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Comprehensive Assessment of Metformin's Impact on the mTOR Signaling Pathway in Spinal Cord Injury Treatment

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Abstract

Metformin is one of the safest drugs used to control blood sugar in diabetes. This drug is being investigated by orthopedists as well as by neurosurgeons as it may increase the locomotor recovery after spinal cord injury (SCI). In the present study, the aim was to evaluate a promising pharmacological agent, metformin, which is known to be used in SCI treatment by acting over the mammalian target of rapamycin (mTOR) and some other signaling pathway. Relevant clinical trials were included in the study following electronic database scan. Preclinical and cellular studies have demonstrated that metformin may have antitumoral properties, regulate inflammation, and therefore play a role in the tumor microenvironment, or that it has a therapeutic effect in SCI by acting over mTOR pathway. However, Level of I clinical trials should be performed to determine the effectiveness of metformin in SCI, a serious pathology.

Keywords: Metformin; mTOR 1ignalling pathway, rapamycin; spinal cord injury, sirolimus

INTRODUCTION

Spinal cord injury (SCI) is defined as a medical condition that causes temporary or permanent damage to the spinal cord functions. According to the report drafted by the American National Center for Health Statistics in 2014, the incidence of SCI is approximately 12,500 new cases in each year, males are made up 80% of SCI cases, and the age range is between 33,5 years and 42 years (National SCI Statistical Center). Neurological problems caused by primary and secondary cord injury after SCI affect the quality of life of patients. Moreover, this medical condition may lead to psychosocial problems and constitute a financial burden on the healthcare economy of countries. Gradually, this may turn into a significant health problem that has both individual and social dimensions (Devivo, 2002).

The post-traumatic damage can be divided into primary and secondary damage. The primary damage occurs at the moment of impact and cannot be prevented. The secondary damage initiates systemic, local, biochemical and electrolytic changes in the spinal cord. Different symptoms occur in the case of complete and incomplete injury. Some motor and sensory function may be preserved below the injury level in the incomplete injury. A complete injury leads to permanent loss of all motor and sensory function below the level of injury.

Today, surgical interventions such as neural decompression / spinal stabilization are performed top reserve residual neurological functions, reduce secondary damage and treat the damaged nerve tissue. Other than these, pharmacological treatment modalities are being investigated for the reduction of secondary injury, including inflammation, edema, ischemia and chronic processes. Although the use of many pharmacological agents other than corticosteroids, which is clinically used, is in their infancy, they are promising for the future (Kemerdere, 2015).

The administration of high-dose methylprednisolone in the early stage has been demonstrated to increase spinal cord blood flow and facilitate the healing process even though its mechanism of action remains unknown **(**Chen et al., 2018**).**

In a study **(**Demopoulos et al., 1980**)**, where the SCI was reported to be associated with free radicals, other than steroids, a variety of pharmacological agents, the effectivity of which were demonstrated through experimental and clinical studies, were suggested to be used to prevent secondary tissue damage.

Gangliosides are localized on the outer surface of the cell membrane in synaptic areas. They perform the

synthesis of growth factors by their ability to modulate protein kinase-C, reduce the release of excitatory amino acids, or inhibit the formation of nitric oxide (Constantini and Young, 1994; Ma et al., 2017).

Central nervous system tissues normally contain considerable amounts of antioxidants such as glutathione, ascorbate and alpha-tocopherol acetate. These endogenous antioxidants retain free radicals. The levels of antioxidants such as ascorbate, alpha-tocopherol acetate, ascorbic acid, retinoic acid, and ubiquinone are reduced in spinal cord injuries (Riffel et al., 2018; Walker et al., 2018; Zhang et al., 2015). Many pharmacological agents such as arachidonic acid modulators, monoamine modulators, glutamate receptor antagonists, calcium channel blockers, growth factors are being tested for the treatment of spinal cord injuries (Caglar et al., 2018; Pallottie et al., 2018; Russell et al., 2016).

