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Role of Erythropoeitin in Prevention of Chemotherapy-induced Peripheral Neuropathy

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Abstract: Neurotoxicity complicates the use of several commonly administered chemotherapeutic agents (platinum based alkylating agents, taxanes and vinca alkaloids), with chemotherapy-induced peripheral neuropathy being the most common manifestation. Structural damage to the peripheral nervous system results in positive symptoms, e.g., allodynia, hyperalgesia and pain with unpleasant features as burning and shooting. Patients are unable to complete full or optimal treatment schedules. The pathophysiologic basis of nerve injury in chemotherapy-induced peripheral neuropathy is incompletely understood and appears to be unique for each class of the chemotherapeutic agents. Erythropoeitin (EPO), a well-established hematopoietic factor, is a very effective and widely used treatment for anemia in cancer patients undergoing chemotherapy. It also possesses generalized neuroprotective and neurotrophic properties. Co-treatment of chemotherapy and erythropoietin has been proposed for preventing or reversing the disabling peripheral neuropathy induced by the different chemotherapeutic agents. This study first describes the pathophysiological background of the clinically relevant chemotherapeutic agents-inducing peripheral neuropathy. Secondly, the possible mechanisms that might underlie the neuroprotective effect of erythropoietin in chemotherapy-induced neuropathy. Further clinical trials of EPO in cancer patients receiving chemotherapy and suffering from neurological symptoms seem to be warranted in the future. This might improve the quality of life in cancer patients.

Key words: Erythropoeitin, chemotherapy, neuropathy, neurotoxicity

INTRODUCTION

Peripheral neuropathy is a common and potential dose-limiting complication of cancer chemotherapy. Involvement of the peripheral nervous system may be in the form of purely sensory and painful neuropathy, which occurs after therapy with cisplatin, oxaliplatin and carboplatin, or mixed sensory-motor neuropathy which may be accompanied by dysfunction of the autonomic nervous system, that results after therapy with vincristine, taxanes, suramin and other drugs (Quasthoff and Hartung, 2002; Sioka and Kyritsis, 2009). Neurotoxicity depends on the cumulative dose and the type of drug used. In some cases neuropathy can arise after a single drug application (Arne-Bes, 2004). The recovery from symptoms is often incomplete and a long period of regeneration is required to restore function.

The focus of the present review is to highlight the various mechanisms studied so far through which individual chemotherapeutic agents induce peripheral neuropathy. Improved understanding of the pathophysiologic mechanisms underlying neuropathy will inevitably assist in the development of appropriate neuroprotective approaches. Up to now, no drug is available to reliably prevent or cure chemotherapy-induced neuropathy (Quasthoff and Hartung, 2002). Recently much interest has focused on the neuroprotective effect of Erythropoietin (EPO) in the peripheral and central nervous system. Thus, this review will outline the protective effect of EPO with regard to chemotherapy-induced peripheral neuropathy.

PATHOPHYSIOLOGIC MECHANISMS INVOLVED IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Axonal degeneration: The Platinum-based agents seem to affect the axons, myelin sheath, neuronal cell body and the glial structure of the neuron (Stillman and Cata, 2006). These platinum drugs have the propensity to enter the DRG and peripheral nerves (Sul and Deangelis, 2006) as opposed to the brain, as these drugs have poor penetration through the blood-brain barrier (McKeage et al., 2001). It was previously thought that platinum drugs entered the DRG through passive diffusion (Wang and Lippard, 2005), although current data indicate the presence of metal transporters that may be involved in their entrance into the cell (Safaei, 2006).

