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The dark side of an essential amino acid - L-arginine in spinal cord injury.

Céline Erens[1], Jana Van Broeckhoven[1], Annelies Bronckaers[2], Stefanie Lemmens[1*], Sven Hendrix[1,3*†]

1. Department of Immunology and Infection, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium.

2. Department of Cardio and organ systems, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium.

3. Medical School Hamburg, 20457 Hamburg, Germany.

*Equally contributing senior authors

† To whom correspondence should be addressed: Sven Hendrix; Medical School Hamburg, Am Kaiserkai 1, 20457 Hamburg, Germany; E-mail: sven.hendrix@medicalschool-hamburg.de

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Céline Erens, Department of Immunology and Infection, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium; +32 11 26 92 01, celine.erens@uhasselt.be

Jana Van Broeckhoven, Department of Immunology and Infection, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium; +32 11 26 92 01, celine.erens@uhasselt.be

Annelies Bronckaer, Department of Cardio and organ systems, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium; +32 11 26 92 01, celine.erens@uhasselt.be

Stefanie Lemmens, Department of Immunology and Infection, Hasselt University, Biomedical Research Institute BIOMED, Agoralaan, 3590 Diepenbeek, Belgium; +32 11 26 92 01, celine.erens@uhasselt.be

Sven Hendrix, Institute for Translational Medicine, Medical School Hamburg, Am Kaiserkai 1, 20457 Hamburg, Germany; +49 40 36122640, sven.hendrix@medicalschool-hamburg.de

Abstract

L-arginine is a semi-essential amino acid involved in a variety of physiological processes in the central nervous system (CNS). It is key in the survival and functionality of neuronal cells. Nonetheless, L-arginine also has a dark side; it potentiates neuroinflammation and nitric oxide (NO) production, leading to secondary damage. Therefore, modulating the L-arginine metabolism is challenging, because both detrimental and beneficial effects are dependent on this semi-essential amino acid.

After spinal cord injury (SCI), L-arginine plays a crucial role in trauma-induced neuroinflammation and regenerative processes via the two key enzymes: nitric oxide synthase (NOS) and arginase (ARG).

Studies on L-arginine metabolism using ARG and NOS inhibitors highlighted the conflicting role of this semi-essential amino acid. Similarly, L-arginine supplementation resulted in both negative and positive outcomes after SCI. However, new data indicates that arginine depletion substantially improves spinal cord regeneration after injury. Here, we review the challenging characteristics of L-arginine metabolism as a therapeutic target after SCI.

Introduction

Spinal cord injury (SCI) is a chronic disabling condition for which no effective clinical treatment exists.[1] The initial trauma causes cell damage and disruption of the blood-spinal cord barrier resulting in substantial immune cell infiltration.[2] In the first instance, this inflammatory response can be seen as beneficial, as it clears cellular debris and initiates tissue remodeling necessary for wound healing.[3] However, the strong pro-inflammatory microenvironment quickly potentiates a long-lasting type 1 immune response, characterized by excessive production of cytokines, nitric oxide (NO), and associated reactive nitrogen species (RNS), inflicting secondary damage and creating unfavorable conditions for tissue repair.[4-6]

One key component crucial for both tissue repair and immune cell function is L-arginine.[7,8] Macrophage responses and T-lymphocyte functionality are dependent on the availability of this amino acid.[9,10] They mainly potentiate the dominant type 1 immune response and are largely responsible for the L-arginine catabolism after SCI. As L-arginine is necessary to potentiate inflammation, it is not surprising that its depletion has emerged as a powerful immunosuppressive tool.[11] Nevertheless, L-arginine supplementation also showed to promote wound healing in patients.[12] Thus, L-arginine depletion may reduce potentially detrimental neuroinflammation, while supplementation may promote wound healing. As a result of these contradictory findings, it remains unclear whether L-arginine exerts primarily beneficial or detrimental effects after SCI.[13-16] In this review, we describe the challenging characteristics of L-arginine metabolism as a therapeutic target after SCI and report a recent study of our lab that attempts to answer this question.

L-arginine, a substrate for two differential immune responses.

L-arginine is considered a semi-essential or conditional amino acid. For healthy adults, the level of endogenous L-arginine synthesis is sufficient to meet metabolic demands. It is predominantly derived from protein turnover, responsible for up to 85% of the circulating arginine in the fasting state.[17] However, in certain physiological and pathological cases

(e.g., growth, pregnancy, trauma, or infection), the requirement of L-arginine exceeds the production capacity. Accordingly, it needs to be acquired through dietary intake.[18]

Several enzymes are involved in the metabolism of L-arginine, making it one of the most metabolically versatile amino acids. It can be catabolized by four different groups of enzymes: nitric oxide synthases (NOS), arginases (ARG), arginine decarboxylase (ADC), and arginine:glycine amidinotransferase (AGAT).[19] The metabolites differ tremendously (NO, urea, citrulline, creatine, agmatine, glutamate, proline, etc.) and form precursors for several important biological processes in mammalian physiology like collagen production involved in wound healing or a NO-mediated bactericidal response (Figure 1).[8,19] The arginine metabolism has been reviewed in more detail elsewhere.[19]

