The role of pharmacotherapy in modifying the neurological status of patients with spinal and spinal cord injuries\textsuperscript{\dag}

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ARTICLE INFO

Article history:
Received 17 July 2014
Accepted 2 September 2014
Available online 1 October 2015

Keywords:
Spinal cord injuries
Methylprednisolone
GM1 ganglioside
Apoptosis
Calpain
Naloxone

ABSTRACT

The aim here was to conduct a review of the literature on pharmacological therapies for modifying the neurological status of patients with spinal cord injuries. The PubMed database was searched for articles with the terms "spinal cord injury AND methylprednisolone/GM1/apoptosis inhibitor/calpain inhibitor/naloxone/tempol/tirilazad", in Portuguese or in English, published over the last five years. Older studies were included because of their historical importance. The pharmacological groups were divided according to their capacity to interfere with the physiopathological mechanisms of secondary injuries. Use of methylprednisolone needs to be carefully weighed up: other anti-inflammatory agents have shown benefits in humans or in animals. GM1 does not seem to have greater efficacy than methylprednisolone, but longer-term studies are needed. Many inhibitors of apoptosis have shown benefits in in vitro studies or in animals. Naloxone has not shown benefits. Tempol inhibits the main consequences of oxidation at the level of the spinal cord and other antioxidant drugs seem to have an effect superior to that of methylprednisolone. There is an urgent need to find new treatments that improve the neurological status of patients with spinal cord injuries. The benefits from treatment with methylprednisolone have been questioned, with concerns regarding its safety. Other drugs have been studied, and some of these may provide promising alternatives. Additional studies are needed in order to reach conclusions regarding the benefits of these agents in clinical practice.

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O papel da farmacoterapia na modificação do estado neurológico de traumatizados vértebro-medulares

RESUMO

O objetivo deste trabalho foi fazer uma revisão da literatura sobre a terapia farmacológica para a modificação do estado neurológico de traumatizados vértebro-medulares. Foi feita uma na base de dados Pubmed por artigos com os termos "spinal cord injury AND
Introduction

Spinal and spinal cord injuries are among the most devastating traumatic situations and are responsible for high morbidity and mortality rates. The consequences of these injuries include reduction of motor and sensory capacity and perturbations of intestinal, urinary and sexual functioning.1 The impact of these problems becomes greater through absence of satisfactory therapy for modifying these patients’ neurological status.1

The pathogenesis of spinal cord injuries can be divided into two phases. The primary injury occurs immediately, and is characterized by compression, bruising or, rarely, complete breakage of the spinal cord. The secondary lesions arise over the course of several days and involve a variety of processes, such as inflammation, edema, ischemia, hemorrhage, electrolytic imbalances, release of arachidonic acid, excitotoxicity due to glutamate, apoptosis and lipid peroxidation. These phenomena lead to expansion of the primary lesion and cavitation of the spinal cord.1,2 Pharmacological therapies for spinal and spinal cord injuries have the aim of inhibiting these processes or stimulating spinal cord regeneration.

Methylprednisolone (MP), which is frequently used in treating acute spinal cord injuries, showed evidence of benefits in the National Acute Spinal Cord Injury Survey (NASCIS) II and NASCIS III studies.3,4 However, these findings have not been reproduced in other studies and use of MP is becoming increasingly controversial, because of the risk of potentially serious complications, in comparison with the modest benefits.1 This has led to efforts toward developing new drugs that might improve neurological functioning in cases of these diseases.1

The objective of this study was to conduct a review of the literature on pharmacological therapy for spinal and spinal cord injuries.

Materials and methods

A search for articles was conducted in the PubMed database, using the terms “spinal cord injury AND methylprednisolone/GM1/apoptosis inhibitor/calpain inhibitor/naloxone/tempol/tirilazad”, in Portuguese or in English, published over the last five years. Some older studies were also included because of their historical importance.

The pharmacological groups were divided according to their capacity for interfering in the physiopathological mechanisms of secondary lesions.

Drugs that inhibit inflammation

Subsequent to spinal and spinal cord injury, inflammation and hydrolysis occur in the spinal cord, which results in destruction of neurons and microvessels.4 The main function of these drugs is to inhibit or modify the local inflammatory response.

