Possible Correlation between Paclitaxel Chemotherapy, Vitamin D and Peripheral Neuropathy

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Abstract

Background: Cancer patients who receive anticancer treatment develop peripheral neuropathy in up to 60% of cases. Several cytotoxic agents such as epothilones (ixabepilone), platinum compounds (cisplatin, carboplatin, and oxaliplatin), proteasome inhibitors (bortezomib), taxanes (paclitaxel and docetaxel), vinca alkaloids (vincristine and vinblastine), and immunomodulatory drugs (thalidomide) are able to induce a peripheral neuropathy (CIPN). Paclitaxel induces axonal transport disruption via microtubule stabilization, changes in morphology and function of mitochondria, and inflammation. These pathological changes cause symmetrical damage of axons and nerve fiber loss. While a number of reports exist describing the pleiotropic effects of vitamin D and its role in the development of cardiovascular disease, diabetes, and various cancers, less attention has been paid to the effects of vitamin D on the development and function of the nervous system. There is evidence indicating the role of vitamin D in regulating the development and function of nerve cells, The involvement of vitamin D in the function of the central nervous system is supported by the presence of the enzyme 25(OH)D3-1a-hydroxylase, responsible for the formation of the active form of vitamin D, as well as the presence of vitamin D receptors in the brain, mainly in the hypothalamus and dopaminergic neurons of the substantia nigra. The neuroprotective role of vitamin D3 involves the synthesis of proteins binding calcium (Ca2+) ions (e.g., parvoalbumin) and thus maintaining cellular calcium homeostasis, which is very important for brain cell function. Moreover, 1,25-(OH)2D3 administration was shown to down-regulate L-type voltage-sensitive Ca2+ channel expression in rat hippocampal cultures.

Keywords: Paclitaxel Chemotherapy, Vitamin D, Peripheral Neuropathy

Introduction

Cancer patients who receive anticancer treatment develop peripheral neuropathy in up to 60% of cases**.** Several cytotoxic agents such as epothilones (ixabepilone), platinum compounds (cisplatin, carboplatin, and oxaliplatin), proteasome inhibitors (bortezomib), taxanes (paclitaxel and docetaxel), vinca alkaloids (vincristine and vinblastine), and immunomodulatory drugs (thalidomide) are able to induce a peripheral neuropathy (CIPN). The occurrence of pain in hands and feet in a “glove and stocking” pattern is one of the most common dose-limiting factors in treatment **(1).**

Besides pain, CIPN presents with numbness, loss of vibration sense due to the affection of large caliber sensory neurons, as well as dysesthesia and cold and mechanical hypersensitivity, functions that

are executed by small A_- and C-fibers. It is anticipated that due to an overall increase of survival, the prevalence of CIPN will increase in the next decade. Paclitaxel is an antineoplastic agent most used in ovarian, breast, and prostate cancer treatment. Symptoms such as pain and numbness in hands and feet are predominately sensory due to paclitaxel

accumulation in the dorsal root ganglia (DRG). The DRGs mainly consist of sensory neuron cells and are highly susceptible to paclitaxel accumulation due to a more permeable blood-nerve barrier.Paclitaxel induces axonal transport disruption via microtubule stabilization, changes in morphology and function of mitochondria, and inflammation. These pathological changes cause symmetrical damage of axons and nerve fiber loss.With the occurrence of paclitaxel-induced peripheral neuropathy, the treatment schedule of patients usually needs to be altered—e.g., via dosage reduction or treatment stop—to prevent further progress of neuropathic symptoms **(2)**.

Paclitaxel

Paclitaxel was first extracted and isolated from the bark of the Pacific yew tree (Taxus brevifolia) in 1971 **(2).**It became more viable with semi-synthetical production from a paclitaxel precursor from the European yew tree's needles.

Currently, paclitaxel is one of the most used taxanes, besides other formulations such as docetaxel, cabazitaxel, and nab-paclitaxel. Taxanes are commonly used to treat ovarian, breast, small- and non-small-cell lung, prostate, stomach, esophageal, bladder, pancreas head and neck cancer, as well as Kaposi's sarcoma and melanoma **(3).**

Structurally paclitaxel is a diterpenoid pseudoalkaloid with a taxane ring as its nucleus (C47H51NO14). Due to its hydrophobic properties, a vehicle must be used for proper administration. Usually, a 50/50 solution of dehydrated ethanol and a polyethoxylated castor oil, Kolliphor EL, is **used**. it can induce acute hypersensitivity reactions with symptoms of dyspnea, flushing, rash, chest pain, tachycardia, hypotension, angioedema, and generalized urticaria [20]. To prevent these possible side effects, patients need to be pre-treated with antihistamines and corticoids **(4)**.

It is neurotoxic itself due to the induction of axonal swelling and degeneration of DRG neurons and vesicles **(2)**.

There is another paclitaxel formulation, nanoparticle albumin-bound paclitaxel (nabpaclitaxel), which is commonly used. Like CreEL-paclitaxel, nab-paclitaxel mainly accumulates in neurofilament 200-positive large-caliber neurons and less in Isolectin B4-, or calcitonin gene-related peptide-positive small-caliber neurons. Sensory nerve conduction studies demonstrated altered sensory dysfunction between the two formulations. It indicates that different "carriers" may impact the severity of neuropathy induced by paclitaxel

via different tissue uptake. Antineoplastic Mechanism of Paclitaxel Paclitaxel and other taxanes can impair the increased abnormal cell proliferation and mitosis rate of tumor cells. During mitosis, microtubules are essential players in the segregation process of chromosomes into the daughter cells**.** Especially during the interphase, microtubules act as tracks for organelles and the nucleus **(5).**

Microtubules are formed by _- and _-tubulin heterodimers to act as intracellular dynamic cytoskeletal polymers.