In the immune system, L-arginine is a precursor for two complementary yet substantially different inflammatory responses. Either it is hydrolyzed by ARG to ornithine, influencing cell proliferation, collagen production, and tissue regeneration via polyamine synthesis.[19] Alternatively, NOS converts L-arginine to NO, which is indispensable in the defense against pathogens (Figure 1).[20] The expenditure of the amino acid by the two pathways is directed by substrate availability and the type of immune response. ARG is primarily associated with a type 2 immune response, and its expression suppresses NO production.[21] Conversely, a type 1 immune response enhances L-arginine uptake for NOS-mediated NO production.[21] Both pathways deplete extracellular arginine concentrations and have a major impact on the local wound milieu, suggesting that it is an indispensable amino acid after trauma to the spinal cord.

The arginase - arginine pathway

Two isozymes of ARG have been described: ARG I and ARG II. Both enzymes catalyze the same biochemical reaction yet differ in their expression profile, transcriptional regulation, and subcellular compartmentation.[22,23] ARG I is a cytoplasmic enzyme primarily expressed in the liver as a component of the urea cycle. In the central nervous system (CNS),

however, expression of this isozyme also occurs in neurons. The resulting L-ornithine production is associated with the formation of glutamate.[24] ARG II is expressed in mitochondria and is mainly confined to the kidney, prostate, and small intestine.[22] Here, ARG II is involved in regulating water reabsorption and endothelial cell function.[25,26] CNS neurons also express ARG II. The related polyamine production is essential for cell growth, survival, and neuronal development.[24,27-29]

Apart from the constitutive expression of both isozymes, expression of ARG I and/or II can be induced in granulocytes, myeloid- and dendritic cells by several inflammatory mediators in rodents. For ARG II, these factors were identified as interferon-γ (IFN-γ), LPS, and 8-bromo-cAMP.[30,31] ARG I expression can be induced by several T helper 2 cytokines, such as interleukin (IL)-4, IL-13, and IL-10 via the transcription factors *signal transducer and activator of transcription* (STAT)6 and STAT3.[21] These cytokines potentiate an anti-inflammatory type 2 immune response and a pro-regenerative immune cell activation. Generally, the expression of ARG I in murine macrophages is used as a marker for the associated alternative macrophage polarization. Nevertheless, toll-like receptor (TLR) agonists lipopolysaccharide (LPS), transforming growth factor-β (TGF-β), and IL-6 too can induce ARG I translation, making it hard to solely use ARG I as a maker for alternative activation into practice.[21,30]

In humans, ARG I expression can be induced in innate lymphoid cells[32] and ARG II in macrophages and neutrophils during inflammation[33]. Moreover, ARG I expression is mainly apparent and constitutively expressed in neutrophils and erythrocytes, where it is released upon activation or cell death.[34-36] The correlated L-arginine deprivation in the microenvironment can alter the local immune response, impeding T-cell proliferation and NO production involved in inflammation.[34,36] Consequently, ARG expression in humans can also be modulated by immune processes and likewise plays a major part in immunomodulation. The critical involvement of ARG-mediated immunosuppression is

observed in several diseases, including sepsis[37] and cancer[38]. Therefore, it is tempting to expect a critical role of ARG in SCI.

The NOS – arginine pathway

For NOS, three isozymes have been identified: NOS I (neuronal NOS; nNOS), NOS II (inducible NOS; iNOS), and NOS III (endothelial NOS; eNOS). The NOS enzymes are all encoded by distinct genes and differ in regulation and distribution, yet all catalyze the same reaction, the release of NO through the incorporation of molecular oxygen into L-arginine.[19] NOS I and III are constitutively expressed, and their activity is calcium-dependent.[39] The expression of NOS I is primarily found in neurons, where it is thought to mediate cell signaling and stimulate synaptic plasticity.[40] NOS III is principally expressed in the endothelium and blood cells like erythrocytes, leukocytes, and platelets , making NOS III the main source of vascular NO.[41] Besides its well-known key role in vasodilatation, vascular NO acts to inhibit platelet aggregation and epithelial adhesion of leukocytes.[42,43]

The major inducer of NO after trauma is NOS II or iNOS. The transcription of NOS II is inducible and evoked by pro-inflammatory mediators and T helper 1 cytokines (e.g., IFN-γ, LPS, IL-1β, or tumor necrosis factor-α; TNF-α) via the transcription factors STAT1 and nuclear factor (NF)-κβ.[44,45] Anti-inflammatory cytokines, on the other hand, are able to suppress NOS II transcription.[46] Unlike NOS I and III, the enzymatic activity of NOS II is not controlled by calcium availability, enabling the production of large amounts of NO even at low intracellular calcium concentrations.[47] The induction of NOS II has been primarily described in macrophages. It can be found in the CNS under pathological conditions in various cells, including neurons and glial cells.[48,49] The NO produced in this context can directly cause cellular dysfunction or cell death by mutations, DNA strand breaks, lipid peroxidation, and/or protein nitration/oxidation.[50,51]

