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# REVIEW

# Recent advances in arginine metabolism: roles and regulation of the arginases

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As arginine can serve as precursor to a wide range of compounds, including nitric oxide, creatine, urea, polyamines, proline, glutamate and agmatine, there is considerable interest in elucidating mechanisms underlying regulation of its metabolism. It is now becoming apparent that the two isoforms of arginase in mammals play key roles in regulation of most aspects of arginine metabolism in health and disease. In particular, work over the past several years has focused on the roles and regulation of the arginases in vascular disease, pulmonary disease, infectious disease, immune cell function and cancer. As most of these topics have been considered in recent review articles, this review will focus more closely on results of recent studies on expression of the arginases in endothelial and vascular smooth muscle cells, post-translational modulation of arginase activity and applications of arginase inhibitors *in vivo*.
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**Keywords:** arginase; arginase inhibitors; arginine; cancer; endothelial; genetic knockout; nitric oxide; polyamines; vascular

**Abbreviations:** ABH, 2(S)-amino-6-boronohexanoic acid; ADMA, asymmetric dimethyl-L-arginine; BEC, S-(2-boronoethyl)-L-cysteine; CAT-1, cationic amino acid transporter-1; DDAH, dimethylarginine dimethylaminohydrolase; DFMO, α-difluoromethylornithine; iNOS, inducible isoform of NOS; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NMMA, $N^G$-monomethyl-L-arginine; NO, nitric oxide; NOHA, $N^G$-hydroxy-L-arginine; nor-NOHA, $N^\omega$-hydroxy-nor-L-arginine; NOS, nitric oxide synthase; SDMA, symmetric dimethyl-L-arginine; shRNA, short hairpin RNA; TNFα, tumour necrosis factor-α

## Introduction

There is widespread interest in arginine because it is involved in multiple metabolic processes that play important roles in a very wide range of physiological and pathophysiological conditions. Although the roles of arginine as an intermediate in the urea cycle and as a precursor in creatine biosynthesis have been familiar to biochemists for many years, there was an explosion of interest in this amino acid beginning 20 years ago, stemming from the recognition that arginine is the source of the nitrogen atom in the biosynthesis of nitric oxide (NO) (Hibbs *et al.*, 1988; Palmer *et al.*, 1988). As most readers are probably well aware of, this discovery has resulted in the unintended and unwelcome consequence of an ongoing flood of spam emails advertising arginine supplements, primarily involving exaggerated claims for enhancing sexual performance.

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The NO-centric view of arginine has been so predominant that the complexities of arginine metabolism can be easily overlooked. Briefly, the free arginine pool is derived from diet, endogenous synthesis and turnover of cellular protein (Figure 1) (Morris, 2004; Wu and Morris, 2004). Endogenous synthesis is comprised of de novo synthesis from the carbon skeleton of glutamine and of the enzymatic recycling of citrulline produced in reactions catalyzed by the NO synthase (NOS) and dimethylarginine dimethylaminohydrolase (DDAH) enzymes. Despite erroneous claims in some biochemistry textbooks, the urea cycle accounts for very little or no net synthesis of arginine. In healthy adults, endogenous synthesis provides sufficient arginine that it is not required in the diet, but it cannot provide enough arginine to meet metabolic requirements in certain conditions, including inflammation, dysfunction of small bowel or kidney, or in neonates and premature infants. Thus, arginine is classified as a semi-essential or conditionally essential amino acid (Abumrad and Barbul, 2004; Flynn *et al.*, 2002).

Arginine is used as substrate by five sets of enzymes, leading to its incorporation or transformation into the products shown in Figure 1. Interestingly, charged $tRNA^{Arg}$ is required not only for protein synthesis but also plays a role in protein

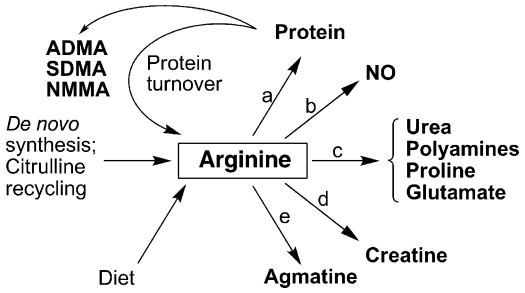


**Figure 1** Overview of mammalian arginine metabolism. Sources of the free arginine pool are indicated, as well as the various end products of arginine metabolism. In addition to arginine, the methylated arginine derivatives asymmetric dimethyl-L-arginine (ADMA), symmetric dimethyl-L-arginine (SDMA) and $N^G$-monomethyl-L-arginine (NMMA) are released upon the turnover of post-translationally methylated proteins. Enzymes that use arginine as substrate are: (a) arginyl-tRNA synthetase; (b) NO synthases; (c) arginases; (d) arginine:glycine amidinotransferase; and (e) arginine decarboxylase. Not shown are the various transporters that are required for movement of arginine across plasma and mitochondrial membranes.