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Vincristine, which produces peripheral neuropathy in humans that is accompanied by painful parasthesia and dysthesias (Pal, 1999), was found to cause disorganization of the axonal microtubule cytoskeleton, as well as an increase in the caliber of unmyelinated nociceptive sensory axons (Tanner *et al.*, 1998). A distal to proximal degeneration of axons, or dying back is a common pattern of vincristine-induced neuropathology. Vincristine, when directly applied to DRGs, had no effect on axonal survival or growth, whereas its application to axons produced axonal toxicity that resulted in progressive axonal degeneration (Ravula *et al.*, 2007; Silva *et al.*, 2006). It was also observed that exposure of axons to vincristine produced an initial period of hyperexcitability in the cell bodies, suggesting that a signal is transmitted from the distal axon to the soma during the progression of vincristine-induced axonal degeneration.

Paclitaxel and vincristine are thought to exert their antitumor activity largely by binding to β-tubulin and disrupting mitotic spindle formation in actively dividing cells. Paclitaxel stabilizes β-tubulin polymerization, whereas vincristine inhibits spindle assembly (Jordan and Wilson, 2004). Axonal microtubules are composed largely of β-tubulin and it has generally been accepted that the neurotoxicity caused by paclitaxel and vincristine is caused by disruption of microtubule structure that impairs axoplasmic transport and leads to a dying-back neuropathy (Siau and Bennett, 2006).

With regard to the vincristine effect on both sensory and motor neurons, it was reported that it caused minor changes in Schwann cells and/or myelin sheaths in the form of myelin sheath vacuolization. Myelin loss was also detected in a very few myelinated fibers found in the cross sections of the sciatic nerve of vincristine-treated rats (Jaafer *et al.*, 2006).

In addition, a well recognized toxicity of taxanes is peripheral neuropathy. It was found to produce a symmetric, axonal predominantly sensory distal neuropathy with less prominent motor involvement. A dying back process starting from distal nerve endings followed by effects on Schwann cells, neuronal body or axonal transport changes and a disturbed cytoplasmic flow in the affected neurons is the most widely accepted mechanism of taxanes neurotoxicity (Argyriou *et al.*, 2008).

Apoptosis: Degenerating neurons display morphological features consistent with active cell death (Rzeski *et al.*, 2004). Platinum-based compounds have long been associated with DRG damage and neuronal cell death (McKeage *et al.*, 2001). They were found to undergo aquation, which is a key step in the drug forming a

complex with the target DNA. The result of this hydrolysis is the formation of a positively charged molecule that then cross-links to DNA, forming the DNA/platinum adducts (Zhu *et al.*, 2005). The amount of DNA cross-links in DRG neurons at a given cumulative dose was found to be significantly correlated with the degree of neurotoxicity (Dzagnidze *et al.*, 2007). Apoptosis has been observed in DRG neurons following cisplatin treatment both *in vitro* and *in vivo* and is correlated with increased platinum-DNA binding in the DRG neurons (McDonald *et al.*, 2005). Although, the mechanism of the transition from a platinum-DNA adduct to neuronal apoptosis is not fully understood, one proposal has suggested that the DNA repair machinery is unable to repair the damaged DNA (Suk *et al.*, 2005). Another one is that the DNA-cross-links bind with High Mobility Group (HMG) proteins that are able to block transcription factors, thus preventing both transcription and replication. This block in cellular processes may be responsible for sending out DNA damage signals that result in initiation of apoptosis (Siddik, 2003). It has also been suggested that the platinum-DNA adducts interfere with the normal function of cellular proteins such as binding or interactions with other proteins (Raymond *et al.*, 1998). Gill and Windebank (1998a) demonstrated that cisplatin-induced neuronal death is preceded by up-regulation of expression of proteins associated with entry into the cell cycle.