These anti-pathogenic mechanisms are well preserved. Despite the importance of NO in physiological processes, high concentrations of NO may harm healthy cells and contribute

to tissue injury. Similarly, NO has been considered as one of the main mediators of secondary damage after SCI.[52]

ARG and NOS, one substrate to share

Both NOS and ARG effectively compete for L-arginine despite the affinity of ARG being a 1000-fold lower (ARG K_m = 3.3 mm vs. NOS K_m = ± 16 µm).[53,54] This can be explained by the V_{max} of ARG, which is around 1000-fold greater than that of NOS enzymes (V_{max} = 1400 µmol/min vs. V_{max} = 1 µmol/min per mg at a physiological pH respectively).[53,55] Consequently, an upsurged NOS expression can deprive ARG of L-arginine and vice versa. The metabolism of arginine is, however, not always a matter of substrate competition. The addition of exogenous L-arginine, for example, induces ARG expression.[56] To further complicate the picture, multiple cross-inhibitory interactions between both antagonizing pathways exist. For example, ARG-mediated arginine depletion can suppress NOS translation and dimerization necessary for NOS enzyme function.[57,58]

Polyamines generated from arginine can also inhibit NO synthesis to warrant and support cell growth.[59] Conversely, NO can inhibit ornithine decarboxylase (ODC) by nitrosylation, one of the enzymes involved in polyamine synthesis.[60]

Finally, NO production by NOS generates NG-hydroxyl-L-arginine (NOHA) as an intermediate, a potent natural inhibitor of ARG, thereby reducing arginine availability for urea synthesis.[61,62]

These counter-regulating mechanisms do occur in the SCI pathology as ARG and NOS expression overlap in time. Correspondingly, understanding the main regulatory mechanisms and taking advantage of the ARG/NOS dichotomous outcome is considered to be a potential mechanism to dampen the NO-mediated side effects and affect the disease outcome.

The L-arginine metabolism after SCI

Several pathological conditions are linked with argininemia, for example in severe trauma patients where it has a relevant impact on the prognosis.[63] The arginine deficiency state is

caused by a surged amino acid catabolism and a reduced *de novo* synthesis and dietary uptake.[63,64] SCI is mainly caused by a traumatic incident (e.g., motor vehicle accidents, work-related injuries) and therefore is also characterized by a decreased systemic L-arginine.[65]

Importantly, it is demonstrated that in trauma solely induced to the spinal cord, local intraspinal L-arginine levels are increased directly after injury and correlate with the injury severity.[66,67] This elevated L-arginine concentration significantly decreases later in time.[68] The initial rise of L-arginine may be explained by a rapid opening of the blood-spinal cord barrier or arginine released from injured cells.[69] Eventually, the onset of chronic NOS and temporal ARG expression will boost NO release and deprive the local L-arginine reserve.[68] Therefore, supplementation of L-arginine can abolish the deprived state, yet should be considered carefully in relationship to detrimental NO production.

Nutritional supplementation of L-arginine is already used in trauma patients, shortening hospital stays.[70] Research on the effects of L-arginine supplementation in SCI is mostly performed in rodents. The results were heterogeneous (Table 1). On one hand, several studies indicated a beneficial impact. An acute functional improvement and a reduction in axonal degeneration, vascular injury, or motor neuron loss were noted.[13,14,71] On the other hand, a worsened outcome was observed by others.[15,16] A possible explanation for these conflicting results might be the use of L-arginine by either ARG or NOS. The ARG-mediated L-arginine conversion leads to polyamine production promoting neuronal survival, while NOS-mediated L-arginine consumption enhances secondary damage (Figure 1).

New data from our laboratory appear to solve a long-held problem in L-arginine research. Until now it was unclear whether it is more important for wound healing or as a driver of detrimental neuroinflammation and NO production. Our study revealed an overall detrimental impact of L-arginine after SCI. Systemic L-arginine depletion via the application of recombinant ARG I resulted in an improved functional outcome after a T-cut hemisection injury in mice. Histopathological analysis showed a reduction in neuronal cell

death in the spinal cord grey matter. Further examination indicated that these results were concomitant with a diminished intraspinal NO concentration and $CD4^+$ T cell accumulation. Moreover, the systemic/splenic immune response was altered as Th1 and Th17 cell numbers were significantly decreased. L-arginine is crucial for polyamine production, hence T cell proliferation. Consequently, two potential mechanisms were suggested to underlie the functional improvement after SCI: 1) the local L-arginine depletion led to a reduced NO production by NOS-expression inflammatory cells. In turn a decreased NO-induced neuronal cytotoxicity occurred, explaining the increase in neuronal survival. 2) L-arginine deprivation hampered detrimental T-cell proliferation and the systemic immune response ameliorating theoutcome in this SCI mouse model.[72] In this way, L-arginine depletion impacts several pathways that are depicted in Figure 1.