The therapeutic effect of metformin in the intervertebral disc degeneration has been demonstrated by*in vitro* and *in-vivo* studies. This pharmacological agent has been reported to prevent, decelerate and downgrade the disc degeneration, and suggested to reduce the aging of nucleus pulposus cells and apoptosis. In addition, metformin is a promising agent since it increases the expression of anabolic genes such as type II collagen and ACAN and reduces the expression of catabolic genes such as matrix metalloproteinase-3 and ADAMTS-5**(**Chen et al., 2016**).**

In this review, the aim was to evaluate a promising pharmacological agent, metformin, which is known to be used in SCI treatment by acting over the mammalian target of rapamycin (mTOR) and some other signaling pathway.

MATERIALS AND METHODS

A comprehensive and systematic literature search of electronic databases, including the National Library of Medicine at the National Institutes of Health, and PubMed was performed. Keywords used were as follows: “metformin,” “mTOR signaling pathway,” “rapamycin,” “sirolimus,” and “spinal cord injury”.

The headings and abstracts of all the experimental and clinical studies related to the inhibition of the mTOR signaling pathway by metformin in SCI were reviewed. The full texts of the appropriate studies were retrieved according to the headings and abstracts, and then the decision of whether to include or exclude these studies was made after a comprehensive review (Karaarslan et al., 2018[a]; Karaarslan et al., 2018[b]; Topuk et al., 2017).

Letters to the editor, bibliographies, reviews, and meta-analyses were excluded from the study. Critical appraisal checklists were used to assess and analyze the quality of the studies (Akgun et al., 2018; Ali Gumustas et al., 2016; Yilmaz et al., 2016). Following this assessment, a consensus was reached in the event of disagreement. The obtained data were summarized, and the findings were presented in a clear and understandable manner using tables. The present study was conducted using the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Akgun et al., 2018; Ali Gumustas et al., 2016; Karaarslan et al., 2018[a]; Karaarslan et al., 2018[b]; Topuk et al., 2017; Yilmaz et al., 2016). The results were presented using *Microsoft Office Excel (v.2013)*.

RESULTS

Two studies were retrieved using the keywords “metformin,” “mTOR signaling pathway,” and “spinal cord injury”(Guo et al., 2018; Wang et al., 2016). Twelve studies were retrieved using the keywords “metformin,” “mTOR signaling pathway,” “rapamycin,” “sirolimus,” and “spinal cord injury”. The same two studies **(**Guo et al., 2018; Wang et al., 2016**)** retrieved during two separate scanning were eliminated, the remaining 10 studies were evaluated (Afshari et al., 2018; Ge et al., 2018; Lin et al., 2015; Murad et al., 2015; Nash et al., 2016; Oda, 2017; Wang et al., 2017; Wang et al., 2018; Zhang et al., 2017). However, three studies that were not associated with the subject of interest were excluded (Lin et al., 2015; Murad et al., 2015; Nash et al., 2016).

The full texts of the remaining nine studies **(**Guo et al., 2018; Afshari et al., 2018; Ge et al., 2018; Murad et al., 2015; Nash et al., 2016; Oda, 2017; Wang et al., 2016; Wang et al., 2017; Wang et al., 2018; Zhang et al., 2017) were examined. The results suggested that metformin may have neuroprotective and anti-inflammatory effects that reduce hyperalgesia in neuropathic pain and injured nerves. However, no clear data were retrieved about whether it can be used against SCI by acting on which signaling pathway.

DISCUSSION

In meta-analyses or systematic reviews, it is important that the articles selected by the researchers should be presented in a way that includes all relevant, impartial and accurate data. Therefore, systematic reviews and meta-analyses are increasingly accepted and published by Biomedical Journals (Akgun et al., 2018; Ali Gumustas et al., 2016; Karaarslan et al., 2018[a]; Karaarslan et al., 2018[b]; Topuk et al., 2017; Yilmaz et al., 2016**).**

Metformin is a derivate of an active natural product, galegine, which is an alkaloid and has an antihyperglycemic effect, isolated from the plant *Galega officinalis.* It has been used in the treatment of diabetes mellitus since 1957 (Ursini et al., 2018). It shows its effect by stimulating AMPK in the liver and muscles. It suppresses gluconeogenesis in the liver and has a

peripheral effect. Metformin increases the level of Glucagon-like peptide-1 (GLP-1) (Wu et al., 2014). It makesa slight decrease in the insulin level when used in the treatment of diabetes mellitus, which also helps to lose weight. Hence, metformin is known to be used by *non-diabetic individuals* to lose weight.