Their dynamic stems from the ability to polymerize and depolymerize. Polymerization can also be described as rapid growth, and depolymerization can be characterized as shrinkage. Due to these properties, the cell can rapidly reorganize its cytoskeleton. Both _- and _-tubulin are bound to guanosine triphosphate (GTP). GTP-bound tubulin units are incorporated at the microtubule ends during the polymerization.

This process makes the microtubule grow and form a stabilizing cap. Afterward, GTP gets hydrolyzed into GDP. This hydrolyzation process releases energy and destabilizes the microtubules and the microtubules splay apart at the end **(6).**

Paclitaxel is able to interact with _-tubulin, which interferes with the dynamic process**.** Through small openings in the microtubule lattice, paclitaxel can enter and bind to _-tubulin This causes a strengthening between the tubulin subunit's lateral contacts**.** Hence, the depolymerization is suppressed and the microtubules are stabilized. **(6).**

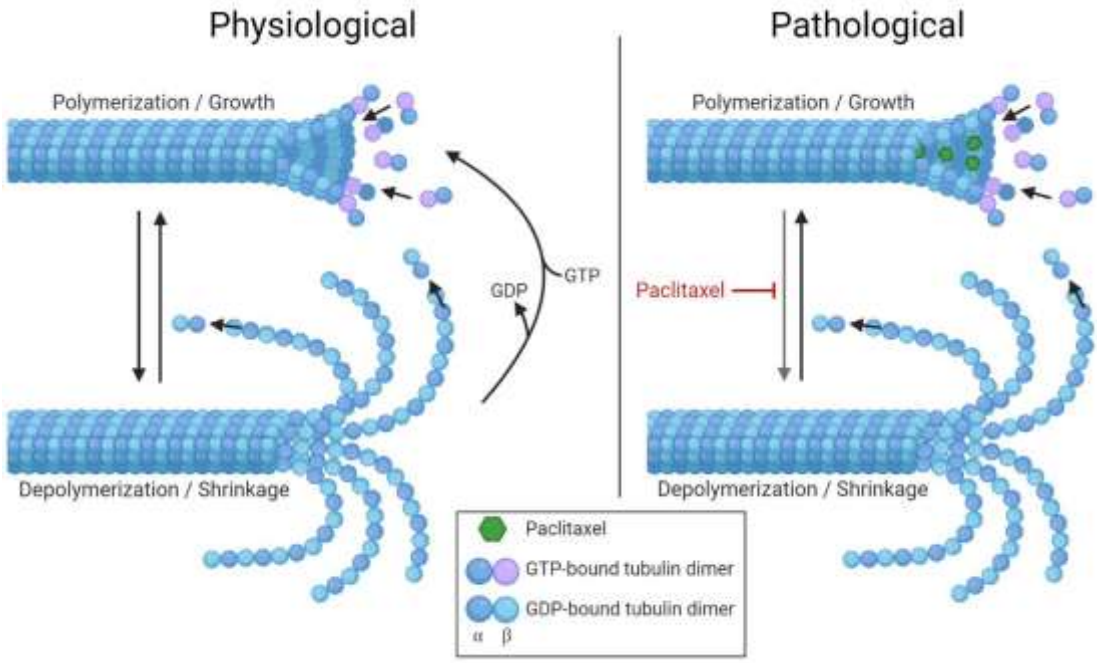

Figure 1. Paclitaxel enhances microtubule stability. Under physiological conditions, guanosine triphosphate (GTP)-bound tubulin dimers get incorporated at the growing end of the microtubules. This structure is supposed to form a stabilizing cap of GTP-bound tubulin. The conformation change in tubulin dimers is due to the GTP getting hydrolyzed into guanosine diphosphate (GDP), and this destabilizes the microtubule lattice. Through the loss of the GTP-tubulin cap, the microtubules are getting depolymerized. This process of microtubule depolymerization gets prevented by paclitaxel binding to _-tubulin. Due to the interference with paclitaxel, mitosis is arrested between the metaphase and anaphase called the G2/M phase This leads to membrane potential reduction in mitochondria and causes the opening of the permeability transition pore channel. Proapoptotic factors release subsequently induces apoptosis, Moreover, antiapoptotic effects are impaired, as paclitaxel is able to bind directly to B-cell lymphoma 2 (BCL2) **(7).**

4. Paclitaxel-Induced Peripheral Neuropathy

Paclitaxel-induced peripheral neuropathy is associated with a length-dependent axonal sensory neuropathy .The induced neurotoxicity depends on paclitaxel dosage and infusion time and can be fostered by underlying conditions or co-treatment with other drugs **(8)**.

This makes the severity and occurrence of paclitaxel-induced peripheral neuropathy hard to predict. First symptoms such as numbness, tingling, and/or allodynia in the patient's fingers and toes can be observed 24 to 72 h post-injection. Numbness and tingling can reach up to the lower leg and wrists of patients in a "glove and stocking" like manner. These symptoms occur in up to 97% of all treated patients, especially when the cumulative dose exceeds 1400 mg/m2, and about 60% of all treated patients manifest chronic paclitaxel-induced peripheral neuropathy **(9)**.

Due to the microtubule-stabilizing properties of paclitaxel, it damages peripheral axons symmetrically, and described symptoms are the phenotypic correlations of an axonal dying back pattern. In severe cases, axonal degeneration occurs along with secondary demyelination. Axonal injury can be seen in nerve conduction studies by a reduction or a complete loss of sensory nerve action potentials, Paclitaxel-induced impairment is most prominent in large myelinated A_-fibers (**10)**.