The results of this study seem to be in conflict with the supplementation studies and revealed that L-arginine depletion is beneficial for recovery after SCI. Therefore, an opposite and thus detrimental effect might have been expected after L-arginine supplementation. Surprisingly, the supplementation studies showed either beneficial or detrimental effects. However, it is important to note that the experiments concerning L-arginine depletion were performed with n= 16-22 mice/group, while the supplementation studies were performed with a substantially lower number of experimental animals (n= 6-10 animals/group).[13-16] Importantly, in the supplementation studies, the authors mainly focused on acute (2-24 h post-injury) intraspinal responses, while we investigated several time points including the acute, subacute, and early chronic phase (hours to 4 weeks post-injury). Therefore, the unexpected findings in the supplementation studies have to be interpreted with care.

ARG expression after spinal cord injury

In SCI animal models, only a temporal increased ARG expression is observed. The ARG expression is mainly localized in the perilesional spinal cord with a pique around 3 days after SCI, consistent with the short-lasting type 2 immune response.[73,74] Quickly

thereafter, both mRNA and ARG I protein levels diminished to pre-injury levels. As previously mentioned, local pro-inflammatory signals quickly induce a phenotype switch, promoting a type 2 to a type 1 immune response. As ARG I is a typical type 2 response marker, the dominant pro-inflammatory microenvironment also diminishes its expression. Resident cells like activated astrocytes, neurons, and microglia are responsible for only a small portion of the temporal ARG I expression.[73] The temporarily upsurged expression of ARG I is rather the result of the influx of myeloid cells, responsible for up to 70% of the ARG I expression 3 days post-injury.[73-75]

Little is known about the change in ARG II expression after trauma to the spinal cord. To our knowledge, only one study evaluated the expression of ARG II after SCI. Imagana *et al.* showed a marked decreased ARG II protein expression 48 h after injury induction.[76]

NOS expression after spinal cord injury

Excessive release of NO is consistently reported after SCI in rodents, with an initial pique immediately after trauma and a second wave 3 days later.[52,77,78] The release of RNS and protein nitration occurs in a similar pattern.[52,79] These temporal post-traumatic changes in NO concentrations are linked with the transient expression of the different NOS isoforms in the CNS. An upregulation of NOS I and III was repeatedly found hours to one day after SCI.[77,80] NOS II expression and activity were observed as early as 3 h post-injury with a pique 7 days after SCI.[79-81] This progressive NOS II expression is attributed to the initial activation of resident glial and/or epithelial cells and the evolving post-traumatic inflammatory reaction that lasts for weeks.[80,82] The mechanisms for NOS I and III upregulation, on the other hand, are not completely understood. N-methyl-D-aspartate (NMDA) receptor activation by glutamate is known to stimulate Ca^{2+} influx, allowing NOS I activation via calmodulin.[83,84] Yet, severe ischemia, widespread depolarization, and the release of large amounts of excitotoxic amino acids might also contribute to the upregulation of the constitutive NOS isoforms.

Modulating the arginine metabolism after SCI

Regulating ARG expression/function in SCI

In SCI, increasing evidence suggests that pro-inflammatory immune cells dominate the lesion site chronically, despite the induction of wound healing genes in the injured spinal cord.[75,85] The persistent presence of pro-inflammatory macrophages and the lack of a permissive pro-regenerative environment contribute to secondary damage. Due to the role of ARG in polyamine production and its association with the type 2 immune response, ARG may be considered a key factor enabling the healing process after trauma. Yet only Zhang and colleagues provide evidence that ARG I is essential for the histopathological outcome after SCI. They studied the effect on functional outcome in SCI after transplanting alternatively activated macrophages (RAW246.7 cells) silenced for ARG I compared to wild-type alternatively activated macrophages. A reduced functional recovery, axon myelination, and expression of axonal plasticity genes were observed 6 weeks after trauma in the ARG I silenced treatment group (Table 2).[86] Thus, ARG I silencing has a negative impact on the SCI pathology, underlining its key role in regenerative processes. Several other studies already showed the importance of ARG I and the involved polyamine production in neuronal survival and axonal regeneration.[27-29] Moreover, ARG I induction in neurons even was sufficient to overcome axonal growth inhibition in a myelin-enriched milieu, similarly as found in SCI.[87]

Besides the associated polyamine production, regulation of the local NO production through L-arginine availability by ARG is also an important mechanism to curtail neuronal cell death.[72,88] The majority of studies on altering the L-arginine metabolism focus on NOS inhibition. No clinical trials on the arginase pathway in the context of SCI were performed.