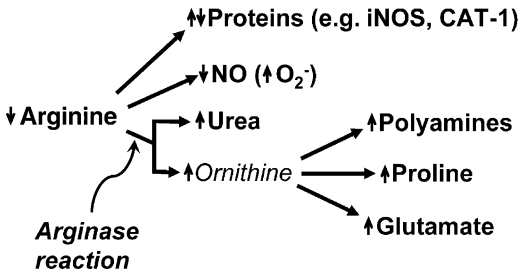


**Figure 2** Consequences of increased arginase activity. Up and down arrows indicate increases or decreases in amount or rate of synthesis of the indicated biochemical products. Depending on the specific protein, reduced arginine availability may result in either decreased translational efficiency (e.g. iNOS) or increased translational efficiency (e.g. CAT-1) of the corresponding mRNA (reviewed in Morris, 2007).

degradation. Transfer of the arginine from the charged tRNA to N-terminal Asp, Glu or Cys of proteins by arginyltransferase targets the modified proteins for degradation by the N-end rule pathway (Tasaki and Kwon, 2007). The greatest diversity of end products arises from catabolism of arginine by arginase (Figure 1). The hydrolysis of arginine by arginase produces urea and ornithine, which in turn can serve as precursor for synthesis of polyamines, proline or glutamate. Thus, in some cells it is arginase rather than ornithine decarboxylase that is limiting for polyamine synthesis (Li *et al.*, 2001; 2002; Wei *et al.*, 2001). Moreover, there are significant secondary consequences of reductions in the availability of arginine due to its catabolism by arginase, including reductions in the NO synthesis [and increased superoxide production by NOS when arginine levels are sufficiently low (Xia *et al.*, 1996)] and either increases or decreases in expression of specific proteins [e.g. the cationic amino acid transporter CAT-1 and inducible isoform of NOS (iNOS), respectively] (Figure 2) (Morris, 2006). Consideration of the $K_m$ and $V_{max}$ values for NOS and the arginases indicates that the arginases can effectively compete with NOS for arginine under physiological conditions (Wu and Morris, 1998; Santhanam *et al.*, 2008). The complexity of the outcomes shown in Figure 2 poses a considerable challenge to understanding the precise roles of the arginases in specific organs and cell types in health and disease.

Adding to this complexity is the fact that two arginase isoforms are expressed in mammals: arginase I (cytosolic) and arginase II (mitochondrial) (Jenkinson *et al.*, 1996). These isoforms are the products of distinct genes located on different chromosomes. The arginase genes are regulated independently, can be expressed in many different cell types and are inducible by a wide range of agents and in many pathophysiological conditions (Mori and Gotoh, 2000; Morris, 2000; Mori, 2007). In the recent years the arginases have become implicated in many disease processes, including various types of vascular disease, pulmonary disease, infectious disease, immune cell dysfunction and cancer (Bansal and Ochoa, 2003; Kim *et al.*, 2004; Lange *et al.*, 2004; Mori and Gotoh, 2004; Bronte and Zanovello, 2005; Maarsingh *et al.*, 2008; Rodriguez and Ochoa, 2008). As it is not possible to consider all these topics here, this article will focus primarily on selected recent findings, with emphasis on expression of the arginases in endothelial and vascular smooth muscle cells, post-translational modulation of arginase activity and applications of arginase inhibitors *in vivo*. Various aspects of functional roles of the arginases in vascular biology, including atherosclerosis, have been reviewed recently and thus will not be considered here (Morris, 2005; Huynh and Chin-Dusting, 2006; Yang and Ming, 2006; Durante *et al.*, 2007; Santhanam *et al.*, 2008).