Methylprednisolone

MP is the best known and most studied anti-inflammatory drug for attempting to block this process, and thus forms the paradigm.

The NASCIS studies proposed to administer MP at high doses (loading dose of 30 mg/kg of weight and maintenance dose of 5.4 mg/kg/h), for 24 h if the treatment was started not more than 3 h after the injury, or for 48 h if it was started between 3 and 8 h after the injury.1,4

In victims of complete cervical rupture, increased levels of interleukin (IL) 6, IL-8, macrophage chemoattractant protein-1, neutrophil activating peptide-2, intercellular adhesion molecule-1 (ICAM-1), soluble Fas, tissue inhibitors of metalloproteinase-1 and matrix metalloproteinases (MMP) 2 and 9 have been observed.5 Treatment with MP after spinal
cord injury has been found to result in significant reduction in the activity of caspases 3, 6, 8 and 9 for a period of up to seven days after the occurrence of the injury. MP induces interaction of the glucocorticoid receptor with HIF-1α, which results in transactivation of erythropoietin in oligodendrocytes, but not in cortical neurons, which may explain its efficacy in lesions of the white matter and inefficacy in lesions of the gray matter. MP inhibits the death of oligodendrocytes induced by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) in a manner dependent on the signal transducer and activation of transcription (STAT) 5 gene. On the other hand, the neural growth factor increases in patients treated with MP.

However, other experimental studies have found negative effects from MP. In vitro, significant inhibition of the proliferation of neuron progenitor cells that were incubated with MP for at least five days was observed, along with alterations to the expression of 143 genes, among which some are involved in regulating cell proliferation and apoptosis.

MP was found to chronically reduce proliferation and activation of microglia and macrophages and the number of oligodendrocyte progenitor cells, and to inhibit proliferation of neuron progenitor cells of the hippocampus and medulla.

In animals treated with MP, a significant reduction in ciliary neurotrophic factor (a neuroprotective molecule) was observed 12 and 24 h after spinal cord injury. At six, 48 and 72 h, no statistically significant differences were found.

In therapeutic terms, rats treated with MP have shown significant improvements in neurological function, with reduced latency and threshold and increased evoked motor potential. In another animal study, no significant improvement in neurological recovery or in the quantity of injured tissue was observed through treatment with MP. A review evaluated the validity of animal trials for studying treatments for spinal cord injuries. Among the studies included, 34% found that MP had beneficial effects, 58% did not find any such effects and 8% had mixed results, and there were inconsistent results between species and within each species. This review concluded that there was a need to develop validated methods for analyzing these treatments.

In humans, the results have been contradictory: according to Andrade, there was no relationship between application of the NASCIS II protocol and the patients’ evolution. However, among patients treated using MP and surgery, there was greater motor recovery than among patients who did not undergo this treatment.

A meta-analysis on three studies on humans indicated that treatment with MP that was started not more than 8 h after the injury significantly improved neurological functioning. One of these studies concluded that treatment with MP for 48 h provided additional benefit, especially if the treatment was only started beyond the first 3 h.

Although evidence of greater incidence of complications or mortality through treatment with MP is not always reported, the complications resulting from its use have generally been due to immunosuppressant effects (infections) and metabolic effects (hyperglycemia). A statistically significantly greater number of complications was observed among patients with complete ruptures. A significantly greater risk of complications in general was seen among patients treated with high doses of MP (>5000 mg), along with significantly greater incidence of ulcers or gastric hemorrhage. No significant differences in intrahospital mortality were found between a group that received high doses of MP and a control group.

Among patients hospitalized in intensive care, it was seen that the risk of infection (especially respiratory infection) and hyperglycemia was significantly greater among patients treated with MP. There was no statistically significant difference in mortality and no differences in neurological functioning were seen at the time of release from hospital, between treated and untreated patients.

Benefits from treatment with MP were thus only observed in the NASCIS studies and in one or two other studies, and it was seen that treatment with high doses of MP increased the risk of infection and consequently lengthened hospitalization and dependence on mechanical ventilation. It was concluded that until there was more evidence, use of steroids for treating spinal cord injuries should be suspended, considering that the benefits from the treatment might not compensate for the adverse effects associated with this.