This is reflected by symptoms such as the impaired sensation of vibration or touch. Further, a high concentration of paclitaxel leads to a loss of intraepidermal nerve fibers (IENFs). These nerve fibers enter the epidermis as A_- and C-fibers. Loss of those fibers is reflected in taxol-induced hyperalgesia and pain sensation in patients. In comparison, treatment with lower doses of paclitaxel induces an increase in IENFs diameter and a degeneration of terminal arbor.

This overall leads to a nerve fiber loss in the epidermis, manifesting in symptoms of thermal hyperalgesia and mechanical allodynia **(11).**

An important molecule that executes axonal degeneration is SARM1 (Sterile Alpha and TIR Motif Containing 1).

It is activated in demyelinated axons and its NADase, which downstream can initiate the process of axonal self-destruction, A class of isoquinoline small molecules has been described to reversible inhibit SARM1 NADase leading to protection against traumatic injuries and mitochondrial damage **(12).**

It has been recently described that these molecules were also able to prevent the loss of IENFs and partially protect axonal functions in a paclitaxel-induced peripheral neuropathy animal model **(2)**.

5. Mechanisms of Neuronal Injury and Neuronal Dysfunction

5.1. Altered Microtubules Dynamics

Paclitaxel is a highly effective chemotherapeutic agent due to the stabilization of microtubules in cancer cells. However, this stabilization also does affect microtubules of sensory neurons in the DRG and axons of the PNS. Over the last decades, a number of in vitro and in vivo models for paclitaxel-induced neuropathy have been established **(13).**

There is evidence that paclitaxel impairs the transport of proteins, organelles, nutrients, neurotransmitters, and **mRNA** Downstream of impaired mitochondrial transport lay the undersupply of ATP, which is needed for axonal transport. This way, axonal transport is further decreased. Further, impaired mitochondrial transport promotes the breakdown of the ion gradient in the axolemma, which is crucial for electrochemical impulses. Hence, microtubule stabilization leads to loss of axonal transport, which promotes axonal degeneration or axonopathy and ends in peripheral neuropathy **(14).**

Moreover, the impairment of axonal trafficking of RNA transport granules inhibits bclw translation, which reduces the expression of Bcl2 family member Bclw (Bcl2l2). The Bcl-2 homology (BH)4 domain of Bclw is able to bind inositol 1,4,5-trisphosphate receptor (IP3R)1. IP3R lays upstream of axonal degradation via the release of cytoplasmic calcium, leading to increased calcium flux into mitochondria and the proteolysis of calpain, a calcium-dependent enzyme. Due to the impairment of axonal trafficking during paclitaxel treatment, IP3R is not bound and able to induce axonal degeneration **(15).**

Mitochondria and Oxidative Stress Paclitaxel treatment impairs not only the axonal transport of mitochondria but also their morphology and function. Mitochondria of myelinated fibers and unmyelinated Cfibers and paclitaxel treatment swell up and vacuolize with the fragmentation of cristae due to the opening of the mitochondrial permeability transition pore, These changes correlate with the pain-like behavior in rats. Furthermore, the paclitaxel-induced alterations in the permeability transition pore opening also cause changes in calcium flow. Calcium flow changes induce deficiencies in the mitochondrial respiratory chain, which leads to ATP deficits. These ATP deficits are detectable during paclitaxel-induced pain sensation and persist even after the peak of pain sensation **(16).**

It is thought that impairment of mitochondrial function moreover results in degeneration of terminal arbors and further fostering the generation of neuropathic pain Furthermore, the response to oxidative stress is impaired by paclitaxel treatment, further increasing the ATP deficit**.** It was observed that co-treatment with antioxidants reduced mitochondrial dysfunction, intraepidermal nerve fiber loss, and pain. Mitochondrial damage and reactive oxygen species (ROS) are closely dependent since mitochondrial damage cause the production of ROS, i.e., H2O2 formation. ROS, in turn, cause damage to the mitochondria, inducing DNA fragmentation and mitochondrial membrane potential loss **(17).**

Hence, ROS are crucial players in the oxidative stress reaction and are increasingly expressed after paclitaxel treatment**.** Along with elevated ROS levels, manganese activity, copper-zinc SOD activity, and glutathione (GSH) peroxidase antioxidant enzyme activities are induced by paclitaxel. Notably, Fidanboylu and colleagues could demonstrate that a non-specific ROS scavenger, N-tert-Butyl-_-phenylnitrone (PBN), could prevent the development of paclitaxel-induced peripheral neuropathy.There is also evidence that epidermal matrix-metalloproteinase 13 (MMP-13) promotes degeneration of unmyelinated nerve fibers following paclitaxel treatment. In zebrafishes that are exposed to paclitaxel, H2O2 reactive species are increased in basal keratinocytes, originating from damaged mitochondria. This upregulates MMP-13, which contributes to matrix degradation and degeneration of axons in the

epidermis. While MMP-13 inhibition did not alter mitochondrial damage, it was able to prevent axon degeneration **(18).**

In general, matrix-metalloproteinases (MMPs) are regulated by ROS: expression of MMP-2 has been increased in a breast cancer cell line after being exposed to a mitochondrial ROS inducer. In prostate cancer cells, increased H2O2 upregulated MMP-3 expression by inhibiting the MMP-3 suppressor. Inflammation and Pain Paclitaxel induces inflammation by cytokine and chemokine release and infiltration of non-resident macrophages into the DRGs, and this inflammatory reaction results in neuropathic pain. Most prevalent are increased interleukin-1_ (IL-1_), IL-8, and tumor

necrosis factor _ (TNF-_) expression . An anti-TNF-_ agent or an IL-1 receptor agonist was able to prevent paclitaxel-induced pain . The same goes for IL-8 and its receptors, inhibition of those reduced nociception as well as mechanical and cold hypersensitivity **(19).**

