

The role of L-arginine metabolism in neurocritical care patients

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Abstract

Nitric oxide is an important mediator of vascular autoregulation and is involved in pathophysiological changes after acute neurological disorders. Nitric oxide is generated by nitric oxide synthases from the amino acid L-arginine. L-arginine can also serve as a substrate for arginases or lead to the generation of dimethylarginines, asymmetric dimethylarginine, and symmetric dimethylarginine, by methylation. Asymmetric dimethylarginine is an endogenous inhibitor of nitric oxide synthase and can lead to endothelial dysfunction. This review discusses the role of L-arginine metabolism in patients suffering from acute and critical neurological disorders often requiring neuro-intensive care treatment. Conditions addressed in this review include intracerebral hemorrhage, aneurysmal subarachnoid hemorrhage, and traumatic brain injury. Recent therapeutic advances in the field are described including current randomized controlled trials for traumatic brain injuries and hemorrhagic stroke.

Key Words: arginine; brain injuries, traumatic; cerebral hemorrhage; dimethylarginine; nitric oxide; stroke; subarachnoid hemorrhage

Introduction

Intracerebral hemorrhage (ICH), aneurysmal subarachnoid hemorrhage (SAH), and traumatic brain injury (TBI) are common neurocritical care diseases with incidences ranging from 6.1/100,000 per year for SAH, between 19.6 and 51.8/100,000 per year for ICH, and up to 73/100,000 for severe traumatic brain injury and are despite the deeper understanding of pathological mechanisms and improved specialized care still associated with high morbidity and mortality (Majdan et al., 2016; An et al., 2017; Iaccarino et al., 2018; Tatlisumak et al., 2018; Busl et al., 2019; Etmian et al., 2019; Maegle et al., 2019). Besides distinct pathophysiological differences between these diseases, a common feature is that after the initial event of injury, secondary cerebral damage can be mediated by various pathological pathways including inflammation, oxidative stress, excitotoxicity, depolarization, decreased cerebral blood flow, blood-brain barrier disruption, intracranial hypertension and cytotoxicity of blood components (Lawton and Vates, 2017; van Lieshout et al., 2018; Wilkinson et al., 2018; Ng and Lee, 2019; Mohme et al., 2020; Weiland et al., 2021).

One pathway of interest is the nitric oxide (NO) metabolism, given the important role of NO as a mediator of vascular autoregulation and the established association between NO disturbances and cerebrovascular disease as well as brain injury (Cherian et al., 2004; Garry et al., 2015). L-arginine serves as a substrate for different enzymatic pathways, which includes the production of NO and L-citrulline catalyzed by NO synthases (NOS). L-arginine metabolism is therefore a central element of the NO pathway (Figure 1). Different types of NOS have been identified, which include the inducible form (iNOS) as well as with the endothelial NOS (eNOS) and neuronal NOS (nNOS) two constitutive forms (Garry et al., 2015).

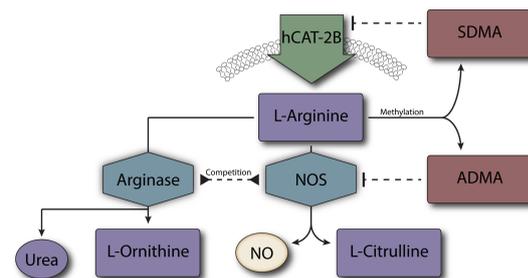


Figure 1 | The flow chart depicts key pathways of L-arginine metabolism and endogenous mechanisms of interference.

ADMA: Asymmetric dimethylarginine; hCAT-2B: human cationic amino acid transporter 2B; NO: nitric oxide; NOS: nitric oxide synthase; SDMA: symmetric dimethylarginine.

Dimethylarginines, which are products of L-arginine methylation and include asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), have been identified as risk markers in a range of primarily cardiovascular diseases (Böger, 2006; Tain and Hsu, 2017). Dimethylarginines have multifunctional roles with ADMA acting as a reversible competitive inhibitor of NOS leading to endothelial dysfunction (Böger et al., 1998) as well as oxidative stress (Li et al., 2011) and SDMA competing with L-arginine for the human cationic amino acid transporter 2B (hCAT-2B) (Closs et al., 1997; Böger, 2006). ADMA generation from protein-incorporated L-arginine is catalyzed by protein arginine methyltransferases (Leiper and Vallance, 1999). ADMA is metabolized to citrulline by dimethylarginine dimethylaminohydrolases (DDAH) whereas SDMA is eliminated by renal excretion (Leiper and Vallance, 1999; Bode-Böger et al., 2006).

An alternative enzymatic pathway of L-arginine metabolism is mediated by arginases I and II, which are located in the cytosol

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and the mitochondria, respectively, and lead to the products L-ornithine and urea (Morris, 2002). Consequently, arginase is a direct competitor with NOS. Another metabolizing enzyme of L-arginine is the Arginine:glycine amidinotransferase (AGAT), which exhibits a promiscuous activity either using glycine or L-lysine as co-substrates to generate L-ornithine and either guanidino acetic acid or L-homoarginine, respectively (Davids et al., 2012).