In an article on first-aid measures and treatment for spinal and spinal cord injuries with fracturing, the authors reported that they were currently only using MP (using the NASCIS II protocol) among patients with incomplete spinal cord injuries, within the first 8 h after the injury, given that they had not observed any benefits from this therapy among patients with complete rupture.

A review conducted in 2013 concluded that MP could not be considered to be a standard treatment for patients who had suffered spinal or spinal cord injuries, but that it should be kept as an option until new treatments of proven efficacy emerged.

**Tirilazad**

Tirilazad is a 21-aminosteroid that acts in a manner similar to corticoids, with inhibition of lipid peroxidation but without immunosuppressant or metabolic effects. A review reported that tirilazad had beneficial effects in the NASCIS studies, but emphasized that there was a need to develop new antioxidant therapies that were safer and more effective.

**Drugs that interfere with edema, ischemia and membrane equilibrium**

After injury, edema, ischemia and altered equilibrium of the cell membranes of the spinal cord are observed.

**GM1**

GM1 is a ganglioside that intervenes in these processes, through raising the neurotrophic factor levels and reducing neuron destruction.

A study on rats compared the effect of MP with that of GM1. MP resulted in significant improvement of neurological function in relation to the control group. The group treated with GM1 also showed better motor function, but the difference was not statistically significant. Combined treatment significantly
improved motor function but in a less marked manner than seen with MP alone.\textsuperscript{23} In humans, no evidence of reduced mortality through treatment with GM1 has been found.\textsuperscript{24}

Apoptosis inhibitors

Apoptosis is an important component of secondary spinal cord lesions, which contributes toward loss of neurons and oligodendrocytes.\textsuperscript{25} Several drugs inhibit apoptosis through a variety of mechanisms, and these include inhibition of caspases,\textsuperscript{26} inhibition of several intracellular signaling routes\textsuperscript{26,27} and reduction of oxidative stress.\textsuperscript{28} A vast group of drugs inhibits calpain, an endoprotease that promotes apoptosis in several types of cells through proteolysis of proteins in the cytoskeleton, membrane and myelin.\textsuperscript{29}

Caspase inhibitors

After experimental injury in rats, it was found that caspases 3, 8 and 9 became activated, especially in neurons and oligodendrocytes. Intrathecal injection of Boc-d-fmk, a nonspecific inhibitor of caspases, resulted in improvement of motor function observed on the 21st and 28th days after the experimental spinal cord injury. Use of z-DEVD-fmk, a selective inhibitor of caspase 3, led to functional improvement only on the 21st day after the injury.\textsuperscript{25} In another study, use of z-DEVD-fmk resulted in a lower degree of histological alterations seen 24 h after the injury, with reduced apoptosis and significant improvement of motor function.\textsuperscript{30} In a comparison between treatment with magnesium sulfate, an antagonist of N-methyl-D-aspartate (NMDA) receptors, and use of z-LEDH-fmk, along with combined treatment with both of these, a histological but nonfunctional improvement was seen. No statistically significant differences were identified between these two drugs.\textsuperscript{31} A group studied the effects of M50054, an inhibitor of caspase-3, in fish with the capacity to regenerate the central nervous system. A significant reduction in apoptosis was observed over the medium to long term, among the existing neurons, recently formed cells and recently differentiated neurons, in association with faster functional recovery among the treated fish.\textsuperscript{32}

Calpain inhibitors

The calpain inhibitor MDL28170 improved the survival of Schwann cells, both in vitro, after exposure to hydrogen peroxide, and in vivo, after transplantation in the injured spinal cord.\textsuperscript{25} Treatment with a single intravenous dose of this agent, or with a daily dose administered intraperitoneally, resulted in improvement of motor function, but not of the extent of the lesion. Combination of these two forms of administration improved both of the variables.\textsuperscript{29} Removal of calcium from the medium or inhibition of calpain using MDL28170 avoided the myelin retraction induced by exposure to glutamate.\textsuperscript{34}