4.2 Regulating NOS expression/function in SCI

Several deleterious biochemical processes are identified after SCI, including an increase of intracellular calcium[89], lipid peroxidation[90], and an overproduction of reactive oxygen species (ROS) and RNS.[91] The release of large quantities of NO after SCI (1000x more; µM) is considered as one of the inducers of these processes which initiate secondary injury.[52]

Extensive studies were performed to improve functional and histopathological recovery by diminishing detrimental intraspinal NO production. The beneficial impact of non-selective NOS inhibitors on functional recovery in spinal cord-injured animals has been described.[78,92-94] Ameliorated spinal cord reflex potentials and repression of injury evoked hypersensitivity were found.[92-94] Correspondingly, administration of NO scavengers improved the histopathological outcome by a decrease in DNA laddering.[95] On the other hand, supplementation of L-arginine increased NO production and reversed the observed beneficial effects of broad-spectrum NOS inhibitors.[96] Together these data indicate the damaging impact of NO in SCI pathology.

As all isoforms of NOS are involved in response to SCI, the usage of non-selective NOS inhibitors seems to be justified, yet their effects on SCI repair are quite variable. Multiple studies also indicated no effects[97-99] or even worsened results on outcome measurements (e.g., deteriorated motor function and hypertension).[93,100,101] Similar conflicting data after general NOS inhibition are also found in cerebral ischemia studies and suggest a complex role of NO in post-injury tissue reactions.[102] Thus, a neuroprotective role of NO in SCI certainly cannot be excluded. It is known, for example, that NO can be beneficial for wound healing, may function as an anti-oxidant, or has anti-excitotoxic effects.[103] Furthermore, under physiological conditions, NO modulates acetylcholine release for the proper functioning of the neuromuscular junction[104] and is involved in regulating the local microcirculation.[100] Thus, complete abolishment of NO may hamper motor function and blood flow. Moreover, pretreatment with a NO donor prior to spinal ischemia already showed to accelerate the recovery of polysynaptic reflex potentials.[105]

In summary, the contradicting results underline the complexity of NO in the SCI pathology, as the NOS inhibition performed here can be both detrimental or beneficial. Therefore, NO production must rather be targeted in a time-dependent manner, preferentially with specific NOS inhibitors.

Timing is essential when general NOS inhibition is applied after SCI in rats. Hamada *et al.* showed that pretreatment (15 min. before injury) with N(ω)-nitro-L-arginine methyl ester (L-NAME) was detrimental to functional and histopathological outcome parameters. Conversely, L-NAME treatment starting subacutely after SCI (6 h after injury) ameliorated motor recovery.[93] Others also observed no or negative effects when general NOS inhibition was performed as a pretreatment or within minutes after SCI induction, causing reduced blood flow or neuronal loss.[97,98,100] As NOS I and III expression is increased acutely after SCI, it has been suggested that NO formation by these isozymes has a protective effect. Moreover, the associated low NO concentration is contended to prevent NF-κβ activation since the decreased expression of both NOS enzymes, and NF-κβ activation are temporally correlated.[81] Correspondingly, maintaining the physiological level of NO is seen as favorable e.g., for increased blood flow or through suppressing NOS II expression by preventing NF-κβ activation.

While it is generally considered that low NO concentrations are advantageous and high concentrations of NO to be harmful in CNS trauma, some nuance must be made concerning SCI. For example, direct application of L-NMMA, a broad spectrum NOS inhibitor, was beneficial after SCI. The acute depletion of low NO levels through constitutive NOS inhibition resulted in fewer apoptotic cells.[78]

The controversies about the effects of general NOS inhibition could be explained by the lack of treatment specificity. The half-life of NO is relatively short (± 2 sec.), and only local structures/cells will come in contact with the produced NO. Consequently, the distinction between the different NOS isoforms is of great importance due to their differences in their spatial and/or temporal expression profile. The acute changes in the NOS I and III expression after SCI are similar, yet the function of both enzymes differ. Therefore, acute treatment or pretreatment with broad-spectrum NOS inhibitors can have variable results, depending on the binding capacity of the compound with the different NOS isoforms.