Chemokine receptors that are upregulated in the DRG after paclitaxel treatment are C-X-C chemokine receptor type 4 (CXCR4) and receptor for advanced glycation end products (RAGE). The increased expression of those receptors goes along with an increased macrophage accumulation in the sciatic nerve. Interestingly paclitaxel-induced allodynia could be prevented with CXCR4 and RAGE antagonists. Further, chemokines C-X-C motif chemokine ligand 1 (CXCL1), C-X-C motif chemokine ligand 12 (CXCL12), and C-X3-C motif chemokine ligand 1 (CX3CL1) are reported to be involved in the immune reaction following paclitaxel exposure. On the one hand, inhibition of CXCL1 was able to reverse paclitaxel-induced mechanical allodynia **(20).**

On the other hand, CXCL1's receptor CXCR2 and PI3K have been found to be upregulated after paclitaxel treatment and may contribute to mechanical hypersensitivity**.** CXCL12 expression is increased after paclitaxel treatment and correlates with increased excitatory postsynaptic currents in the spinal dorsal horn neurons. CXCL12 could induce mechanical allodynia since inhibition of the CXCL12 signaling pathway improved paclitaxel-induced mechanical allodynia **(20).**

Lastly, paclitaxel induces an upregulated expression of CX3CL1 in spinal neurons. Inhibition of this chemokine was reported to reduce macrophage-neuron interactions**.** CX3CL1 possibly lies downstream of transcriptional factor NF-_B activation and histone acetylation. At the same time, NF-_B itself is a key player in thermal hypersensitivity in paclitaxel-induced peripheral neuropathy. As another part of the innate immune system, the complement system was linked via complement component 3 (C3) activation to paclitaxel-induced peripheral neuropathy, In vitro, paclitaxel enhanced C3 activation, and in vivo knock-out of C3 ameliorated paclitaxel-induced touch sensitivity and increased intradermal nerve fibers **(21).**

Cannabinoid receptors 1 (CB1) and 2 (CB2) were discovered to possibly play a role in the immune reaction, microglia activation, and pain sensation after paclitaxel treatment. Increased expression of CB2 **(21).**

Alongside chemokine (C-C motif) ligand 2 (CCL2) and IL-6, IL-4, and IL-10 expression after paclitaxel treatment is associated with a dysregulation of microglia in the dorsal horn . CB1 and CB2 agonists inhibited spinal glial activation and IL-1_, IL-6, TNF-_, and CCL2 up-regulation. Further, they also modulated spinal p38 MAPK and NF-_B activation**.** These effects prevented cold and mechanical allodynia **(21).**

Besides paclitaxel-induced microglial activation in the dorsal horn, an astrocyte activation, independent from microglia activation, in the spinal cord is detectable **(1).**

The main players of inflammation in the DRG are accumulated macrophages. Initially, an upregulation of MMP-3 after paclitaxel exposure is detected. MMP-3 can break down the extracellular matrix and attract macrophages. This is followed by an upregulation of CD163, a macrophage marker, and C11b, a monocyte and macrophage marker. CD11b is further implied in pathogen recognition, phagocytosis, and cell survival. The accumulated macrophages in the DRG express pro-inflammatory markers desensitizing primary sensory afferent resulting in neuronal and glial damage. It is most likely that altered neuronal and glial physiology disrupts spinal dorsal horn input and generates neuropathic pain **(22).**

Another macrophage attracting chemokine, monocyte chemoattractant protein 1 (MCP-1), is increasingly expressed in small nociceptive DRG neurons as well as spinal astrocytes of paclitaxel treated rats. Increased MCP-1 expression leads to an upregulation of its cognate receptor, C-C chemokine receptor type 2 (CCR2), in large and medium-sized

myelinated neurons. While increased overexpression of CCR2 induced increased calcium spikes in CCR2-positive neurons, macrophage depletion prevented increased CCR2 expression and reversed intra-epidermal nerve fiber loss and mechanical hypersensitivity. The activation of spinal astrocytes and satellite glial cells (SGC) is thought to be a secondary response to macrophage activation and neuronal damage. Paclitaxel induced an activation state in SGC and Schwann cells detectable in an upregulation of the transcription factor 3 (ATF3) **(23).**

Further, paclitaxel treatment leads to increased gap junction coupling between the SGC, promoting ATP and neurotransmitter expression by the SGC. While SGC are essential players in neuronal homeostasis, increased ATP and neurotransmitter expression paradoxically could induce neuronal death. Furthermore, the previously described increased expression of TLR4 could navigate TNF-_ release of SGC and thus inflammation, pain sensation, and transient receptor potential (TRP) channel activation. For example, TRPA1 and TRPV1 have already been implicated to be key roles in the induction of paclitaxel-induced cold hypersensitivity **(24).**

5.4. Drug Transporters Involvement in Paclitaxel Translocation

In the last few years, organic-anion-transporting polypeptides (i.e., OATP1B1 and OATP1B3) are increasingly recognized to be important molecules for translocation of paclitaxel across the plasma membrane. While hepatocytes are known to use OATPs to translocate paclitaxel, their expression and role in the peripheral nervous system are not well known. Recently, it has been shown that OATP1B2, the mouse homolog to OATP1B1 and OATP1B3, is expressed in mouse DRG and that pharmacological inhibition and knock-out of OATP1B2 ameliorated paclitaxel-induced peripheral neuropathy **(25)**.

This indicated that OATP1B2 could be a driving force of paclitaxel accumulation in the DRG neurons. Moreover, OATP1B3 has been found to be expressed on ovarian and associated cancer cell lines **(25)**.