Other calpain inhibitors have also been studied. SJA6017 significantly reduced the degree of tissue damage and apoptosis and significantly improved motor function.\textsuperscript{35} In rats, administration of calpain inhibitors led to formation of abnormal axon extremities that were swollen and did not present microtubules. This suggests that activation of calpain is necessary for effective regeneration.\textsuperscript{36}

Drugs that interfere with other routes

Spinal cord injury activates autophagy and apoptosis in neurons and astrocytes. Inhibition of autophagy using 3-methyladenine has been found to result in worsened neurological function, while its stimulation with rapamycin has the opposite effect. These results suggest that stimulation of autophagy has anti-apoptotic and neuroprotective effects.\textsuperscript{37}

Treatment with aminoguanidine, an inhibitor of inducible nitric oxide synthase (iNOS), has been found to lead to improvement of motor function of the hind limbs of rats, reduce mortality and reduce neuronal morphological alterations. This treatment has also been shown to lead to reduction of dephosphorylation of the pro-apoptotic phosphorylated Bcl-2-associated death promoter (pBAD) protein and reduction of iNOS expression, which results in lower release of cytochrome c and mitochondria and reduces the degree of apoptosis.\textsuperscript{38}

Treatment with butein has been found to attenuate the expression of protein p65 of the nuclear factor κB (NF-κB) and to increase the phosphorylation of the α inhibitor of NF-κB (IκBα). There was also a reduction in myeloperoxidase activity, which translated as lower neutrophil infiltration and less expression of activated caspase-3. From this, it could be concluded that there was a decrease in apoptosis.\textsuperscript{39}

In another study, use of BMS-345541, an inhibitor of the kinase pathway of IκBα (IKK)/NF-κB, avoided neutrophil infiltration through reduction of the expression of the adhesion molecule ICAM-1 and had anti-apoptotic effects through inhibition of caspase 3 and modulation of the expression of Bcl-2 and Bax.\textsuperscript{40}

A study on rats compared the effects of treatment with ginkgolide B with the effects of methylprednisolone and AG490, an inhibitor of the Janus kinase (JAK)/Stat pathway. The animals treated with ginkgolide B or with MP presented significantly better motor function than those of the control group. The treatment with ginkgolide B and AG490 reduced the activation of the JAK/Stat pathway and increased the Bcl-2/Bax ratio, which resulted in an anti-apoptotic effect, with lower expression of caspase-3 and reduction of the number of TUNEL-positive cells. The treatment with ginkgolide B and MP also resulted in greater neuron preservation.\textsuperscript{27}

Inhibition of cyclin-dependent kinase-1 (CDK1) using CR8 or roscovitine resulted in reduction of apoptosis among cultured cortical neurons, especially using CR8. In vivo, administration of CR8 resulted equally in greater neuron survival.\textsuperscript{26}

Another possible therapeutic target is Rho-kinase, which regulates a variety of events such as proliferation, differentiation and cell death. Several studies have shown that inhibiting this favors axon regeneration and neuron survival. Treatment with fasudil, an inhibitor of this enzyme, reduced the histological and functional alterations, activation of astrocytes, activation of the NF-κB pathway, expression of inflammatory mediators, infiltration of neutrophils, expression of mitogen-activated protein kinases (MAPK), activity of Rho-kinase and expression of nitrotyrosine and poly-ADP-ribose (markers of
oxidative and inflammatory damage). Decreased expression of Fas ligands and Bax and increased expression of Bcl-2 have also been observed. In the spinal cord of animals treated with fasudil, no apoptotic cells have been detected.28

In rats, treatment with 17β-estradiol was found to reduce apoptosis of oligodendrocytes, loss of axons and activation of caspases 3 and 9, homologue A of Ras (RhoA), c-Jun N-terminal kinase (JNK) 3 and phosphorylated c-Jun (p-c-Jun) levels, independently of the estrogen receptor. Administration of PEP-1-C3, a fusion protein that inhibits RhoA, was also found to reduce apoptosis of oligodendrocytes, JNK3 activity and p-c-Jun level, which confirmed that this route has a role in inducing apoptosis.41 17β-estradiol reduced the phosphorylation of JNK and apoptosis of spinal neurons after spinal cord injury in rats and reduced the phosphorylation of JNK in spinal cord injury and spinal cord injury in vitro.42 An in vitro study tested the effects of estrogen and of an agonist of estrogen receptor (ER) α (PPT) and an agonist of ERβ (DPN) on motor neurons exposed to TNF-α. All of these led to reduction of apoptosis, induction of phosphorylation of extracellular-sign-regulated kinases (ERK) and increased expression of the respective receptors, with greater expression of anti-apoptotic proteins. The agonists of estrogen receptors inhibited both the intrinsic and the extrinsic pathway of apoptosis.43