It has been shown that mitochondrial dysfunction is one of the routes triggering neuronal death (Won *et al.*, 2002). Based on extensive literature demonstrating effects of platinum-based cancer chemotherapies on mitochondrial dysfunction (Goodisman *et al.*, 2006; Garrido *et al.*, 2008; Heaney *et al.*, 2008; Kim *et al.*, 2008; Melli *et al.*, 2008), we evaluated the different mechanisms involved. Paclitaxel- and vincristine-evoked neuropathic pain has been associated with impaired mitochondrial function (Siau and Bennett, 2006). Flatters and Bennett (2006) suggested that paclitaxel has a direct effect on the mitochondrial membrane, which alters both mitochondrial function and structure. They reported that sensory primary afferent axons from rats with paclitaxel-evoked neuropathic pain have a greatly increased incidence of mitochondria that are swollen, vacuolated and have severely disrupted cristae, which are seen to have collapsed, fragmented and puddled at the periphery of the organelle. Mitochondrial function is critically dependent on molecular exchange between its outer and inner membranes (Kassem and Heikal, 2008); thus, disrupted cristae indicate impaired function (Flatters and Bennett, 2006).

Increased Reactive Oxygen Species (ROS) are the main mitochondrial mechanisms implicated in oxidative

stress that triggers apoptosis (Won *et al.*, 2002; Abd-El-Ghany *et al.*, 2009).

Joseph and Levine (2009) evaluated the contribution of mitochondrial electron transport chains (mETC), which drive ROS production in mitochondria. They found that oxaliplatin-induced mechanical hyperalgesia was antagonized by mETC inhibitors and by antioxidants. These data support a role for mETC driven ROS production in oxaliplatin-induced painful peripheral neuropathy. There is also evidence that oxidative stress may be involved in cisplatin neuropathy (Tredici *et al.*, 1998).

Joseph and Levine (2004) reported that vincristine increased the activity of caspase signalling pathways that ultimately lead to apoptosis and contributed to the generation of neuropathic pain in cancer chemotherapy. It has been shown that cisplatin causes activation of apoptotic caspases through activation of the endoplasmic reticulum stress pathway (Mandic *et al.*, 2003), which is dependent upon activation of caspase 12 (Breckenridge *et al.*, 2003). In addition, activation of extracellular regulated kinases (ERK1/2 kinases) and increased cleavage of caspase 3 were reported (Nowis *et al.*, 2007).

Suramin induces apoptosis of sensory neurons *in vitro* (Gill and Windebank, 1998b). It has been shown that it induces neuronal inclusions that are rich in GM1 ganglioside (Gill *et al.*, 1995). It induces elevation of intracellular ceramide levels in neurons. Ceramide is a key precursor in the synthetic pathway for gangliosides. It has also been shown to be a potential mediator in neurons (Wiesner and Dawson, 1996; Gill and Windebank, 1998b). Ceramide-induced neuronal death may be mediated by activation of nuclear factor-κB (Gill and Windebank, 1998c), which is a nuclear transcription factor (Abd-El- Ghany *et al.*, 2009).

Excitotoxicity: Glutamate mediates excitatory synaptic transmission through the activation of ionotropic glutamate receptors that are sensitive to N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) or kainate. Excess and sustained activation of the ionotropic glutamate receptors has been implicated in the initiation of neuropathologic events leading to a state of chronic hyperexcitability and persistence of exaggerated sensory processing (Hama *et al.*, 1995). It results in fulminant neuronal death, namely, glutamate neurotoxicity or excitotoxicity (Won *et al.*, 2002). The Ca^{2+} influx through NMDA receptors mediates the rapidly-triggered NMDA neurotoxicity, while Na^+ influx contributes to the swelling of the neuronal cell body (Choi, 1987).

Rzeski *et al.* (2004) investigated the neurotoxic effects of common cytotoxic drugs, namely cisplatin, cyclophosphamide, methotrexate, vinblastin and thiotepa, in neuronal cultures and observed a concentration dependent neurotoxic effect, which was potentiated by nontoxic glutamate concentrations, but was ameliorated by a NMDA receptor antagonist as well as AMPA receptor antagonists. In addition it was demonstrated that NMDA receptor antagonists decreased vincristine-induced hyperalgesia (Fukuizumi *et al.*, 2003; Zhu *et al.*, 2004).

Few studies have so far investigated the mechanisms of excitotoxicity induced by chemotherapeutic agents. We detected in our lab that vincristine caused a significant increase in NMDA receptor expression in both sciatic nerves and the spinal cord (Kassem *et al.*, 2009). Another mechanism involved in excitotoxicity could be the impaired mitochondrial calcium uptake caused by chemotherapeutic agents as will be discussed later.