Disturbances of the NO pathway can lead to different pathophysiological sequels, including most prominent impairment of cerebral blood flow regulation (Cherian et al., 2004). Depletion of the substrate L-arginine can lead to oxidative stress due to uncoupling of NOS with oxygen radical formation potentially leading up to the production of the highly toxic oxidant peroxynitrite (ONOO⁻) (Xia et al., 1996; Cherian et al., 2004). Uncoupling of NOS can also be mediated by ADMA (Böger et al., 2000; Wells and Holian, 2007). NO holds anti-inflammatory properties by redox regulation of nuclear factor (NF)-κB (Reynaert et al., 2004). Depletion of NO can therefore lead to an enhanced neuroinflammatory reaction. Arginase has also been shown to promote inflammation by decreasing the cellular content of NO and enhancing the NF-κB pathway (Ckless et al., 2007).

This systematic review discusses the role of L-arginine metabolism in patients suffering from acute and critical neurological disorders often requiring neuro-intensive care treatment. Moreover, recent developments of therapeutic advances in this field are described. We are focusing in particular on the pathophysiological mechanisms in ICH, aneurysmal SAH, and TBI. The mechanisms of L-arginine metabolism in ischemic stroke have previously been discussed by Grosse et al. (2020).

Database Search Strategy

The US National Library of Medicine PubMed database (<https://pubmed.ncbi.nlm.nih.gov>) has been accessed on February 26, 2021, and searched for the following search string: (arginine OR dimethylarginine) AND ('subarachnoid hemorrhage' OR 'traumatic brain injury' OR 'intracerebral hemorrhage' OR 'hemorrhagic stroke'). This search returned 477 articles. Of these, the title and abstract of articles published within the last 10 years (172 articles) were screened independently by the authors for relevant content. Articles covering vasopressin (also called arginine vasopressin, antidiuretic hormone), its derivatives, Agatroban and pituitary hormones were not considered for this review. A total of 33 articles were included in this search strategy. Additional literature identified from these articles or known as relevant to the authors was included as well.

Moreover, ongoing and recently completed clinical trials were identified by searching the US National Library of Medicine ClinicalTrials.gov database (<https://www.clinicaltrials.gov/ct2/home>) using the search term 'arginine' together with either 'intracerebral hemorrhage' (4 studies), 'subarachnoid hemorrhage' (1 study) or 'traumatic brain injury' (4 studies). Trials covering arginine vasopressin, its derivatives and pituitary hormones as well as non-interventional studies were excluded, leading to the inclusion of two studies for this review (ClinicalTrials.gov Identifier: NCT02012582 and NCT03711903).

L-arginine Metabolism in Intracerebral Hemorrhage

Whereas detrimental effects of increased NO and overexpression of NOS in the context of ICH have been demonstrated in animal models, clinical data is sparse and controversial (Li et al., 2011). Similarly, studies focusing on the underlying L-arginine metabolism are scarce and mostly focus on the peripheral vascular compartment. A first study

measuring L-arginine metabolites in the plasma of 49 patients with primary ICH within three days of onset was conducted by Rashid et al. (2003). The authors observed significant reductions in L-arginine, L-citrulline, and L-ornithine, compared to healthy controls. This was accompanied by reduced levels of NO end products (nitrate and nitrite). Moreover, patients who could be discharged home demonstrated higher levels of NO end products, L-arginine, L-citrulline and serine than patients with in-hospital mortality or discharge to another institution. In a cohort presented by Wanby et al. (2006) plasma of 22 hemorrhagic stroke patients was collected on the first day after admission and analyzed for L-arginine, ADMA, and SDMA. Decreased levels of L-arginine and a reduced L-arginine/ADMA ratio, as an indicator for relative L-arginine deficiency, but no changes in dimethylarginine concentrations were associated with ICH. These findings were partially in contrast to a study of Worthmann et al. (2017), analyzing blood samples ≤ 24 hours and 3 and 7 days after ICH onset in 20 patients. In this cohort, ADMA was significantly elevated in ICH patients compared to controls at all time points. No change in SDMA concentration was detected. L-arginine showed a trend for lower levels at earlier time points but without statistical significance. Moreover, the authors observed elevated levels of SDMA and early ADMA in patients with the unfavorable outcomes and an independent association was confirmed for SDMA at the ≤ 24 hours and 3-day time points.