Chicken embryos have the capacity to regenerate the spinal cord until the 13th day of the embryonic period. Peptidylarginine deiminase 3 is a calcium-dependent protein that has been implicated in loss of this capacity. Treatment with CI-amidine, a calcium chelant, has been found to reduce apoptosis and the extent of spinal cord injury in chicken embryos until their 15th day of development.44

In a study on mice, apocynin, an inhibitor of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, gave rise to reduced inflammation, extent of spinal cord damage, infiltrating neutrophils, adhesion molecule expression, NF-κB expression, nitrotyrosine and poly-ADP-ribose formation, pro-inflammatory cytokine levels, MAPK activation and apoptosis. An improvement in motor function was also seen.45

Pretreatment with U0126, an inhibitor of MAPK kinases (MEK), was found to lead to inhibition of phosphorylation of ERK1/2, reduction of apoptosis and greater neuron survival. Inhibition of MEK induced phosphorylation of I-κB, favored binding of NF-κB to AND and increased the expression of apoptosis-inhibiting cellular protein-2. A statistically significant improvement of motor function was observed in the limbs affected.46

SP600125, an inhibitor of JNK, was found to produce increased levels of p-BAD and the dimer BAD/14-3-3, decreased dimerization of BAD with Bcl-XL and Bcl-2, and reduced release of cytochrome c. There was also greater preservation of the morphology of the mitochondria and diminished apoptosis.47

Rolipram, an inhibitor of phosphodiesterase-4, was found to induce growth of neurites and axon regeneration, unlike MP, but it did not reduce neuron death in vitro, or the levels of chondroitin sulfate proteoglycans, which was observed with MP. Combined treatment had an effect on these variables that was more intense than monotherapy. Both of these drugs significantly diminished the volume of the lesion, and this was more markedly so in combination. Only the treatment with both of these agents resulted in a significant functional improvement.48

Naloxone

Dynorphin A, an endogenous opioid that shows increased levels after spinal cord injury, has neurotoxic effects and reduces the arterial flow. Naloxone, an antagonist of opioids, has been used in some studies to counteract these effects.3 However, in the NASHC II study, naloxone did not show any neuroprotective effect.3

Antioxidant drugs

Oxidative damage caused by reactive oxygen and peroxynitrite species is an important process in secondary lesions. It leads to perturbation of ionic homeostasis, mitochondrial dysfunction, potentiation of excitotoxicity and microvascular lesions.52

Tempol is an antioxidant that reduces the levels of these substances and diminishes inflammation through inhibiting COX-2.49

In several studies, tempol has been shown to reduce the oxidative damage mediated by peroxynitrite and the mitochondrial respiratory dysfunction.50,51 Reductions in the degradation of cytoskeleton proteins have also been observed when treatment was administered within the first hour after the injury,51 along with reduction of COX-2 expression.52 The area of the spinal cord that was irreversibly damaged was also seen to be reduced through using tempol.49

In a study in which the effect of tempol was compared with that of the uncoupling protein 2,4-dinitrophenol, the latter preserved the functioning of synaptic and nonsynaptic mitochondria, whereas tempol only had an effect on nonsynaptic mitochondria.52

Another antioxidant, edaravone, was compared with MP in a trial using rats. It was found that MP had greater effect regarding motor recovery than edaravone when the treatment was administered within eight hours after the spinal cord injury, while the opposite was seen when the treatment was given more than eight hours afterwards. There was greater expression of Bcl-XL in the group treated with edaravone, independent of the timing of the treatment. When the treatment was administered within 8 h of the injury, there was greater reduction of the expression of caspase-3 in the animals treated with MP, while beyond 8 h, only edaravone gave rise to a significant reduction.53