The need for targeted treatment is supported by studies in which specific NOS inhibitors and knock-out (KO) animal models were used. Specific acute NOS I inhibition using Nomega-Nitro-L-arginine (L-NNA) or 7-nitro indazole (7-NI), for example, attenuated cell injury, bladder incontinency, chronic allodynia, and improved functional recovery after SCI.[94,106,107] Accordingly, NOS I deficient mice had improved hindlimb motor function, demonstrating that the early NOS I upregulation found after SCI might be detrimental.[108] Glutamate-mediated NMDA activation can explain the deleterious effect of NOS I in the SCI pathology. NMDA receptor activation mediates Ca^{2+} influx stimulating NO production by NOS I, contributing to the glutamate-evoked neurotoxicity.[109]

On the other hand, NOS III expression is recognized to be beneficial after SCI. Multiple studies using NOS III KO mice clarified that NOS III plays a crucial role in the protection against ischemic neuronal vulnerability in stroke by mediating vasodilation and ameliorating blood flow.[110,111] As post-traumatic ischemia in the spinal cord occurs directly after SCI, counteracting the reduction of spinal cord blood flow plays a significant role in minimizing secondary damage. General NOS inhibitors would inhibit these positive effects of NOS III on SCI repair. Numerous research reports confirmed that the use of these drugs is accompanied by an increase of the mean arterial pressure due to a hampered NO-mediated vasodilation.[94,96,101,112]

The situation for NOS II inhibition is more complicated. Acute antagonism of NOS II with pharmacological[113-115] or molecular inhibitors[113,116], or through genetic KO[117,118] is favorable for SCI repair. A reduced neutrophil infiltration, lipid peroxidation, and less neuronal cell death were observed. These findings support other reports on the involvement of NOS II in the progression of secondary injury after CNS trauma.[119,120] However, there is ample evidence that in neurological diseases (e.g., experimental autoimmune encephalomyelitis) and CNS trauma (e.g., traumatic brain injury), the complete ablation of NOS II, especially in the chronic phase of the pathology, is deleterious.[121,122] Also in SCI, NOS II KO led to an exacerbated outcome over time. KO mice had less preserved white matter within the spinal cord and exhibited 35% fewer perilesional blood vessels compared to wild-type mice 6

weeks post-injury.[123] Moreover, the initial improved functional outcome in the transgenic mice after SCI disappeared later in time.

The length and timing of NOS II inhibition were crucial and may point out the very distinctive role of NOS II and NO over time. Why late NOS II inhibition can lead to detrimental effects is not entirely understood. Yet NOS II expression is observed in both vascular smooth muscle cells and neurons after SCI.[77,79,80] Therefore, one explanation may be that excessive and long-term inhibition might result in microvascular injury. Furthermore, the role of NOS II expressed in neurons after SCI is unknown and might be detrimental. Moreover, NO is also involved in T cell apoptosis and memory formation, and NOS II inhibition might impede this process.[124] Therefore, more research, for example, using conditional and cell type-specific KO mice, is necessary to specify the roles of NO and NOS II over time to enable treatment optimization.

In contrast to the large number of preclinical studies, only a few studies have been performed on SCI patients. In these studies, NOS inhibition was primarily applied to alleviate hypotension and the associated reduced blood flow rather than to improve the functional and histopathological outcome in these patients.[101,112] Further investigations are needed to evaluate NOS inhibitors as a therapeutic approach in a clinical setting.

Conclusion and future perspectives

The L-arginine catabolism is highly activated after SCI by a strong ARG and NOS expression. Studies on L-arginine supplementation provided insight into the dual role of the amino acid after SCI. On one hand, L-arginine is important for wound healing, neuronal functioning, and survival. On the other hand, it potentiates secondary damage by forming an essential mediator for neuroinflammation and NO production. This conflicting role became clear when L-arginine supplementation both could worsen or ameliorate the histopathological recovery after SCI. New findings however assign an overall detrimental role to the amino acid, as L-arginine depletion improved SCI recovery.

Conflict of interest

There does not exists a conflict of interest for any of the authors.

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Table 1: Overview of the impact of L-arginine supplementation on SCI.

Model	Treatment	Effects	Cell type studied	Ref.
Spinal cord ischemia – Wistar rat	L-arginine 200 µg/kg/min i.v. for 20 min. after ischemia	≅ Blood glucose level, PCO_2, and PO_2 ↓ Rats with paraplegia ↑ Intraspinal HIF-1α expression 24 h after SCI ↑ Spinal motor neuron survival 24 h after SCI	Neurons	[13]
Spinal compression – Rabbit	L-arginine 750 µg/kg/min i.v. for 30 min. before SCI	↓ Edema formation 24 h after SCI ↓ Degeneration of myelinated axons 24 h after SCI ↓ Vascular injury 24 h after SCI	Neurons	[14]
Spinal compression – Rabbit	Topical L-arginine application via cotton 1.7 mg/kg for 5 min. post-SCI	↓ Edema formation 24 h after SCI ↓ Degeneration of myelinated axons ↓ Vascular injury 24 h after SCI	Neurons	[71]
Spinal cord ischemia – Wistar rat	L-arginine 100 mg/kg i.p. prior to ischemia	↑ Intraspinal MDA levels 2 h after SCI ↑ Intraspinal NO levels 2 h after SCI ↓ Intraspinal VEGF levels 2 h after SCI	Not specified	[15]
Spinal contusion – Long Evans rat	L-arginine 300 mg/kg/dose either 1 h, 24 h or 1x/day after SCI	↓ Functional recovery for all treatment schemes 8 weeks after SCI ≅ Spared white matter 8 weeks after SCI	Not specified	[16]
Spinal hemisection – C57BL/6J	Recombinant ARG I 50 mg/kg every 3 days after SCI	↑ Functional recovery 4 weeks after SCI ↓ Number of intraspinal $CD4^+$ T cells 4 weeks after SCI ↓ Splenic Th1 and Th17 cell number 4 days after SCI ↓ Intraspinal nitrite levels 7 days after SCI ↓ Number of Cl. Caspase 3^+ $NeuN^+$ intraspinal neurons 4 weeks after SCI ↓ Number of NF/Iba-1 contacts 4 weeks after SCI	Neurons Astrocytes Microglia/macrophage $CD4^+$ T cells	[72]