Metformin has been known to reduce the aging of nucleus pulposus cells and apoptosis, increase the expression of anabolic genes such as type II collagen and ACAN, and decrease the expression of catabolic genes such as matrix metalloproteinase-3 and ADAMTS-5 **(**Chen et al., 2016**).** Studies investigating its effects of preventing, decelerating and downgrading the disc degeneration have gained popularity. Metformin has recently been reported to suppress cell proliferation and to increase apoptosis and autophagy by inducing the mTOR pathway (Ma et al., 2018).

Guo et al. reported that secondary injuries such as apoptosis and cell autophagy significantly affected the healing process of motor function. They highlighted that metformin was an oral anti-diabetic agent commonly used for type 2 diabetes in the world and that this agent showed its effects by inhibiting apoptosis and promoting autophagy in the nervous system. In that study, where the role of metformin in the treatment of SCI was reported to be unknown, the authors denoted that motor function evaluated using the Basso, Beattie, and Bresnahan (BBB) locomotor assessment scale was significantly higher in rats treated with metformin after injury. In addition, they revealed that metformin increased the number of surviving neurons in the spinal cord lesion after performing Nissl staining. More important than these results, they emphasized that apoptosis markers were reduced in live mammalian subjects treated with metformin following SCI (Guo et al., 2018)

Wang et al. (2016) suggested that inflammatory and autophagy responses in SCI, which is one of the most severe nervous system disorders characterized by high morbidity and disability, playeda significant role in the development of SCI. In that study (Wang et al., 2016), where metformin, the first-line drug for type-2 diabetes, was reported to have anti-inflammatory and anti-apoptotic effects as well as autophagy support in the nervous system, the authors investigated the neuroprotection effects of metformin on post-SCI mice. They reported that the BBB scores and pathological staining revealed that the function and amount of motor neurons were protected by metformin after SCI. They expressed that this pharmacological agent increased the expression of Beclin-1 and LC3B-II, and reduced the phosphorylation levels of the mammalian target of mTOR protein and p70S6K after SCI.In addition, they reported that metformin significantly decreased the expression of NF-$_K$B. Furthermore, they emphasized that the activation of caspase 3was decreased and the bcl-2 level was increased significantly in patients treated with metformin (Wang et al., 2016)

In a study where metformin was reported to have neuroprotective and anti-inflammatory effects that reduce hyperalgesiain neuropathic pain and injured nerves, metformin's effects on SCI neuroinflammation and its sensory and locomotor complications were evaluated (Afshari et al.ç, 2018).The obtained results were compared with minocycline which is used as an anti-neuroinflammation therapy in SCI.In doing so, T9 vertebra laminectomy was performed in 48 male mice in an animal model of SCI.After the subjects were divided into the operative group and the five treatment groups, the treatments comprised normal saline as a vehicle control group, minocycline 90 mg/kg and metformin at doses of 10, 50 and 100 mg/kg. Subsequently, behavioral tests for locomotor scaling, neuropathic pain, and weight changes were assessed and compared over a period of 28 days (Afshari et al.ç, 2018).At the end of the study, tissue samples were obtained from the subjects to evaluate the neuroinflammatory changes. The authors reported that 50 mg/kg of metformin increased the locomotor ability and decreased the sensitivity to mechanical allodynia. They also emphasized that metformin caused weight loss and that it significantly decreased neuroinflammation given the TNF-α and interleukin-1β levels (Afshari et al.ç, 2018).Therefore, they concluded that metformin might be used as an alternative therapeutic agent for SCI (Afshari et al.ç, 2018).