An in vivo and in vitro study evaluated the effect of MP and MnTBP (an antioxidant) on the production of reactive oxygen species. In vivo, both agents gave rise to diminution of hydrogen peroxide production, but only MnTBP significantly reduced the quantity of superoxide. In vitro, MnTBP revealed a capacity to capture both reactive oxygen species, but MP did not have any effect on either of them. Treatment with MnTBP significantly reduced the levels of markers of protein nitration and lipid peroxidation, compared with the control. Both of these drugs gave rise to significant improvements in
neurological functioning, and this was more markedly so with MnTBAP.54

Other drugs

A study on mice evaluated the effect of cocaine and amphetamine-regulated transcript (CART) peptides, alone or in combination with MP. It was found that both of these drugs improved motor function. The effect of MP was boosted through concomitant administration of CART, even at a dose that would be sub-effective as monotherapy. CART and MP also reduced the numbers of astrocytes with positive marking for GFAP and astrocytic hypertrophy. Histological abnormalities were reduced in similar manners by CART, MP and the combined treatment.55

In a study on rats, the effects of MP and magnesium were evaluated. Administration of magnesium within eight hours after the injury resulted in a significant improvement in motor function, in relation to the placebo group and to groups that received magnesium after 12 or 24 hours. MP, magnesium and their combination significantly reduced the loss of white matter, but the combination was not shown to be better than the separate treatment. None of the treatments had any significant effect on the myelin index.56

The Nogo-66 receptor is activated by three myelin molecules and it inhibits the growth of neurites. A study in which combined treatment consisting of MP and NEP1-40 (an antagonist of Nogo-66) was used resulted in a greater increase in survival of neurons and oligodendrocytes and significantly better motor recovery than among animals treated with only one of these drugs.57

In a retrospective study on humans, it was found that there was a more significant improvement in autonomy in relation to activities of daily living through combined treatment consisting of MP and erythropoietin than with MP alone.58

In a study on rats that evaluated the effects of MP and dexmedetomidine, both of these treatments significantly reduced the levels of TNF-α and IL-6, along with the infiltration of neutrophils.59 In another study, dexmedetomidine caused greater elevation of paraoxonase and IL-6, along with greater reduction of hemorrhage, than MP. Both of these agents reduced edema and necrosis to equal degrees.60

Discussion

The evidence relating to the efficacy of MP is contradictory, while evidence regarding its negative effects has been accumulating. In the light of the present knowledge, use of MP for treating spinal and spinal cord injuries needs to be carefully weighed up. Other anti-inflammatory agents have shown benefits for humans or animals, but further studies are required in order to come to conclusions regarding its efficacy and safety in clinical practice.

GM1 does not seem to have greater efficacy than MP. Given that the effects of this drug may only be shown later on, long-term studies are needed in order to identify the benefits of GM1.23

Apoptosis is a complex process, with many intervening factors, and thus, drugs that inhibit it through various mechanisms have been developed. The ones that have been studied most seem to be caspase inhibitors and calpain inhibitors. Many of these drugs have shown benefits in in vitro studies or in animal models.

Naloxone did not show any neuroprotective effect in the NCSIS II study.

In the studies that have been conducted, tempol was shown to inhibit the main consequences of oxidation at the spinal cord level and is a promising form of therapy. Other antioxidants seem to have effects that are superior to that of MP, but further studies are needed in order to confirm their efficacy.

Final remarks

In order to address the enormous impact of spinal cord injuries and the lack of effective therapeutic options for secondary lesions, there is an urgent need to find new treatments that make it possible to improve the neurological status of patients with spinal and spinal cord injuries. The benefits of treatment with methylprednisolone have been questioned, and there are concerns regarding its safety. Other drugs that intervene in inflammation or that have other mechanisms have been studied. Some of these may be promising alternatives to methylprednisolone. Additional studies are needed in order to reach conclusions regarding the benefit of these agents in clinical practice.

Conflicts of interest

The authors declare no conflicts of interest.

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