Abbreviations: CD: cluster of differentiation; HIF-1α: hypoxia-inducible factor 1α; Iba1: ionized calcium-binding adapter molecule 1; i.p.: intraperitoneal; i.v.: intravenous; MDA: malondialdehyde; NeuN: neuronal nuclei; NF: neurofilament; Th: T helper cell; VEGF: vascular endothelial growth factor

Table 2: Overview of the impact of ARG I expression in the context of SCI.

Model	Treatment	Effects	Cell type studied	Ref.
Spinal contusion - Sprague Dawley rat	Intraspinal injection of RAW246.7 cells with or without ARG I silencing	↓ Functional recovery 4 weeks after SCI ↓ Number $NeuN^+$ cells ↓ Expression of N-WASP, Arp2, Tau, and Cdc42 4 weeks after SCI	Neurons	[86]
Neuronal cell culture	ARG I supplementation	↑ Neuronal survival after glutathione depletion as a result of a halt in protein synthesis	Neurons	[27]
Compression injury-Sprague Dawley rat	Polyamines s.c. 1x/day	↑ Neuronal survival 30 days post-injury	Neurons	[28,29]
Neuronal cell culture	ARG I transduction and/or ARG I inhibition	↑ ARG I expression after BDNF or cAMP treatment ≅ Neurite length on MAG-enriched milieu compared to control when transduced with ARG I ↓ Neurite length after ARG I inhibition on MAG-enriched milieu ↓ Neuronal survival after BDNF deprivation in the presence of ARG I inhibitor because of increased NO production ↑ Neuronal survival after BDNF deprivation and ARG I overexpression	Neurons	[87,88]

Abbreviations: Arp2: actin-related protein 2; BDNF: brain-derived neurotrophic factor; cAMP: cyclic adenosine monophosphate; cdc42: cell division control protein 42; MAG: myelin-associated glycoprotein; NeuN: neuronal nuclei; N-WASP: Wiskott-Aldrich syndrome protein; s.c.: subcutaneous

Table 3: The impact of NOS inhibitors, knockout, NO scavengers or NO donors on SCI.

Model	Treatment	Effects	Cell type studied	Ref.
Spinal ischemia – cat	L-NMMA 10 mg/kg i.v. before ischemia	↑ Recovery of polysynaptic reflex potentials	Neurons	92
Spinal compression – rat	L-NMMA 0.05 mg after SCI, intraspinal	↓ $TUNEL^+$ cells 1 day after SCI ↓ [NO]intraspinal 4 hours after SCI	Not specified	78 95
Spinal compression – rat	L-NMMA 3 mg/kg i.p. 4x after SCI	↑ Mean arterial blood pressure, reversible with L-arginine 10 days after SCI ↑ Functional recovery 10-30 days post-injury, abolished with L-arginine addition ↓ Latency for hind paw stimulation, abolished with L-arginine addition 10 days post-injury	Not specified	96
Spinal compression – Guinea pig	L-NMMA 10 mM intrathecal	≅ Somatosensory evoked potential 1 hour to 3 days post-SCI	Neurons	99
Spinal compression – Wistar rat	L-NAME 0.1 mg/kg i.v. 4 x after SCI	↑ Functional recovery 14 days post-injury	Not specified	93
	L-NAME 30 mg/kg 15 min. before SCI	↓ [NO]intraspinal directly after SCI ↓ Functional recovery 2 days post-injury ↑ increased MPO intensity	Not specified	93
Spinal hemisection – Sprague Dawley	L-NAME 50-100 µmol/kg i.p.	↓ Hypersensitivity ≅ Spontaneous activity 30 min. after treatment ↑ Mean blood pressure ↑ Mean arterial blood pressure	Neurons	94
Spinal hemisection – Wistar rats	L-NAME 2.5 µg topically	≅ Edema formation 5 hours after SCI	Not specified	97
Spinal hemisection – Sprague Dawley	L-NAME 50mg/kg i.v. 30 min. prior to SCI	≅ Amplitude of spinal cord evoked potentials 5 hours after SCI ≅ Edema formation	Neurons	98
Undefined – patients	L-NAME 1.55 or 2.7 mg/kg i.v. over 1 hour	↓ Heart rate in both treated healthy controls and SCI patients ↑ (2-3x) blood pressure in treated SCI patients compared to treated healthy control ≅ Blood vessel diameter and conductance	Not specified	101
Undefined - patients	L-NAME 1 mg/kg i.v.	↑ Mean arterial blood pressure ≅ Heart rate	Not specified	112
Spinal compression –	carboxy-PTIO	↓ [NO] intraspinal 4 hours after SCI ↓ DNA laddering 1 day post-injury days	Not specified	95