## Arginases in vascular smooth muscle cells

As described in the following section, immunohistochemical studies have indicated that most of the arginases in blood vessels are present in the endothelium. However, vascular smooth muscle cells do express low levels of arginase, and arginase expression in these cells can be induced by interleukin-4 (IL-4) and IL-13 (Wei *et al.*, 2000), transforming growth factor-β (Durante *et al.*, 2001), lysophosphatidycholine (Durante *et al.*, 1997) and mechanical strain (Durante *et al.*, 2000). Rather than modulating NO synthesis, arginase in vascular smooth muscle cells appears to function primarily to enhance synthesis of polyamines and proline for cell proliferation and collagen synthesis, respectively (Durante *et al.*, 2007). Thus, elevated arginase expression in vascular smooth muscle cells may be an important factor in development of intimal hyperplasia and vascular stiffness in response to injury or during ageing. In proof-of-principle experiments, simply overexpressing arginase I in cultured vascular smooth muscle cells resulted in increased polyamine synthesis and cell proliferation (Wei *et al.*, 2001), supporting the notion that elevated arginase expression may play a role in the initiation or progression of intimal hyperplasia following vascular injury (Marinova *et al.*, 2008).

## Arginase expression in endothelial cells

Arginase is constitutively expressed in endothelial cells, but the expression of the specific isoforms differs among

**Table 1** Arginase isoforms detected in endothelial cells of various mammalian species

| *Species* | *Arginase isoform* | *References* |
|---|---|---|
| Human | I and II | (Bachetti *et al.*, 2004; Xu *et al.*, 2004; Marinova *et al.*, 2008) |
| | II | (Ryoo *et al.*, 2006; Topal *et al.*, 2006; Yang *et al.*, 2006a) |
| Rat | I | (Lewis *et al.*, 2008) |
| | I and II | (Buga *et al.*, 1996; Berkowitz *et al.*, 2003; Suschek *et al.*, 2003; Johnson *et al.*, 2005) |
| Mouse | I | (Bivalacqua *et al.*, 2007; Gao *et al.*, 2007) |
| | II | (Lim *et al.*, 2007) |
| Pig | I | (Zhang *et al.*, 2001; 2004; Hein *et al.*, 2003; Thengchaisri *et al.*, 2006) |
| | II | (Lee *et al.*, 2007; Zharikov *et al.*, 2008) |
| Cow | I | (Stanley *et al.*, 2006; Romero *et al.*, 2008) |
| | I and II | (Chicoine *et al.*, 2004; Nelin *et al.*, 2005) |

Because of differences in the sensitivity and specificity of the various detection methods used (Western blot, immunohistochemistry, real-time polymerase chain reaction), the report of only one arginase isoform in a particular study does not necessarily indicate that the other isoform is not also expressed. Some differences in patterns of isoform expression listed here also may reflect characteristics of endothelial cells isolated from different vascular beds, disease conditions or variable contamination with vascular smooth muscle cells.

mammalian species (Table 1). Although some arginase I has been detected in human endothelial cells, the predominant isoform is arginase II. There is strong evidence that constitutive levels of arginase activity in endothelial cells limit NO synthesis and NO-dependent vasodilatory function (e.g. Zhang *et al.*, 2001; Berkowitz *et al.*, 2003; Lim *et al.*, 2007). A wide range of agents can induce arginase expression when administered to cultured endothelial cells, including lipopolysaccharide (LPS) (Buga *et al.*, 1996), tumour necrosis factor-α (TNFα) (Gao *et al.*, 2007), LPS + TNFα (Bachetti *et al.*, 2004; Chicoine *et al.*, 2004; Nelin *et al.*, 2005), thrombin (Ming *et al.*, 2004; Topal *et al.*, 2006; Yang *et al.*, 2006a; Lewis *et al.*, 2008), high glucose (Romero *et al.*, 2008), oxidized low-density lipoprotein (LDL) (Ryoo *et al.*, 2006) and $H_2O_2$ (Thengchaisri *et al.*, 2006). Conditions that result in elevated endothelial arginase expression *in vivo* include hypertension (Zhang *et al.*, 2004; Johnson *et al.*, 2005), ischaemia–reperfusion (Hein *et al.*, 2003), intimal hyperplasia (Loyaga-Rendon *et al.*, 2005) and ageing (Berkowitz *et al.*, 2003). Other than effects of plant compounds such as genistein (Nelin *et al.*, 2005) and cocoa flavanols (Schnorr *et al.*, 2008) or simvastatin (Romero *et al.*, 2008), there is little information regarding agents that suppress arginase expression or prevent its induction in endothelial cells. Arginase activity is greatly diminished in endothelial cells of the spontaneously diabetic BB rat, compared with cells from the non-diabetic BB strain (Wu and Meininger, 1995); the arginase isoform(s) in these cells were not identified. Seemingly in contrast, arginase activity and arginase I expression were elevated in aortas of streptozotocin diabetic rats (Romero *et al.*, 2008), but it was not determined whether the increased expression occurred in the endothelium and/or vascular smooth muscle. The latter group also found that incubation of bovine coronary endothelial cells with high (25 mM) glucose for 24 h resulted in increased arginase activity without increasing arginase I protein levels (Romero *et al.*, 2008), indicating an increase in catalytic efficiency of the arginase (see also the following section). Additional studies are required to elucidate the basis for the apparent discrepancies in results of these two studies.