A recent study examined, besides L-arginine metabolite concentrations in the plasma, also the levels in the cerebrospinal fluid (CSF) of 25 ICH patients over a time course of up to 11 days (Mader et al., 2021). With regard to plasma concentrations compared to control patients, reductions of L-ornithine, L-lysine, and L-citrulline were measured, consistent with the findings of Rashid et al. (2003). Similar to Worthmann et al. (2017), L-arginine showed only a trend to decrease without statistical significance. Intrathecally, an early rise in L-citrulline levels with unchanged L-arginine concentrations was measured, which is suggestive for an early increase in NOS activity. At later timepoints, increasing CSF concentrations of ADMA were measured while accumulating L-arginine and decreasing L-citrulline levels were indicative for a reduction of NOS back to baseline. This observation could be well explained by the known inhibitory effect of ADMA on NOS (Vallance et al., 1992). In contrast to SDMA in the plasma, SDMA levels in the CSF were elevated throughout the observation period, which could also contribute to a later decline in NOS activity by competition with L-arginine for cellular hCAT-2B-mediated uptake leading to intracellular L-arginine depletion (Closs et al., 1997). Moreover, signs for an increase in arginase activity were reported with over time rising levels of L-ornithine and a persistently elevated L-ornithine/L-arginine ratio. Given the direct competition of arginase with NOS for the common substrate L-arginine, this may be another mechanism leading to a reduction of NOS activity back to baseline in the subacute phase. Moreover, rather elevated L-lysine and low L-homoarginine concentrations were possible indicators for reduced AGAT activity. In contrast to the work of Worthmann et al. (2017), no association between dimethylarginines and outcome was detected in this study cohort. Instead, early CSF concentration of L-arginine was found to be an independent predictor of outcome. Patients who were dead or in a persistent vegetative state after six months demonstrated lower CSF L-arginine levels at early timepoints, identifying manipulation of intrathecal L-arginine concentration as a potential therapeutic target. The cationic arginine-rich peptides poly-arginine-18, which is an 18-mer of L-arginine, and its D-enantiomer have recently been evaluated in a collagenase ICH model in rats (Liddle et al., 2019). Intravenous and/or intraperitoneal administration in the acute phase of bleeding has been shown to be safe without exacerbation of hematoma volume. But no reduction of lesion volume or improvement of functional outcome was observed.

L-arginine Metabolism in Aneurysmal Subarachnoid Hemorrhage

A major complication of aneurysmal SAH is cerebral vasospasm (CVS)/delayed cerebral ischemia (DCI), which contributes substantially to the morbidity and mortality of this disease (Lawton and Vates, 2017). An increase in ADMA in the peripheral blood and CSF after SAH was detected in several studies. In a cohort of 20 SAH patients, ADMA concentration of the peripheral blood was not different from control patients in the first 3 days after onset but increased within the first week and remained elevated after 3 months (Rodling-Wahlstrom et al., 2012). In a cohort of 56 SAH patients, plasma levels of ADMA were measured over 10 days and were already significantly elevated within the first 48 hours compared to sex- and age-matched healthy donors (Lindgren et al., 2014). ADMA levels showed then a further increase at later time points (97–240 hours). Interestingly, this increase in ADMA started after proinflammatory markers CRP and IL-6 had peaked, suggesting a potential relationship with systemic inflammation. Patients with higher-grade SAH (defined as Hunt & Hess > 2) demonstrated higher ADMA concentrations and a decreased L-arginine/ADMA ratio. Looking at peak ADMA levels and nadir L-arginine/ADMA ratio, no association with impaired cerebral circulation or functional neurological outcomes was detected. But patients with good functional outcome demonstrated a higher peak L-arginine/ADMA ratio.

Studies in primates revealed that CVS seems to be associated with a reduction in NOS expression (Pluta et al., 1996) as well as reduced CSF levels of nitrite, nitrate, and L-citrulline but increased concentrations of ADMA (Jung et al., 2004). Similar findings were obtained in a cohort of 18 SAH patients (Jung et al., 2007). Patients suffering from SAH demonstrated higher CSF levels of ADMA than control patients. ADMA further increased in patients with CVS and correlated with CVS degree. Measurements of ADMA and vasoconstrictor endothelin-1 in the CSF of 24 patients with SAH showed again that ADMA but not endothelin-1 concentration was correlated with CVS (Jung et al., 2012). But neither correlated with ischemic cerebral lesions. Interestingly, measurement of monomethylated L-arginine, which is also a strong inhibitor of NOS, in serum and CSF showed no correlation with CVS or nitrite but correlated with occurrence and size of ischemic lesions (Jung et al., 2013). Measurement of ADMA concentrations in the CSF of a cohort of 20 SAH patients for 2 weeks revealed an increase on days 3–5, peaking around 7–9 days, and remaining elevated until 12–14 days after SAH (Li et al., 2014). Interestingly, higher ADMA levels at days 7–9 were associated with both the detection of CVS in transcranial Doppler ultrasonography as well as poor outcome (Karnofsky Performance Status scale < 60) after 2 years.