Disturbance of sodium channel function: Excitability studies have shown alteration in Na^+ channel function in toxic and metabolic neuropathies (Krishnan and Kiernan, 2005; Kiernan *et al.*, 2005a) and in patients with genetic mutations in Na^+ channels (Kiernan *et al.*, 2005b).

Oxaliplatin is a novel chemotherapeutic agent effective against advanced colorectal cancer (De Gramont *et al.*, 2000; Goldberg *et al.*, 2004). Oxaliplatin differs from other platinum compounds (cisplatin and carboplatin) in that it uniquely produces an acute painful Hyperexcitability Syndrome (HES) accompanied by cold-induced paresthesia, dysesthesia and myotonia.

DRG neurons express a diverse range of Na^+ channel isoforms which are selectively affected by oxaliplatin in experimental models (Adelsberger *et al.*, 2000). Gamelin *et al.* (2002) suggested that oxaliplatin may act on specific isoforms of the voltage-gated Na^+ channel to increase the excitability of sensory neurons, an action inhibited by the Na^+ channel blocker carbamazepine. Black *et al.* (2004) added that, short periods of toxin exposure induce Na^+ channel isoform expression changes in DRG neurons.

Krishnan *et al.* (2006) also demonstrated acute abnormalities of nerve excitability in patients treated with oxaliplatin. Specifically the changes in Na^+ channel-dependent variables, refractoriness and relative refractory period duration, suggest that this acute form of neurotoxicity is mediated through an effect on axonal voltage-gated transient Na^+ channels. Kowalski *et al.* (2008) was also able to demonstrate that HES is the result of peripheral nerve hyperexcitability as a consequence of the development of a functional channelopathy of axonal

Na$^+$ channels, which may be caused by a Ca^{2+} level imbalance. Furthermore, it was reported that the only Na$^+$ channel found at the node of Ranvier in the peripheral nervous system co-localizes with the Na$^+$/Ca^{2+} exchanger at sites of axonal injury (Craner *et al.*, 2004). Alteration in axonal Na$^+$ concentration may trigger reverse flow of the Na$^+$/Ca^{2+} exchanger which activates damaging Ca^{2+}-mediated processes leading to activation of destructive biochemical cascades and axonal degeneration (Craner *et al.*, 2004; Vucic *et al.*, 2007). Reduction in membrane-bound Ca^{2+} may contribute to axonal hyperexcitability, which underlies parasthesia, cramp and tetany (Mogyoros *et al.*, 2000), common symptoms immediately following oxaliplatin infusion.

Dysregulation of cellular calcium homeostasis: An increase in cytosolic Ca^{2+} concentration mediates a wide range of neuronal functions including membrane excitability, neurotransmitter release, synaptic plasticity, gene expression and excitotoxicity (Berridge *et al.*, 2000).