Another candidate for paclitaxel transport across the cell membrane could be organic anion transporter 2 (OAT2). Interactions between OAT2 and paclitaxel have been observed**,** further underlined by increased accumulation of radiolabeled paclitaxel in OAT2 expressing oocytes, However, OAT2 has three transcript variants and the corresponding variant has not been identified yet. OAT2 transcript variant 2 was able to translocate paclitaxel but is not expressed on the examined cell lines, while OAT2 tran script variant 1 also had paclitaxel as its substrate in disregard on which cell type it is expressed **(18).**

Overall, it is crucial to understand the accumulation mechanism of paclitaxel in the sensory neuron, which could be achieved with further studies of drug transporters. Understanding and manipulating the transport process could prevent or ameliorate paclitaxelinduced peripheral neuropathy **(26).**

6. Summary

Paclitaxel is a potent antineoplastic agent that in tumor cells induces cell death via microtubule stabilization. However, it can cause chemotherapy-induced peripheral neuropathy in up to 97% of all treated patients. Typically, paclitaxel induces a length-dependent axonal sensory neuropathy correlating with the dose, infusion time, underlying conditions, and co-treatment with other drugs. Most prevalent are the symptoms of numbness and tingling in the feet and hands of the patients. While it acts as a microtubule-stabilizing and apoptosis-inducing agent in cancer cells, these properties can symmetrically damage peripheral axons and induce an axonal dying back pattern, mainly affecting A_-fibers. The microtubule stabilization impairs the transport of proteins, organelles, nutrients, neurotransmitters, and mRNA in the PNS. ATP undersupply and paclitaxel-induced morphological changes of mitochondria correlate with increased pain sensation. Typical inflammation markers in the PNS after paclitaxel treatment are IL-1_, IL-8, and TNF-_, which are thought to elicit pain sensation. Other markers involved in the immune response include CXCR4,RAGE, CXCL1, CXCL12, CX3CL1, and C3. Interestingly, cannabinoid receptor agonists could ameliorate paclitaxel-induced neuropathic pain through the involvement in the immune reaction. Microglia and astrocytes independent from each other mainly get activated in the spinal cord. At the same time, SGC are the glial cells activated in the PNS, which in return can increasingly express TNF-_ and neurotransmitters promoting neuronal death. Moreover, CD11b and CD163 positive macrophages infiltrate the DRG attracted by MMP-3 and MCP-1, which further can induce neuronal degeneration. **(26).**

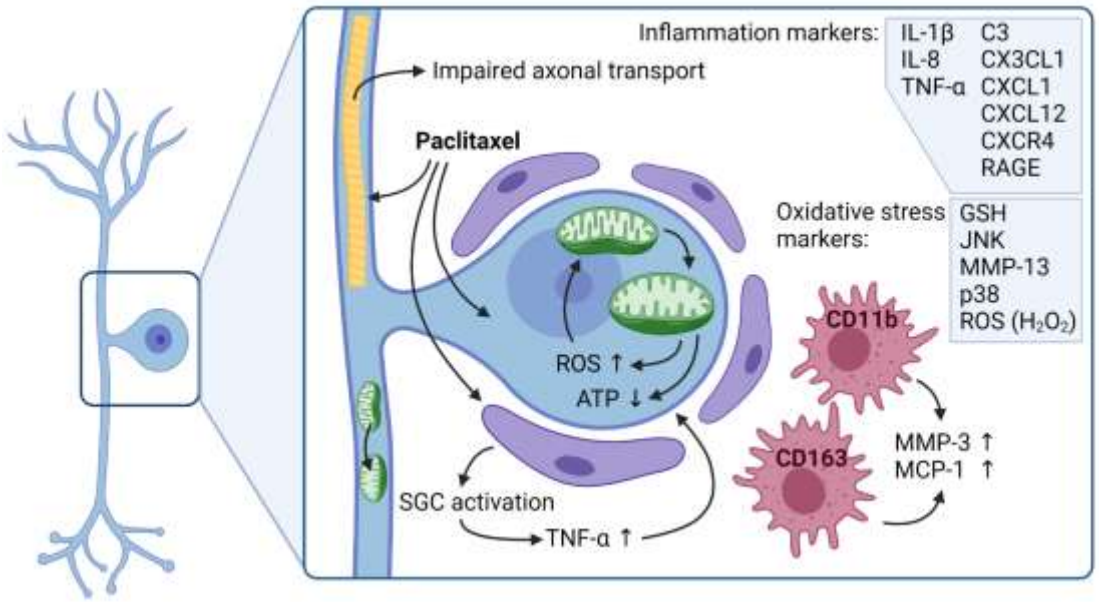

Figure 2. Pathomechanisms of paclitaxel-induced peripheral neuropathy in the peripheral nervous system. ATP—adenosine triphosphate, C3—complement component 3, CD11b—cluster of differentiation 11B, CD163—cluster of differentiation 163, CX3CL1—C-X3-C motif ligand 1, CXCL1—C-X-C motif chemokine ligand 1, CXCL12—C-X-C motif chemokine ligand 12, CXCR4—as C-X-C chemokine receptor type 4, GSH—glutathione, IL—interleukin, JNK—c-Jun N-terminal kinase, MCP-1—monocyte chemoattractant protein 1, MMP—matrix-metalloproteinase, RAGE—receptor for advanced glycation end products, ROS—reactive oxygen species, SGC—satellite glial cell, TNF-_—tumor necrosis factor; Created with BioRender.com