In a cohort of 111 SAH patients, a low plasma L-arginine/ADMA ratio was associated with higher mortality rates and, independently from SAH severity measured by the World Federation of Neurosurgical Societies grade, a higher hazard ratio (Staalso et al., 2013). The L-arginine/ADMA ratio correlated negatively with nitrite/nitrate levels and nitrite/nitrate levels showed an inverse relationship with transcranial Doppler flow velocities. However, neither ADMA itself nor the L-arginine/ADMA ratio demonstrated an association with flow velocities. The same study group evaluated early endothelial function with peripheral arterial tonometry in the fingertip in 48 SAH patients (Bergström et al., 2014). Peripheral endothelial dysfunction was expressed as reactive hyperemia index, which increased in SAH patients after day 2 but remained lower in deceased patients. Similar to previous studies (Rodling-Wahlstrom et al., 2012; Lindgren et al., 2014), ADMA and also L-arginine increased after the acute phase of SAH. A positive correlation was found between the reactive hyperemia index and the L-arginine/ADMA ratio, which was

interpreted as impaired endothelial function in the context of L-arginine/ADMA imbalance but reactive hyperemia index showed no association with CVS or DCI.

An evaluation of L-arginine, ADMA, and SDMA in both the peripheral vascular and the intrathecal compartment was performed in a SAH cohort of 34 patients (Appel et al., 2018). Patients with DCI demonstrated a lower baseline plasma L-arginine/ADMA ratio as well as higher CSF concentrations of ADMA and SDMA. A low L-arginine/ADMA plasma ratio at admission was associated with poor functional neurological outcome, at discharge and after 3 months. ADMA in the CSF was confirmed to be independently associated with DCI whereas increased SDMA levels in the CSF were found to be an independent predictor for the poor neurological outcomes. This is in line with a machine learning-driven screen of 138 metabolites in the CSF of 81 SAH patients reporting SDMA, dimethylguanidine valeric acid, and L-ornithine to be associated with poor outcomes at discharge and after 90 days (Koch et al., 2021). Another study including 51 SAH patients further reported higher plasma ADMA and SDMA levels and higher CSF SDMA levels at admission in patients with DCI (Hannemann et al., 2020). Moreover, single nucleotide polymorphisms of the DDAH1 genes were associated with plasma ADMA and carriers of the minor allele rs233112 had a significantly increased relative risk of DCI.

Potential therapeutic manipulations of the L-arginine metabolism have been explored preclinically in several studies for SAH. The effect of L-arginine administration on vasospasm was explored in a rat femoral artery vasospasm model (Akar et al., 2019). The authors described morphometric improvements in animals treated with L-arginine with reduced wall thickness and increased lumen diameter. Reduced pathological changes of the cortical microstructure have been described after intraperitoneal administration of L-arginine aspartate in a rat model of SAH (Netlyukh, 2016). A different study using a prechiasmatic cistern SAH model in rats reported increased levels of ADMA in the CSF over a time course of 14 days peaking around day 5 (Zhao et al., 2015). A positive correlation between ADMA and connexin43 was described. Interestingly, administration of the gap junction inhibitor 18 β -glycyrrhetic acid led to improved basilar artery vessel diameter and neurological score while reducing the CSF level of ADMA. Intraperitoneal administration of L-citrulline, which can serve as a product for new L-arginine synthesis via the argininosuccinate synthase and lyase pathway, was found to improve basilar artery patency and neurological scores in a SAH model in haptoglobin 2-2 transgenic mice (Pradilla et al., 2012).

L-arginine Metabolism in Traumatic Brain Injury

The role of NO in TBI is complex with a time-specific course of brain levels consisting of an initial peak, followed by a potential period of relative deficiency after which a second NO accumulation can be observed (Cherian et al., 2004). Effects of NO pathway disturbances are multifunctional and can even mediate blood-brain barrier disruption as recently demonstrated (Logsdon et al., 2018, 2020). Different studies evaluated the effect of different NOS-inhibitors and administration of L-arginine pre- and post-injury with partially divergent results, as reviewed by Cherian et al. (2004). More recent studies sought to further elucidate the role of underlying L-arginine metabolism including dimethylarginines, further characterize the alterations in humans and evaluate therapeutic effects of polyarginine peptides.

Local ADMA expression was strongly reduced in a controlled cortical impact model in rats (Jung et al., 2014). This was most prominent and lasting within the lesion whereas a more fluctuating time-dependent expression was observed for the perilesional area which interestingly correlated positively

with the neurological status. Changes were also detected for the expression of underlying enzymes with a decrease in arginine methyltransferase 1 expression but an increase in the expression of DDAH1 and DDAH2, which negatively correlated with the neuroscore performance. However, measurement of ADMA concentrations in the CSF within 3 days of TBI in a pediatric study population showed an increase compared to healthy controls (Thampatty et al., 2013). Interestingly, this increase was attenuated by therapeutic hypothermia. An increase in the concentration of ADMA in the peripheral blood was described in a severe TBI cohort of 46 patients starting 72 hours after trauma (Wahlström et al., 2014).