Paclitaxel and vincristine often produce a pain syndrome that compromises their usefulness as anticancer drugs. It was suggested that the pain may be caused by an impairment of neuronal or glial ability to properly regulate intracellular Ca^{2+} (Siau and Bennett, 2006). The use of ethosuximide, a relatively selective T-type Ca^{2+}-channel blocker and gabapentin, an antagonist of Ca^{2+} channels containing the $\alpha_2\delta$ subunit, significantly reduces paclitaxel- and vincristine-evoked neuropathic pain (Flatters and Hartung, 2004; Xiao *et al.*, 2004). It was thus suggested that a possible underlying pathology could be impaired mitochondrial Ca^{2+} regulation, as mitochondria play a key role in intracellular Ca^{2+} homeostasis. The influx of extracellular Ca^{2+} via activated voltage- and ligand-gated Ca^{2+} channels on the cell membrane and the release of Ca^{2+} from endoplasmic reticulum can generate large, localized increases in cytoplasmic Ca^{2+} concentrations. Mitochondria, with their large buffering capacity and close proximity to these structures, can rapidly sequester this free Ca^{2+} (Berridge *et al.*, 2000; Montero *et al.*, 2000). Impaired mitochondrial Ca^{2+} uptake or increased leakage of mitochondrial Ca^{2+} would thus alter the spatio-temporal changes of cytosolic Ca^{2+} concentration, increase propagation of cytosolic Ca^{2+} concentration signals (Boitier *et al.*, 1999) and modulate Ca^{2+}-dependent processes, such as increased exocytosis of neurotransmitters (Montero *et al.*, 2000). Vincristine has been reported to affect Ca^{2+} movement through the mitochondrial membrane, reducing both the amount and rate of Ca^{2+} uptake and decreasing Ca^{2+} efflux (Tari *et al.*, 1986). Paclitaxel was found to cause a rapid decline in mitochondrial membrane potential and, notably, a loss of mitochondrial Ca^{2+} via the mitochondrial permeability transition pore (Kidd *et al.*, 2002).

Vascular neurotoxicity: Dietrich *et al.* (2004) reported that cisplatin, ifosfamide and etoposide produced vascular neurotoxicity, the mechanism of which is still unclear. Epineurial peptidergic terminals mediate a vasodilatory response through CGRP that increases blood flow in the downstream endoneurial compartment (Zochodne and Ho, 1991). We found in our lab that vincristine resulted in a significant reduction of CGRP expression in sciatic nerves and spinal cord (Kassem *et al.*, 2009). Thus, it may be suggested that VCR by decreasing CGRP decreases the blood flow to the nerve producing ischemia. Won *et al.* (2002) showed that ischemia induces excitotoxicity. Thus, one of the mechanisms by which vincristine causes excitotoxicity may be through the induction of ischemia.

Miscellaneous mechanisms: Cavaletti *et al.* (2004) observed a highly significant correlation between the decrease in circulating levels of nerve growth factor and the severity of chemotherapy-induced neurotoxicity in patients treated with cisplatin and paclitaxel.

NO plays a role in nociceptive transmission owing to the localization of neuronal NO synthase in the superficial dorsal horn of the spinal cord (Coderre and Yashpal, 1994). Kamei *et al.* (2005) found that dysfunction of the L-arginine/NO/cGMP cascade in the spinal cord may trigger vincristine-induced thermal hyperalgesia. They suggested that this dysfunction may be due in part to the activation of protein kinase C.

NEUROPROTECTIVE EFFECT OF ERYHTROPOIETIN

Erythropoietin (EPO) is a naturally occurring hormone with multiple effects on a number of different cell types. Recent data have suggested neuroprotective and perhaps even trophic roles for EPO. *In vivo*, EPO protects neurons from cerebral ischemia and traumatic injury (Brines *et al.*, 2000) and reduces the severity of experimental autoimmune encephalomyelitis, spinal cord injury and sciatic nerve compression (Erbayraktar *et al.*, 2003). Bianchi *et al.* (2004) found that EPO both protects against and lowers the severity of experimental diabetic neuropathy.

In this study, we will discuss the potential role of EPO for neuroprotection against chemotherapy-induced neurotoxicity. Several mechanisms of action were proposed for the protective effect of EPO. It was

suggested that EPO ameliorates or prevents neuronal injury by antiapoptotic (Cole *et al.*, 2007), antioxidant (Sakanaka *et al.*, 1998), anti-inflammatory (Agnello *et al.*, 2002) effects in cell culture and animal models of neurological diseases.