Sprague Dawley				
Spinal compression – rats	L-NNA 500 µg/kg/min i.v. for 401 min starting 10 min prior to SCI	↑ Mean arterial blood pressure starting from infusion till 1 hour post-injury ≅ Heart rate	Not specified	[100]
Spinal hemisection – Sprague Dawley	L-NNA 20mg i.p. 1x/day for 7 days starting 30 min before SCI	↑ Functional recovery ↓ Edema formation, swelling, and hemorrhage ↓ SCI-induced BSCB breakdown 5 hours after SCI	Not specified	[106]
Spinal ischemia - cat	Nicorandil 10-300 µg/kg i.v. prior to ischemia	≅ Recovery time of monosynaptic reflex potential ≅ Recovery time polysynaptic reflex potential for 10 and 300 µg/kg ↓ Recovery time of polysynaptic reflex potential for 100 µg/kg	Neurons	[105]
Spinal hemisection – Sprague Dawley	7-NI 75 – 300 µmol/kg i.p.	↓ Hypersensitivity ↑ Functional recovery ≅ Spontaneous activity 30 min. after treatment	Not specified	[94]
Spinal transection – Sprague Dawley	Spermidine Trihydrochloride 1 µM intrathecal	↓ Bladder incontinency via enlarged bladder capacity and urine storage ≅ Amplitude and duration of bladder contractions	Not specified	[107]
Spinal compression – NOS I KO mice	Not applicable	↑ Functional recovery 10-14 days after SCI ≅ White matter damage 14 days after SCI ≅ Astrogliosis/GFAP intensity	Astrocytes	[108]
Spinal contusion – Sprague Dawley	1400W 10 or 20 mg/kg i.v. or i.p. respectively 3 hours after SCI	↓ NOS II activity, IGg extravasation, and MPO^+ cells 1 day after i.v. treatment ≅ NOS II activity, and IGg extravasation 1 day after i.p. treatment ↑ MPO^+ cells 1 day after i.p. treatment ≅ Atrogliosis/$GFAP^+$ cells and apoptotic neurons for both treatments	Neurons, astrocytes	[113]
Spinal ischemia – Sprague Dawley	Aminoguanidine 150 mg/kg i.p. 1x/day	↑ Functional recovery 3 days post-injury ↑ Neuronal survival ↓[NO]intraspinal till 3 days post-injury ↓ Release of cytochrome C till 3 days post-injury	Neurons	[114]
Spinal contusion – Sprague Dawley	Aminoguanidine 100 mg/kg i.p. 2x/day for 3 days	↑ Functional recovery 8 weeks post SCI ↓ Lesion volume 8 weeks post-injury	Not specified	[115]
Spinal contusion – Sprague Dawley	Mixed base oligonucleotides s.c.	↓ NOS II activity, IGg extravasation, astrogliosis/GFP^+ cells, apoptotic neurons, and MPO^+ cells 1 day after treatment	Neurons, astrocytes	[113]
		↑ Functional recovery 5-10 weeks post-injury ↓ Neuronal cell death 10 weeks after SCI	Neurons	[116]

Spinal contusion – Fischer rats	Mixed base oligonucleotides			
Spinal compression - NOS II KO mice	Not applicable	↑ Functional recovery 24 days post-injury ↓ TUNEL^{+} cells and lesion volume 1 day after SCI	Not specified	[117]
Spinal compression - NOS II KO mice	Not applicable	↑ Functional recovery 14 days post-injury	Not specified	[118]
Spinal contusion - NOS II KO mice		↑ Functional recovery 3-4 weeks post-injury ≅ Functional recovery 5-6 weeks post-injury ↑ Lesion volume 6 weeks post-injury ↓ NF^{+} axons and blood vessels 6 weeks after SCI	Neurons	[123]

Abbreviations: BSCB: blood spinal cord barrier; GFAP: glial fibrillary acidic protein; i.p.: intraperitoneal; i.v.: intraveneous; KO: knockout; L-NAME: L-N^{G}-Nitro arginine methyl ester; L-NNA: Nω-nitro-l-arginine; L-NMMA: NG-Methyl-L-arginine acetate; MPO: myeloperoxidase; NF: neurofilament; s.c.: subcutaneous; TUNEL: terminal deoxynucleotidyltransferase dUTP nick-end labeling; 7-NI: 7-Nitroindazole