Measurement of amino acid concentrations in the rat brain after either mild or severe diffuse traumatic brain injury revealed a reduced L-arginine/L-citrulline ratio already 6 hours after injury and throughout the observation period of 120 hours in severely injured animals but an increased ratio in the mildly injured group (Amorini et al., 2017). The influence of the severity of trauma was also addressed in a clinical study. L-arginine metabolites were measured in the peripheral blood within the first 24 hours in a cohort consisting of mild and severe TBI patients, orthopedically injured patients as well as healthy volunteers (Jeter et al., 2012). Plasma levels of L-arginine, citrulline, ornithine, and hydroxyproline were significantly reduced in the group of severe TBI but no changes were detected in mild TBI patients. Peripheral dimethylarginine plasma levels were unaffected by TBI but an increase in creatine was observed. A metabolomic analysis of brain tissue in rats after a controlled cortical impact revealed that increased ornithine was among four metabolites differentially changed on both days 1 and 3 after TBI (Zheng et al., 2020). Moreover, arginine and proline metabolism were among two pathways identified as significantly affected on day 3 after TBI.

As another relevant component of the L-arginine metabolism, the role of arginase has been further evaluated revealing cell type and subtype-specific implications. Utilizing a genetic approach in a mouse-controlled cortical impact model, the role of overexpression of either arginase I or arginase II in neurons was studied in the context of TBI (Madan et al., 2018). As expected, overexpression of either enzyme subtype led to reduced L-arginine brain levels. Interestingly, animals overexpressing arginase I in neurons demonstrated a significant decrease in lesion size and contusion severity compared to wild-type mice identifying a potential neuroprotective effect of cell-type-specific arginase I expression. In comparison, an induction of systemic endothelial dysfunction after TBI was described to be mediated by arginase I (Villalba et al., 2017). Rats were evaluated for endothelial function in the mesenteric arteries 24 hours after a fluid percussion injury. TBI animals showed diminished vasodilatory and vasoconstrictor responses which were linked to impairment of the NO pathway. Mesenteric arteries of TBI animals demonstrated elevated arginase activity with isoform I being expressed in the endothelium. The vasodilatory function could be rescued by either L-arginine supplementation or arginase inhibition. Increased arginase I levels have also been detected after experimental spinal cord injury (Ahn et al., 2012). Besides constitutive expression in neurons and glial cells also present in control spinal cord tissue, arginase I was additionally detected in macrophages and reactive astrocytes in the core lesion.

The therapeutic potential of cationic arginine-rich peptides for TBI has been evaluated in different recent studies focusing on polyarginine-18 (R-18) (Chiu et al., 2017, 2019, 2020; Batulu et al., 2019). In an *in vitro* excitotoxicity model, R-18 showed strong neuroprotective abilities and reduced neuronal calcium influx (Chiu et al., 2017). Intravenous administration 30 minutes after experimental TBI in rats lead to reduced axonal injury. However, no significant

improvement of hippocampal neuronal loss or functional outcome was detected. In a different rat model of TBI, R-18 reduced neuronal cell apoptosis and brain water content while inhibiting caspases-3/8/9 (Batulu et al., 2019). R-18 treated TBI animals showed lower levels of the pro-apoptotic protein Bax as well as higher levels of the autophagic marker protein LC3 compared to untreated TBI animals. Comparison of R-18 and its D-enantiomer R-18D in a closed-head impact weight drop model in Long-Evans rats revealed that intravenous treatment of R-18D but not R-18 improves sensorimotor and vestibulomotor deficits (Chiu et al., 2019). However, in a subsequent study utilizing a closed-head injury model in Sprague-Dawley rats, no improvement of vestibulomotor functions could be detected and only a trend to improved memory function without statistical significance was observed after R-18D administration (Chiu et al., 2020). Besides L-arginine itself and cationic arginine-rich peptides, other L-arginine-derived compounds have also been identified mediating neuroprotective effects, e.g., agmatine, which is derived from L-arginine by decarboxylation (Kotagale et al., 2019).

Comparison of the Changes in the L-Arginine Metabolism between Different Etiologies

Despite marked differences in the overall pathophysiological processes of ICH, SAH, and TBI, certain similarities can be observed with regard to changes in the L-arginine metabolism. Here we refer to the aforementioned clinical studies and discuss similarities and differences across the different entities (**Table 1**).

Changes in amino acid concentrations

Several studies described systemic reductions in relative (as L-arginine/ADMA ratio) or absolute concentrations of L-arginine and/or related amino acids like L-citrulline and L-ornithine either after disease onset or in association with poor outcome for ICH (Rashid et al., 2003; Wanby et al., 2006; Worthmann et al., 2017; Mader et al., 2021) or TBI (Jeter et al., 2012). Similarly, most clinical data for SAH demonstrated an association between poor outcome or DCI and a relative deficiency in plasma L-arginine (Staalso et al., 2013; Lindgren et al., 2014; Appel et al., 2018) but absolute L-arginine concentrations were reported to not differ from control patients (Jung et al., 2007). Notably, studies have described an increase in systemic L-arginine at later time points after the acute phase, e.g., for ICH (Mader et al., 2021) or SAH (Bergström et al., 2014).

For the intrathecal compartment, only limited data exist for L-arginine concentrations. No changes in CSF L-arginine or L-citrulline concentrations were detected between patients suffering from SAH and controls (Jung et al., 2007). Patients with ICH showed overall a significant increase in CSF L-arginine concentrations after the early phase and an increase in CSF L-ornithine and L-citrulline already early on (Mader et al., 2021). Interestingly, patients with higher early L-arginine concentrations demonstrated a better outcome. Around 10 days after ICH onset, increased L-ornithine was correlated with poor outcomes in the same ICH study. Similarly, elevated L-ornithine was also reported to be associated with poor outcomes in patients with SAH (Koch et al., 2021).