Protective effect of EPO on axonal degeneration: As previously discussed, many chemotherapeutic agents induce peripheral neuropathy through axonal degeneration. Schwann cells were shown to abundantly express EPO receptors (Bruijn *et al.*, 2004). EPO was found to save the myelin sheaths from structural damage of cisplatin (Yoon *et al.*, 2009; Keswani *et al.*, 2004). Keswani *et al.* (2004) also demonstrated that there is an endogenous neuroprotective pathway in the peripheral nervous system that protects sensory neurons against axonal degeneration. Schwann cell-derived EPO is important in this endogenous protective pathway. In a co-culture system, when DRG sensory neurons were injured by axonal transection or by exposure to toxic drugs that cause axonal degeneration, it was observed that periaxonal Schwann cells up-regulated their EPO expression (Keswani *et al.*, 2004). A similar observation was also made in rats when the sciatic nerve was transected; the Schwann cells in contact with the transected axons up-regulated EPO expression within hours and the DRG sensory neurons up-regulated the expression of EPO receptors. Using a variety of experiments, it was possible to show that the injury signal is nitric oxide. Neuronal nitric oxide synthase up-regulates nitric oxide that, in turn, activates the hypoxia-inducible factor-1, a key regulator of EPO in Schwann cells (Hoke, 2006) and provides protection against injury to the neuron and the axon. This endogenous neuroprotective pathway can overcome toxic injury to a certain degree, but can easily be augmented by exogenous EPO, as shown in paclitaxel-induced peripheral neuropathy (Melli *et al.*, 2006). Using microfluidic channels that allow compartmentalized culturing of neurons, it was shown that recombinant human EPO provides neuroprotection against paclitaxel-induced axonal degeneration, whether it is applied to the cell body or the axons directly (Yang *et al.*, 2009). Also in cisplatin-induced peripheral neuropathy, EPO was reported to be protective in rats, as evaluated by electrophysiology (compound muscle action potential) and histologic examination of the number of sciatic nerve fibers. It was found that it spares the number of normal nerve fibers, protects the amplitude and area of compound action potential and may also play a role in active myelinization (Orhan *et al.*, 2004). Furthermore, Yin *et al.* (2010) observed that EPO administration promoted functional recovery and enhanced axonal regeneration after sciatic nerve transsection in the rat by increasing myelin thickness and axon diameter as well as myelinated fiber counts.

Campana and Myers (Campana and Myers, 2001) reported the detection of localized EPO and EPO-receptors in the sciatic nerve and DRG of adult rats. Their data indicate that EPO is produced in the cell bodies and axons in normal DRG and is up-regulated in Schwann cells after painful chronic constriction injury. The distribution of EPO- receptors was different than EPO, indicating that ligand binding was not the sole reason for EPO immunoreactivity in tissues. The presence of EPO and EPO-receptors in axons of normal nerve suggests that they are integral components of neuronal function and that changes after nerve injury may contribute to aberrant cellular activity. The up-regulation of EPO in Schwann cells after nerve injury is not surprising, as it has been shown previously that hypoxic conditions increased EPO mRNA in CNS glia (Masuda *et al.*, 1994; Bernaudin *et al.*, 2000). Enhanced production of EPO at the injury site may regulate several processes. Increased EPO may facilitate retrograde transport of EPO to the sensory neurons of the DRG. Transported EPO/EPO-receptor complexes to specific subpopulations of neurons could potentially regulate signaling cascades involved in aberrant neuronal activity. In addition, increased levels of EPO at the injury site may potentiate Schwann cell proliferation through an autocrine mechanism involving JAK2 activation. After nerve injury, Schwann cells proliferate in the absence of axons producing their own growth factors (Mirsky and Jessen, 1999). Thus, similar to EPO's role in hematopoiesis, EPO may induce dedifferentiated Schwann cells to proliferate.