Model studies have demonstrated that simply overexpressing either arginase I or II in endothelial cells may not only reduce NO synthesis but also can enhance polyamine synthesis and cell proliferation as well as proline synthesis (Li *et al.*, 2001; 2002). From the discussion in this section it is apparent that the precise consequences of arginase expression in endothelial cells will depend upon the animal species, the relative activities of enzymes such as NOS and enzymes involved in ornithine metabolism, whose expression in turn will depend on the particular vascular bed in which the endothelial cells are located and the nature of the various stimuli to which these cells are exposed.

## Post-translational modulation of arginase activity

Although it has not been rigorously evaluated in most cases, arginase activity usually appears to be proportional to the amount of arginase protein, which, in turn, is determined primarily by transcription of the arginase genes. However, several recent studies have reported distinct mechanisms by which arginase activity can be modulated independently of changes in amount of arginase protein. Santhanam *et al.* reported that cysteine residues 168 and 303 in arginase I can undergo S-nitrosylation (Santhanam *et al.*, 2007). In particular, S-nitrosylation of Cys303 stabilizes the arginase trimer, resulting in a sixfold decrease in its $K_m$ for arginine. Cys168 is conserved in mammalian arginases I and II, but there is no cysteine in mammalian arginase II that corresponds to Cys303 in arginase I, suggesting that S-nitrosylation may not alter the activity of arginase II. Thus, this mechanism of modulating arginase activity may not occur in human endothelial cells, which express arginase II but little or no arginase I (Table 1). These authors also presented evidence for S-nitrosylation of arginase I in blood vessels of ageing rats *in vivo*, likely as a consequence of the activity of iNOS. They therefore suggested that this increase in arginase I activity may contribute to endothelial dysfunction in ageing. This appears somewhat paradoxical in view of earlier studies showing that $N^G$-hydroxy-L-arginine (NOHA), an intermediate in NO synthesis and a potent endogenous inhibitor of arginases, can accumulate sufficiently in iNOS-expressing cells to inhibit arginase activity (Buga *et al.*, 1996). Moreover, as arginase II also may be expressed in endothelial cells, depending on species, additional studies will be needed to determine the relative contributions of arginase I and II to endothelial dysfunction. It will be important also to characterize conditions that promote or reverse S-nitrosylation of arginase I, as well as to identify additional physiological or pathophysiological conditions in which arginase activity is regulated by this mechanism.

Following up on observations that elevated serum levels of uric acid are often associated with pulmonary hypertension, Zharikov *et al.* found that uric acid inhibited NO production by activated pulmonary artery endothelial cells and that

this inhibition could be overcome by an arginase inhibitor (Zharikov *et al.*, 2008). Further studies demonstrated that uric acid activated arginase by reducing its $K_m$ for arginine. This effect was seen for arginase activity in lysates of endothelial cells, rat liver and rat kidney, indicating that uric acid has a similar effect on both arginase I and II. As elevated serum levels of uric acid are associated also with pre-eclampsia (Bainbridge and Roberts, 2008; Kenny *et al.*, 2008), these results raise the possibility that uric acid-dependent activation of arginase may play a role in hypertension in this disorder.

Finally, *in vitro* experiments using human erythrocyte lysates or arginase preparations from bovine liver have provided evidence that arginase activity at physiological pH can be enhanced by hydroxyl radicals produced by the Fenton reaction; arginase activity in both these samples is due to arginase I (Iyamu *et al.*, 2008). The precise mechanism underlying this effect has not been identified. These authors speculate that this activation of arginase may contribute to impaired NO homeostasis under conditions of oxidative stress. Although additional studies are needed to determine whether there is evidence for this mechanism of arginase activation in intact cells and to determine whether it applies also to arginase II, it is intriguing to note that $H_2O_2$, which can be converted to hydroxyl radical, was found to induce arginase I expression in porcine coronary arterioles (Thengchaisri *et al.*, 2006). However, arginase activity was not measured in this study, so it was not possible to ascertain whether there was any increase in arginase activity independent of increases in the amount of arginase protein.