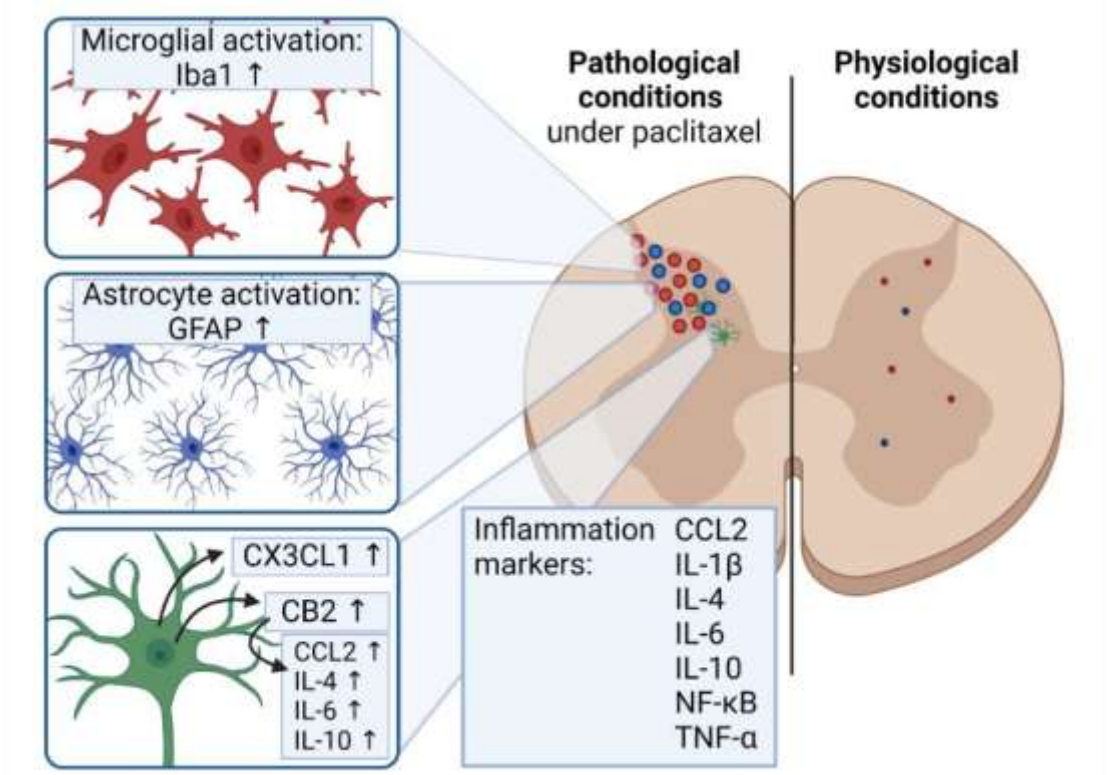

Figure 3. Pathomechanisms of paclitaxel-induced peripheral neuropathy in the spinal cord. CB2—cannabinoid receptor type, CCL2—C-C motif ligand 2, CX3CL1—C-X3-C motif ligand 1, GFAP—glial fibrillary acidic protein, Iba1—ionized calcium-binding adapter molecule 1, IL—interleukin, NF-_B—nuclear factor kappa B, TNF-_—tumor necrosis factor; Created with BioRender.com (accessed on 20 September 2021).

Especially the immune response modulation in in vivo studies with rodents were able to ameliorate or prevent paclitaxel-induced peripheral neuropathy. However, with prolonged survival rates of cancer patients, paclitaxel-induced peripheral neuropathy becomes an increasingly larger problem. Still, thus far, no approaches are available that have reached clinical use to prevent or decrease neuropathy severity

Vitamin D

The discovery of the systemic role of vitamin D opened a new area of research on the role of this vitamin in the modulation of physiological and pathological processes, as well as the prevention and treatment of many diseases **(27).**

While a number of reports exist describing the pleiotropic effects of vitamin D and its role in the development of cardiovascular disease, diabetes, and various cancers, less attention has been paid to the effects of vitamin D on the development and function of the nervous system**.(28)**

The physiological role and sources of vitamin D

The major role of vitamin D in the human body is commonly related to calcium metabolism and bone structure, and vitamin D deficiency is associated with the development of rickets in children and osteoporosis in adults. However, scientific evidence clearly indicates that the biological importance of this vitamin greatly exceeds these aspects. Currently, there is no doubt that vitamin D is involved in a number of processes, that it constitutes an important factor in maintaining health, and that its deficiency is associated with the development of various pathological processes **(29).**

Low levels of vitamin D are considered to be an important factor contributing to the development of cardiovascular diseases, metabolic syndrome and type 2 diabetes mellitus, inflammatory or immune disorders, as well as common cancers In humans, a natural source of vitamin D is its synthesis in the skin upon exposure to sunlight [ultraviolet B (UVB) radiation with a wavelength of 290–315 nm] **(30).**

The level of exposure to sunlight determines the rate of synthesis of vitamin D in the skin. This depends on such environmental factors as geographic latitude, season, time of day, cloud coverage as well as personal characteristics, such as skin pigmentation, age, clothing, typical amount of time spent outdoors, and the use of anti-UV protection. Low exposure to sunlight is associated with a low rate of vitamin D biosynthesis. However, excessive exposure does not result in a further increase in synthesis of vitamin D due to its rapid photodegradation into a variety of biologically inactive photoproducts. Vitamin D is also obtained from the diet. The main dietary sources of cholecalciferol are fatty fish (such as eel, herring, salmon, mackerel), fish oil, and egg yolk, as well as margarines and other products fortified with vitamin D. The presence of vitamin D in foods other than these is limited **(31).**

A specific protein that binds vitamin D, vitamin D binding protein (DBP), transfers it through the circulatory system primarily to the liver, where the first step in the metabolic activation of vitamin D takes place. This involves the enzymatic hydroxylation of carbon 25. The resulting 25-hydroxyvitamin D (25(OH)D) is the main circulating metabolite of vitamin D, with a typical half life of 2 to 3 weeks. **(32).**