Protective effect of EPO against apoptosis and DNA adducts: Raff *et al.* (2002) proposed that dying-back degeneration is a stereotyped response of axons that, under some circumstances, activate a self-destruct program similar to that which occurs in the cell body during apoptosis. Most of the current focus is directed towards the antiapoptotic effect of EPO. In a spinal nerve crush model EPO protected DRG neurons against neuronal apoptosis by enhanced JAK2 phosphorylation (Halstead *et al.*, 2004). Proteins of the bcl-2 family, extracellular signal-regulated kinases and the phosphatidylinositol 3-kinase/Akt seemed to be involved in EPO-activated anti-apoptotic pathways as well (Gregory *et al.*, 1999; Weishaupt *et al.*, 2004). Binding of EPO to its receptor leads to an activation of the phosphatidylinositol 3-kinase/Akt pathway and nuclear factor-κB (NF-κB) pathway via phosphorylation resulting in an up regulation of antiapoptotic proteins including

XIAP and C-IAP2, subsequently blocking the activation of specific cell-death proteases leading to apoptosis (Wang *et al.*, 1998). EPO mediates its neuroprotective properties via cross-talk between JAK2 and the NF-κB (Digicaylioglu and Lipton, 2001).

Nowis *et al.* (2007) suggested that EPO might be an effective cytoprotective agent that reduces cisplatin-induced neurotoxicity. They investigated the direct influence of EPO on cisplatin-induced neurotoxicity against primary cortical neurons isolated from rats. They observed that pre-treatment of neurons with EPO significantly protected these cells. These effects correlated with amelioration of cisplatin-mediated activation of ERK1/2 kinases and decreased cleavage of caspase 3. Similarly to EPO, a selective ERK1/2 inhibitor significantly reduced cisplatin-induced cytotoxicity against neuronal cells.

Effect of EPO on DNA adducts: Cisplatin mediates its antineoplastic activity by formation of distinct DNA intrastrand cross links. The clinical efficacy and desirable dose escalations of cisplatin are restricted by the accumulation of DNA lesions in DRG cells leading to sensory polyneuropathy (Yoon *et al.*, 2009). Yoon *et al.* (2009) investigated in a mouse model by which mechanism recombinant erythropoietin protects the peripheral nervous system from structural and functional damage caused by cisplatin treatment with special emphasis on DNA damage burden. They observed that co-application of EPO with cisplatin did not alter the level of unrepaired cisplatin-DNA lesions accumulating in DRG target cells and inhibited myelin sheaths from structural injuries and resulted in an increased number of intact mitochondria. Their study clearly demonstrated the high neuroprotective efficacy of EPO in the peripheral nervous system with respect to neurophysiological functions and morphological integrity. This neuroprotective effect is not mediated by attenuated DNA adduct formation or accelerated repair but to the mitochondrial protection.

Protective Effect of EPO against excitotoxicity: EPO is neuroprotective in a variety of excitotoxic *in vitro* models (Byts and Siren, 2009). EPO was shown to reduce excitotoxicity in mouse hippocampal slice cultures (Montero *et al.*, 2007) and in newborn mouse brain injury (Keller *et al.*, 2006). Another study showed that the neuroprotective effect of EPO was abolished via an NMDA receptor antagonist (Yazihan *et al.*, 2008). Several mechanisms have been proposed: Won *et al.* (2007) demonstrated that EPO is a potent protector of the spinal GABAergic neurons against kainate excitotoxicity, an effect, mediated by signal transduction involving EPO receptor-dependent tyrosine janus kinase 2 pathway (Yoo *et al.*, 2009). In a model of chronic glutamate excitotoxicity, Naganska *et al.* (2010) reported that EPO exhibited its neuroprotective ability mainly through the prevention of apoptotic neuronal changes. Kawakami *et al.* (2000) have shown that activation of the EPO-receptor modulates Ca^{2+} influx *in vitro*. Inhibition of Ca^{2+} influx upon depolarization directly reduces synaptic vesicle release of glutamate, which acts to reduce the magnitude of neuronal injury (Xu *et al.*, 2009; Kawakami *et al.*, 2000). Other indirect effects of EPO that reduce neuronal injury have also been delineated. For example, EPO treatment increases production of glutathione peroxidase and in this manner ameliorates neuronal damage caused by excitotoxins (Genc *et al.*, 2002).