Changes in dimethylarginine concentrations

Increases in ADMA concentrations in the plasma have been described by several studies for ICH (Worthmann et al., 2017), SAH (Rodling-Wahlstrom et al., 2012; Bergström et al., 2014; Lindgren et al., 2014) and TBI (Wahlström et al., 2014). However, other studies did not detect elevations after ICH (Wanby et al., 2006; Mader et al., 2021) or TBI (Jeter et al., 2012) compared to control patients. Intrathecally, increases in ADMA concentrations were reported for ICH (Mader et al., 2021), SAH (Jung et al., 2007; Li et al., 2014), and pediatric

Table 1 | Key findings of selected clinical studies describing alterations of the L-arginine metabolism in patients suffering from ICH, SAH and TBI

Reference	Entity	Vascular compartment	Intrathecal compartment
Rashid et al., 2003	ICH	<ul style="list-style-type: none"> • Early reductions in nitrate/nitrite, L-arginine, L-citrulline and L-ornithine • L-arginine and L-citrulline associated with feeding status 	
Wanby et al., 2006	ICH	<ul style="list-style-type: none"> • Early decrease in L-arginine concentration but no change in dimethylarginine concentrations 	
Worthmann et al., 2017	ICH	<ul style="list-style-type: none"> • Elevation in ADMA levels • Dimethylarginines increased in patients with poor outcome 	
Mader et al., 2021	ICH	<ul style="list-style-type: none"> • Early reductions in L-citrulline, L-ornithine and L-Lysine • No increase in dimethylarginine levels 	<ul style="list-style-type: none"> • Persistent elevation of SDMA levels • Delayed increase in ADMA levels • Early increase in L-citrulline concentrations • Delayed accumulation of L-arginine and L-ornithine • Early reduction in L-arginine concentration was an independent risk factor for poor outcome
Jung et al., 2007	SAH		<ul style="list-style-type: none"> • Increased ADMA levels
Jung et al., 2012	SAH		<ul style="list-style-type: none"> • ADMA particularly elevated in patients with CVS • ADMA but not endothelin-1 concentration was correlated with CVS
Rodling-Wahlstrom et al., 2012	SAH	<ul style="list-style-type: none"> • ADMA increased during the first week and remained elevated 3 mon later 	
Staalso et al., 2013	SAH	<ul style="list-style-type: none"> • Low L-arginine/ADMA ratio associated with higher mortality 	
Bergström et al., 2014	SAH	<ul style="list-style-type: none"> • ADMA and L-arginine levels increased after the acute phase • Correlation of impaired endothelial function with L-arginine/ADMA ratio 	
Li et al., 2014	SAH		<ul style="list-style-type: none"> • ADMA increase at 3–5 d, peaking around 7–9 d, and remaining elevated until 12–14 d • Correlation of higher ADMA levels with CVS and poor outcomes
Lindgren et al., 2014	SAH	<ul style="list-style-type: none"> • Increased ADMA levels • Higher peak L-arginine/ADMA ratio associated with better outcome 	
Appel et al., 2018	SAH	<ul style="list-style-type: none"> • DCI and poor outcome associated with lower baseline L-arginine/ADMA ratio 	<ul style="list-style-type: none"> • Association of DCI with higher concentrations of ADMA and SDMA • Higher SDMA levels associated with DCI • SDMA and L-ornithine associated with poor outcomes
Hannemann et al., 2020	SAH	<ul style="list-style-type: none"> • Higher ADMA and SDMA levels associated with DCI 	
Koch et al., 2021	SAH		
Jeter et al., 2012	TBI	<ul style="list-style-type: none"> • L-arginine, L-citrulline and L-ornithine concentrations reduced in severe TBI • Dimethylarginine levels unaffected 	
Thampatty et al., 2013	TBI		<ul style="list-style-type: none"> • Increased early ADMA concentrations in a pediatric study population • Increase was attenuated by early hypothermia
Wahlström et al., 2014	TBI	<ul style="list-style-type: none"> • Early increase in ADMA concentrations 	

ADMA: Asymmetric dimethylarginine; CVS: cerebral vasospasm; DCI: delayed cerebral ischemia; ICH: intracerebral hemorrhage; SAH: subarachnoid hemorrhage; SDMA: symmetric dimethylarginine; TBI: traumatic brain injury.

TBI (Thampatty et al., 2013). For SAH, ADMA in the CSF was correlated with CVS or DCI in different studies (Jung et al., 2007, 2012; Li et al., 2014; Appel et al., 2018). In ICH, ADMA was found to be increased in patients with poor outcomes and hematoma enlargement in one study (Worthmann et al., 2017).