This is why the level of 25(OH)D is considered to be an indicator of vitamin D status in the body. Next, 25(OH)D is transported to the kidneys (and to some other tissues, e.g., the skin, and cells of the immune system), where calcitriol (1*a*,25-(OH)2D3) is formed *via* the enzyme 1*a*- hydroxylase (CYP27B1). This is a biologically active form of vitamin D3, considered a hormone. Calcitriol molecules are transported to the cells of various organs, where they influence a number of biological processes by activating receptors for the active form of vitamin D. Moreover, some organs

have the ability to produce locally the steroid hormone 1*a*,25-(OH)2D, which generates cell-specific actions, e.g., proliferation, differentiation or immune regulation **(33).**

Action mechanism of vitamin D

An active metabolite of vitamin D, 1,25-dihydroxycholecalciferol, affects target cell function by regulating gene expression and *via* non-genomic action. In the former, an intracellular vitamin D receptor (VDR), belonging to the family of nuclear receptors, acts as a transcription factor, modifying the expression of a number of genes associated with various metabolic pathways. The other, non-genomic effect of 1,25-(OH)2D3 involves membrane-associated rapid response steroid-binding (MARRS) receptors for vitamin D located in plasma membrane caveolae **(34).**

Via these receptors, the hormonal form of vitamin D regulates cytosolic calcium concentration by releasing calcium (Ca2+) ions from intracellular stores and the influx of calcium ions through calcium channels. It also affects the activity of phospholipase C (PLC), adenylate cyclase as well as Raf and MAP kinase pathways. Vitamin D receptors have been found in cells of various tissues, not only those directly responsible for calcium metabolism. These include pancreas *b* cells, stomach cells, the ovaries, the testes, the thymus, white blood cell precursors, parathyroid tissue, and brain cells. These findings indicate an important role for these receptors, and for vitamin D itself, in regulating various metabolic processes, and underscore the vitamin's pleiotropic effects. **(35)**

The vitamin D receptor gene (*VDR*) is located on the long arm of chromosome 12 (12q13.1), and has several polymorphisms, for example FokI, BsmI, Tru9I, EcoRV, ApaI, TaqI, and Cdx2, which might have biological effects resulting in susceptibility to different diseases. Currently, data are available describing an association between VDR gene variants and risk of diabetes, cancer (cancer of the prostate, colon), osteoporosis, autoimmune disorders (lupus, cirrhosis, hepatitis, Crohn's disease, Graves' disease), and kidney diseases. Specific VDR polymorphisms have also been considered as possible risk factors for developing schizophrenia as well as multiple sclerosis, but this relation still awaits confirmation **(36).**

The neuroprotective effects of vitamin D

There is evidence indicating the role of vitamin D in regulating the development and function of nerve cells, The involvement of vitamin D in the function of the central nervous system is supported by the presence of the enzyme 25(OH)D3-1*a*-hydroxylase, responsible for the formation of the active form of vitamin D, as well as the presence of vitamin D receptors in the brain, mainly in the hypothalamus and dopaminergic neurons of the substantia nigra. Vitamin D is believed to play a similar role to that of neurosteroids. Due to its interaction with the MARRS receptors, the hormonal form of vitamin D affects various intracellular metabolic pathways. Moreover, the enzyme 1*a*-

hydroxylase and the nuclear VDRs are also present in the microglia, i.e., nonneuronal cells of the central nervous system. This suggests both autocrine and paracrine effects for calcitriol on nerve cells **(37).**

The influence of the active form of vitamin D on the nervous system is associated with modifying the production and release of neurotrophic factors such as nerve growth factor (NGF), which is essential for neuron differentiation, as well as increasing the levels of glial cell line-derived neurotrophic factor (GDNF**).(38)**

In addition, vitamin D has been shown to significantly increase the rate of neurite outgrowth when added to hippocampal explants. Moreover, 1,25-dihydroxyvitamin D3 is an important factor modifying the synthesis of such neuromediators as acetylcholine *via* increased gene expression of the enzyme choline acetyltransferase (CAT). Vitamin D has also been found to affect the expression of genes associated with GABA-ergic neurotransmission and to stimulate the expression of tyrosine hydroxylase (TH), responsible for catecholamine biosynthesis. The neuroprotective role of vitamin D3 involves the synthesis of proteins binding calcium (Ca2+) ions (e.g., parvoalbumin) and thus maintaining cellular calcium homeostasis, which is very important for brain cell function. Moreover, 1,25-(OH)2D3 administration was shown to down-regulate L-type voltage-sensitive Ca2+ channel expression in rat hippocampal cultures.**(39)**

This indicates the protective effect of the hormonal form of vitamin D on the brain *via* a reduction in the influx of calcium ions into neurons. It has also been shown, based on studies on immature rats, that vitamin D modulates L-type calcium channel opening *via* nongenomic effects through various kinase pathways and enzyme activities in the cerebral cortex. It is worth emphasizing that maintaining the appropriate level of calcium ions in nerve cells is especially important for their normal function. Physiologically, an increase in calcium (Ca2+) ions in nerve cells contributes to an increased release of glutamic and asparaginic acids that stimulate the N-ethyl-D-aspartate (NMDA) receptors to open the calcium channels, which results in nerve cell depolarization and increased influx of Ca2+ ions through the voltage-dependent calcium channels **(40).**

Increased levels of these ions in cytosol lead to the fusion of synaptic vesicles with the presynaptic membrane and the release of transmitters. Excess calcium in nerve cells can contribute to excitotoxicity because it leads to an increased release of stimulating amino acids and other neurotransmitters, the activation of nitric oxide synthase (NOS), and the formation of reactive oxygen species, as well as the activation of proteases and lipases, leading to plasmic and mitochondrial membrane damage. A disruption in calcium ion transport and high calcium levels triggers the arachidonic acid cascade and enhances lipid peroxidation **(40).**