In a model of vincristine-induced neurotoxicity, we proved in our lab that EPO conferred a neuroprotective effect through inhibition of NMDA receptor expression (Kassem *et al.*, 2009). Although, there are almost no experimental data supporting the efficacy of EPO against excitotoxic damage induced by chemotherapeutic agents, we believe that, on the basis of literature, EPO would prevent chemotherapy-insulted excitotoxicity.

Protective effect of EPO on sodium channel dysfunction or dysregulation of cellular calcium homeostasis: Several neuroprotective strategies have been examined in oxaliplatin-induced neurotoxicity with limited success (Kiernan, 2007; Walker and Ni, 2007). Strategies to modulate axonal Na^+ channels have been attempted with mixed results, suggesting that different approaches may be required specifically to target oxaliplatin-induced modulation of Na^+ channel function. No studies involving the neuroprotective effect of EPO on Na^+ channel dysfunction or on Ca^{2+} imbalance in chemotherapy-induced neurotoxicity have been reported so far.

EPO does not interfere with antineoplastic activity of chemotherapeutic agents: A major concern in the use of neuroprotectant drugs to prevent neurotoxicity is their possible interference with the antineoplastic activity of chemotherapy. A study by Bianchi *et al.* (2006) investigated whether EPO or carbamylated EPO affected the tissue distribution of cisplatin by measuring the concentration of platinum in the peripheral nervous system and in the kidney where it accumulates after cisplatin administration and cisplatin-DNA adducts are present (Meijer *et al.*, 1999). They found no difference in platinum tissue concentrations, supporting the opinion that EPO and carbamylated EPO do not interfere with cisplatin. This was further confirmed by a study that

showed that EPO treatment given concurrently with cisplatin was neuroprotective without influencing the effectiveness of the treatment or tumour growth (Bianchi *et al.*, 2007).

A clinical study performed on patients with advanced lung cancer receiving cisplatin, showed that in the EPO treated group the neurological score was significantly lower in comparison to the untreated group, suggesting that EPO treatment in cancer patients can exert a limiting effect on cisplatin-induced peripheral neurotoxicity (Mangiameli *et al.*, 2002).

Cervellini *et al.* (2009) investigated the effects of EPO on chemotherapy-induced peripheral neurotoxicity by docetaxel, one of the taxane compounds, *in vivo* and whether it interfered with tumor growth or antitumor activity. EPO protected against docetaxel-induced peripheral neurotoxicity and tumor growth. It significantly improved the thermal threshold, tail nerve conduction velocity and intra-epidermal nerve fiber density. EPO did not impair docetaxel antitumor activity.

Erythropoietic action of EPO does not interfere with its neuroprotective effect against chemotherapy-induced neurotoxicity: The erythropoiteic action of EPO is a potential cause of side effects if EPO is used for neuroprotection. Bianchi *et al.* (2006) were able to identify a derivative of EPO (carbamylated EPO) that does not raise the hematocrit value, but retains the neuroprotective action exerted by EPO. They studied the effect of both EPO and carbamylated EPO on cisplatin neurotoxicity *in vivo*. They found that EPO or carbamylated EPO alone had no effect on the normal function of the peripheral nerves but had significant and reproducible neuroprotective effects in cisplatin-induced neurotoxicity. These results were supported both by the neurophysiologic findings showing an improvement of tail sensory nerve conduction velocity and by the histopathologic examination showing a higher density of intra-epidermal nerve fibers in the footpad skin. The authors suggested that there is a direct protective effect of EPO and carbamylated EPO on sensory neurons and/or peripheral nerves through the direct binding to the EPO receptor, which is widely expressed in the peripheral nervous system and overexpressed after nerve injury (Campana and Myers, 2003; Keswani *et al.*, 2004).

In conclusion, these findings widen the spectrum of possible use of EPO as neuroprotectant drug, strongly supporting its effectiveness. However, they also indicate the need for further experimental and preclinical studies to optimize its effectiveness, to determine the exact mechanism and site of action and to clarify the issues of long-term tolerability and safety *in vivo*, with the final aim of identifying the best strategy for clinical application.

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