While SDMA was not elevated in the plasma after ICH (Wanby et al., 2006; Worthmann et al., 2017; Mader et al., 2021), an increase was observed in the CSF (Mader et al., 2021). Plasma SDMA was identified as a predictor for poor outcomes in ICH (Worthmann et al., 2017) but was not confirmed in a different ICH study (Mader et al., 2021). SDMA was also measured in SAH, demonstrating an association between CSF SDMA and DCI (Appel et al., 2018; Hannemann et al., 2020) as well as poor outcomes (Koch et al., 2021).

Pathophysiological considerations

In summary, the heterogeneity in study design and the non-comprehensive nature of data do not allow to point out specific common pathways between different entities with certainty. A common feature reported across different studies

is a reduction in L-arginine concentrations, often associated with an unfavorable clinical course (**Figure 2**). As previously stated, underlying pathophysiological consequences of a depletion of the NOS substrate L-arginine can be impairment of cerebral blood flow regulation (Cherian et al., 2004), oxidative stress via NOS uncoupling (Xia et al., 1996; Cherian et al., 2004) and an increased inflammatory reaction (Reynaert et al., 2004). The potentially detrimental role of elevated CSF L-ornithine described in SAH and ICH can be an indicator for increased arginase activity competing with NOS for L-arginine (**Figure 2**). Indeed, arginase has been reported to promote inflammation by decreasing the cellular content of NO resulting in an enhancement of the NF-κB pathway (Ckless et al., 2007) and to contributing to endothelial dysfunction in different diseases (Durante et al., 2007). In the context of experimental ischemic stroke, arginase inhibition via L-citrulline or L-ornithine administration demonstrated a neuroprotective effect (Barakat et al., 2018).

A detrimental association with intrathecal ADMA concentrations was found predominantly for SAH, where it correlated with CVS and DCI. Presumably, the underlying

mechanism is NOS inhibition by ADMA (Figure 2) leading to impairment of endothelial NO production (Böger, 2006) and potentially uncoupling of NOS (Böger et al., 2000; Wells and Holian, 2007). Explanations for the association of SDMA and DCI in SAH and potentially poor outcome in ICH are less well established. Possibly, SDMA could impair intracellular L-arginine concentrations by competition for hCAT-2B (Closs et al., 1997; Böger, 2006). Moreover, it has been demonstrated that SDMA can cause eNOS uncoupling as well (Feliens et al., 2015) and may be involved in proinflammatory pathways by activation of NF-κB and stimulation of reactive oxygen species production (Schepers et al., 2009, 2011).

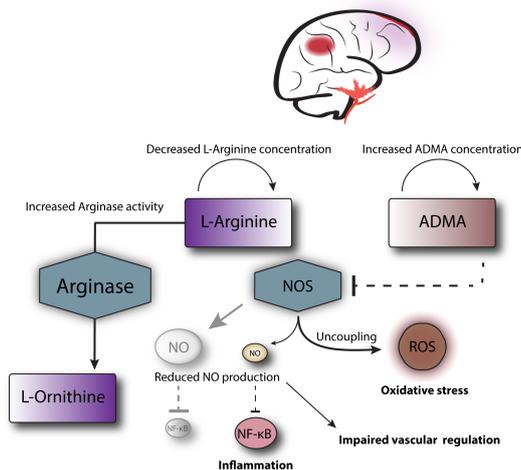


Figure 2 | Potential alterations of the L-arginine metabolism in neurocritical care patients and downstream pathophysiological consequences are illustrated.

ADMA: Asymmetric dimethylarginine; NF-κB: nuclear factor κB; NO: nitric oxide; NOS: nitric oxide synthase; ROS: reactive oxygen species.

Alterations in the L-arginine and NO metabolism may also impact the function of endothelial progenitor cells (EPC), as shown in the context of other diseases. In diabetes, uncoupling of eNOS has been shown to impair EPC function and mobilization (Thum et al., 2007). A decrease in EPC in a model of ischemia/reperfusion injury was demonstrated to be rescued by L-arginine administration (Hsieh et al., 2018).

Recent and Ongoing Therapeutic Clinical Trials

A deeper understanding of the role of L-arginine metabolism in acute neurological diseases has built the basis for the development of new therapeutic strategies (Table 2).

Table 2 | Clinical therapeutic phase 2/3 trials investigating study drugs related to L-arginine metabolism (not considering studies investigating AVP derivatives and pituitary hormones)

ClinicalTrials.gov Identifier	Disease	Phase	Study substance
NCT02012582	TBI	2a	VAS203 (Ronopterin)
NCT02794168	TBI	3	VAS203 (Ronopterin)
NCT03168581	ICH	2	CN-105 (Ac-VSRRR- NH2)
NCT03711903	ICH	2	CN-105 (Ac-VSRRR- NH2)

ClinicalTrials.gov Identifier	Study start	Status (February 2021)	Mechanism of action
NCT02012582	2009	Completed 2012	NOS inhibition
NCT02794168	2016	Completed 2020	NOS inhibition
NCT03168581	2017	Completed 2020	apoE signaling
NCT03711903	2019	Recruiting	apoE signaling

apoE: apolipoprotein E; AVP: arginine vasopressin; ICH: intracerebral hemorrhage; NOS: nitric oxide synthase; TBI: traumatic brain injury.

