: Vascular Endothelial Growth Factor- 1; EPO: erythropoietin; rHuEPO: recombinant human EPO; ACTH: adrenocorticotrophic hormone; MSH: melanocyte stimulating hormon; OHC: outer hair cells; DDTC: Diethyldithiocarbamate; GCPII: Glutamate Carboxypeptidase II; GDNF: Glial cell line-Derived Neurotrophic Factor; MAPK: mitogen activated protein kinase; DRG: dorsal root ganglia.

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