Ge et al. (2018) reported that metforminis a commonly used drug that is capable of activating AMPK. In that study, the signal transducer and the activator of transcription 3 (STAT3) pathways were reported to play a significant role in neuroinflammation. They stated that intraperitoneal injection of metformin (200 mg/kg) for six consecutive days activated AMPK in mice and suppressed the expression of p-STAT3. The authors suggested that metformin alleviated neuropathic pain by inhibiting activation of microglia and astrocytes (Ge et al., 2018).

In a preclinical study (Oda, 2017), where the potential neuroprotective effects of metformin on experimental acrylamide neuropathy were investigated, 24 mice were divided into four equal groups and experiments were performed. Group 1 served as the control group and Group 2 was composed of subjects who orally received metformin. Group 3 was performed intraperitoneal injections of acrylamide and Group 4 was administered both metformin and acrylamide. The author reported that after applying the therapies three times a week for three weeks, acrylamide caused an increase in lipid peroxidation in the brain and spinal cord (Oda, 2017). This result was associated with upregulation of caspase3 and downregulation of bcl2 in the cerebrum, cerebellum, spinal cord, and sciatic nerve. Metformin was shown to

improve lipid perexidation in the brain and spinal cord, reduce caspase 3 activity and upregulate bcl 2 expressions in the cerebrum and sciatic nerve. In the group in which both drugs were administered, it was underlined that metformin improved the neuropathic effects caused by acrylamide in mice (Oda, 2017).

In another study (Zhang et al., 2017[a]), metformin was reported to positively affect functional improvement after SCI but its effect on BSCB was still unknown. It was also reported that metformin could inhibit the loss of tight binding proteins on day three following *in-vivo* SCI, but that there was no significant difference between control and metformin therapy in endothelial cells of these proteins *in vitro.*In that study (Zhang et al., 2017[a]), the role of metformin on matrix metalloproteinase (MMP) -9 and neutrophil infiltration were investigated, andneutrophil infiltration was reported to be the main source of increased MMP-9 in SCI. The authors suggested that metformin reduced MMP-9 production and blocked neutrophil infiltration on the first post-injury period and this might be related to downregulation of ICAM-1(Zhang et al., 2017[a]).

In a study (Zhang et al., 2017[b]), where metformin treatment was reported to reduce apoptosis, leading to improved function recovery in mice, the authors investigated autophagy by means of detecting autophagosomes with transmission electron microscopy and immunofluorescence, as well as autophagy markers with a western blot in each group. In that study, metformin therapy was suggested to decrease the accumulation of p62 and ubiquitin proteins, which led to a stimulative effect of autophagy flux by metformin (Zhang et al., 2017[b]).Based on these findings, the authors emphasized that metformin ameliorated functional recovery through autophagy flow stimulation and that metformin might be a potential drug for SCI treatment (Zhang et al., 2017[b]).

In this study, it was planned to use “fixed-effects” or “random-effects” models to evaluate the heterogeneity between studies in the first place. In this way, it was aimed to make different assumptions about all the results evaluated. Furthermore, it was assumed that there was a fixed value for the results obtained from the entire population, such as fixed-effect meta-analyses, and each study included in the meta-analysis could extract this fixed value of its own sample extracted from the entire population. Or, as in the case of random-effect meta-analyses, the evaluated results were shown not to be associated with a fixed value in each population, but with populations. In the present study, the aim was to collect as much as possible the studies conducted with the aim of investigating the effects of metformin treatment in SCI and to make inferences using the results of these studies. However, a meta-analysis, including the mathematical combination, of the results obtained from the source studies could not be performed since no common data were found, and the results were presented as a systematic review.

CONCLUSION

Taken together, although these findings suggest that metformin acts as a neuroprotective agent following SCI by regulating mTOR / P70S6K signaling pathway, stimulating autophagy and inhibiting apoptosis, clinical and pharmaco-molecular studies should be performed to determine the effectiveness of metformin in SCI, a serious pathology.

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