A study substance closely related to the NO/L-arginine pathway is VAS203 (Ronopterin), which is currently evaluated in the NOSTRA-III study (ClinicalTrials.gov Identifier: NCT02794168), a phase 3 clinical trial evaluating the efficacy of VAS203 in patients with moderate and severe TBI (Tegtmeier et al., 2020). VAS203, 4-amino-(6R,S)-5,6,7,8-tetrahydro-L-biopterin, acts as an inhibitor of NOS (Terpolilli et al., 2009). Notably, compared to other NOS inhibitors like L-arginine-methylester, VAS203 does not inhibit NOS at the L-arginine binding site but at the tetrahydrobiopterine cofactor binding site. This leads to a more NOS-specific binding pattern with a potentially improved side effect profile. The preclinical evaluation showed similar vasoconstrictive effects *in vitro* compared to L-arginine-methyl ester (Terpolilli et al., 2009). Intravenous administration after a controlled cortical impact in mice showed long-lasting prevention of ICP elevation or cerebral blood flow deterioration without affecting the mean arterial blood pressure. A significant improvement of the functional neurological status was observed at 6 days after trauma. VAS203 was shown to partially prevent posttraumatic arteriolar vasodilation suggesting inhibition of excess endothelial NO production as the underlying mechanism of action (Schwarzmaier et al., 2015). Based on these promising preclinical findings, a clinical phase 2a trial, NOSTRA, was conducted (ClinicalTrials.gov Identifier: NCT02012582) (Stover et al., 2014). A total of 32 patients from six European centers were included. VAS203 showed an overall good safety profile except for an association with acute kidney injury in patients treated with the highest dose. No significant effect was observed for intracranial pressure, cerebral perfusion pressure or partial brain oxygen pressure compared to placebo-treated control patients. Nitrate levels were measured via microdialysis but showed high variability and no effect between treatment groups was discernible. Therapy intensity levels increased continuously in the placebo group during the observation period whereas a decrease was observed in the treatment group. Functional neurological outcome after 6 months was improved in VAS203 treated patients compared to the placebo group. The NOSTRA-III study aims to confirm the positive effect on neurological outcomes (Tegtmeier et al., 2020). The actual study completion date was June 30, 2020, and the study has completed multicentric recruitment in Europe with 224 participants.

A second study substance identified from searching ClinicalTrials.gov is CN-105, which is a cationic arginine-rich peptide, a novel class of peptides with potential neuroprotective properties (Edwards et al., 2020). It is currently evaluated for therapeutic use in patients suffering from ICH in two phase 2 clinical trials. The S-CATCH study (ClinicalTrials.gov Identifier: NCT03711903) is currently actively recruiting while the CATCH study (ClinicalTrials.gov Identifier: NCT03168581) has enrolled 38 participants with January 25, 2020, as the actual study completion date. Notably, CN-105 (Ac-VSRRR-amide) is an apolipoprotein E-mimetic peptide assumed to mediate anti-inflammatory and neuroprotective responses via apolipoprotein E signaling (Lei et al., 2016; Guptill et al., 2017; Wang et al., 2021). It is therefore demonstrating a mechanism distinct from the actual L-arginine metabolism primarily addressed in this review. A preclinical study in a murine model of ICH was able to show a decrease in neuroinflammation paired with improved vestibulomotor and neurocognitive performance (Lei et al., 2016). A phase 1 clinical trial confirmed safety in human subjects (Guptill et al., 2017). Preclinical studies in models of TBI and SAH suggested a reduction in neuroinflammation and improved functional outcomes as well (Laskowitz et al., 2017; Liu et al., 2018).

Conclusions

The metabolism of L-arginine is directly related to the production of NO via NOS and metabolic alterations can lead to pathological sequels in acute neurological diseases. The most common critical neurological conditions requiring treatment on a neurocritical care unit are ICH, aneurysmal SAH and TBI. Despite very distinct underlying mechanisms of injury, pathophysiological mechanisms and clinical courses, alterations of the L-arginine metabolism are a common hallmark and appear to be associated with secondary brain injury and/or complications like DCI. Metabolic changes are complex due to specificity for cell type and location, enzyme subtype, timepoint after disease onset, underlying pathology, clinical features and severity of disease, as well as exogenous factors. A common pathological feature between the different diseases observed in several studies is the development of an absolute or relative L-arginine deficiency potentially associated with poor outcomes. Inhibition of NOS by the endogenous metabolite ADMA has been implicated to have detrimental effects particularly in the context of vasospasm after aneurysmal SAH. In contrast, NOS inhibition with the exogenous agent VAS203 showed promising results in a phase 2 clinical trial in TBI patients. Overall, L-arginine and NO metabolism have been identified as relevant pathways in the pathophysiological response in different neurocritical conditions. Future studies may contribute to a more detailed spatiotemporal understanding and help to identify novel therapeutic targets.

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