Vitamin D also stimulates the influx of Ca2+ ions through store-operated calcium entry (SOCE) channels, located in cells such as skeletal muscle cells or lymphocytes. This involves stromal interaction molecule (STIM) proteins that play the role of calcium level sensors in these cells and regulate the SOCE process. It has not been established yet whether vitamin D's involvement in the regulation of these recently discovered paths of calcium influx extends to thenerve cells. Rat neuron culture studies showed that 1,25-(OH)2D3 increases glutathione levels in these cells. The reduced form of glutathione (GSH), supplied into nerve cells by astrocytes, is a fundamental antioxidant protecting cells against reactive oxygen species (ROS) and apoptosis caused by oxidation. This suggests an important neuroprotective effect for the active form of vitamin D3, by counteracting oxidative damage to the central nervous system**.** In addition, vitamin D inhibits the synthesis of inducible nitric oxide synthase (iNOS). In a hypoxic environment, this enzyme becomes activated in neurons, which yields a substantial amount of nitric oxide, high levels of which initiate a cascade of neurotoxicity and neuron death. This is due to the fact that nitric oxide is a precursor of peroxynitrite which in turn leads to the deactivation of a series of enzymes by reacting, for example, with sulfhydryl (-SH) groups as well as by injuring mitochondria and disturbing cellular energy processes **(41).**

The results of many studies suggest an impact of vitamin D on immune system function as well as on the development of inflammation. Suppressive effects of calcitriol on interferon *g* (IF-*g*) or interleukin-2 (IL-2) production by stimulating the synthesis of interleukin-10 (IL-10), also known as the cytokine synthesis inhibitory factor (CSIF), have been demonstrated. Additionally, administration of 1,25(OH)2D3 was shown to inhibit the production of tumor necrosis factor-*a*, interleukin-6, and NO in the EOC13 microglial cell line, indicating direct anti-inflammatory properties for calcitriol on microglia, At the same time, depression, and autoimmune diseases,

including multiple sclerosis, are believed to be associated with overproduction of pro-inflammatory cytokines that disrupt normal brain cell metabolism.**(42)**

Thus, vitamin D, with its immunomodulating effects, may decrease the risk of these processes, There has been growing interest in the potential effect of calcitriol on human glioma cells. Induction of steroidogenic genes by vitamin D was observed in human GI-1 cells [69]. In glioblastoma multiforme (GBM) cell lines (Tx3095, Tx3868, U87, U118, U373), no influence of vitamin D3 on cell growth regulation was found indicating resistance of these cells against antiproliferative effect of calcitriol **(43).**

However, multidirectional action of calcitriol in glioblastoma multiforme, depending on cell environment, and possibly depending on the various molecular profiles involved in metabolizing vitamin D3, was suggested, Therefore, the potential role of vitamin D in human glioma therapeutical concept is still under discussion **(43).**

Vitamin D as a neurohormone

Animal studies have shown that vitamin D deficiency may increase the risk of brain dysfunction. It was reported that vitamin D deficiency significantly affects brain cell differentiation and proliferation during the neonatal period. The timing of correction of vitamin D intake and levels was found to influence persistence of some of these changes and animal behavior. Clinical studies indicate a possible association of vitamin D deficiency with the development of Alzheimer's and Parkinson's diseases. In addition, poor vitamin D status has been implicated in the pathogenesis of dementias **(44).**

It has been shown that 25(OH)D insufficiency (£ 20 ng/ml) was associated with a higher risk of all-cause dementia (AD, stroke with dementia, and other), it has been demonstrated that vitamin D modulates progesterone protection of the brain from traumatic injury. The protective effect of progesterone was reduced in vitamin D deficient animals, and combined vitamin D and progesterone therapy, more effectively than progesterone alone, increased the extent to which spatial and reference memory were safeguarded following bilateral contusions of the medial frontal cortex These findings correspond to data reporting that vitamin D induces progesterone synthesis and progesterone-responsive gene expression in cell cultures. This and other lines of evidence suggest that vitamin D treatment might be critically important or preserving neurocognitive functions among the elderly. However, this hypothesis is still under discussion and need to be confirmed by larger studies . **(45)**

Moreover, a small pilot study investigating vitamin D deficiency and seizure control in epilepsy found that administration of vitamin D3 in patients with pharmaco-resistant epilepsy, and with low (< 30 ng/ml) serum 25(OH)D level, resulted in a median seizure number reduction of 40%. Adequate vitamin D supply may also lower the risk of multiple sclerosis (MS). This chronic demyelinating disease of the central nervous system leads to multifocal nervous tissue damage (axonal demyelination and disintegration) that may lead to spasticity and motor weakness**.** Seasonal vitamin D deficiency was observed to lead to symptom exacerbation. On the other hand, it was found that physical activity secondary to outdoor exercise and sunlight exposure in patients with relapsing-remitting MS was positively correlated with 25(OH)D serum levels which were higher than in inactive MS patients, In addition, patients with established MS and lower vitamin D levels are at higher risk for subsequent relapse **(46) .**

The influence of high treatment doses of vitamin D on MS patients was also studied. Twelve patients in an active phase of multiple sclerosis were given progressively increasing doses of vitamin D3: from 700 to 7,000 μg/week (from 28,000 to 280,000 IU/week) along with 1,200 mg elemental Ca/day**.** After 28-weeks of treatment, the number of gadolinium enhancing lesions per patient (assessed with a nuclear magnetic resonance brain scan) was found to decrease significantly. It has been postulated that the neuroprotective effects of vitamin D3 and its impact on the immune system may inhibit processes that lead to CNS damage, or act indirectly by activating restorative processes **(47).**

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