## Inhibition of arginase activity or expression *in vivo*

Several potent arginase inhibitors have been developed and become commercially available within the past few years (Figure 3). Older inhibitors had low potency and thus were more likely to have side effects because of the high concentrations required. For example, norvaline, which has sometimes been used as an arginase inhibitor, is a good substrate for branched chain aminotransferases (Davoodi *et al.*, 1998). NOHA, which is an intermediate in NO synthesis, is a potent endogenous inhibitor of the arginases (Boucher *et al.*, 1994; Daghigh *et al.*, 1994; Buga *et al.*, 1996). However, its utility as an arginase inhibitor is complicated by the fact that it also is a precursor for synthesis of NO not only by NOS enzymes but also by cytochrome P450 enzymes (Boucher *et al.*, 1992). α-Difluoromethylornithine (DFMO) has been used as a nonspecific arginase inhibitor for *in vitro* (e.g. Ming *et al.*, 2004; Santhanam *et al.*, 2007; Lewis *et al.*, 2008) and *in vivo* experiments (Demougeot *et al.*, 2005), although an effect of DFMO on arginase activity was not directly evaluated in most studies. It is important to note that DFMO is a very poor inhibitor of arginase ($K_i$ = 3.9 ± 1.0 mM) (Selamnia *et al.*, 1998), whereas it is well-established as a potent irreversible inhibitor of ornithine decarboxylase (Metcalf *et al.*, 1978). As concentrations of DFMO required to significantly inhibit arginase activity in intact cells are very high (e.g. 10 mM) (Selamnia *et al.*, 1998), there are concerns about nonspecific effects of such high concentrations, as well as concerns about effects of inhibiting polyamine synthesis. As arginase is subject to inhibition by its product ornithine (Hunter and Downs, 1945; Selamnia *et al.*, 1998), observations that DFMO concentrations in the micromolar range can enhance NO-dependent vascular responses may be secondary to intracellular accumulation of ornithine due to the DFMO inhibition of ornithine decarboxylase. Because of these considerations, use of DFMO as an arginase inhibitor will not be discussed in detail here.

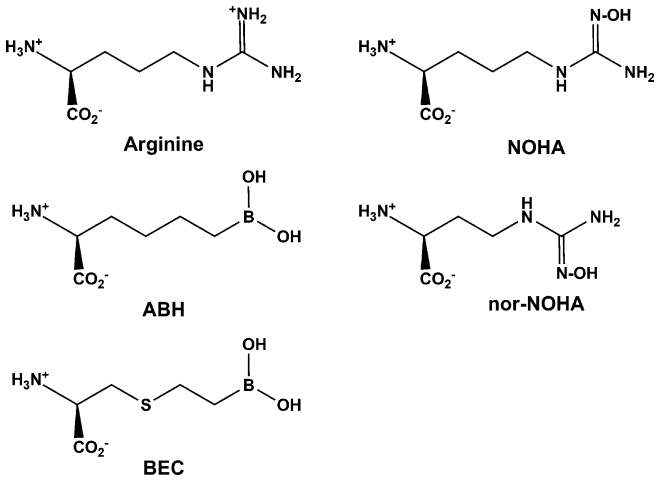


**Figure 3** Structures of arginine and arginase inhibitors. Values of $K_i$ for these inhibitors are in the sub-micromolar to micromolar range (Christianson, 2005). ABH, 2(*S*)-amino-6-boronohexanoic acid; BEC, S-(2-boronoethyl)-L-cysteine; NOHA, $N^G$-hydroxy-L-arginine; nor-NOHA, $N^{\omega}$-hydroxyl-nor-L-arginine.

The arginase inhibitors shown in Figure 3 are competitive inhibitors and do not inhibit the NOS enzymes at concentrations that inhibit the arginases. These inhibitors and their interactions with active site residues of arginase have been reviewed elsewhere (Christianson, 2005). Unfortunately, none of these inhibitors exhibit sufficient differences in affinity for the two arginase isoforms to provide isoform-selective inhibition in cultured cells or *in vivo*. Comparative studies using intact cells or tissues to systematically evaluate effects of multiple arginase inhibitors have been lacking. The exception is a recent study that used isolated rat aorta and mesenteric arteries to compare vascular effects of several arginase inhibitors (Huynh *et al.*, 2009). These investigators found that only S-(2-boronoethyl)-L-cysteine (BEC) and NOHA – but not $N^{\omega}$-hydroxy-nor-L-arginine (nor-NOHA), L-valine, DFMO or norvaline – significantly reversed vascular tolerance to acetylcholine, suggesting increases in NO bioavailability. Of concern was the finding that BEC and NOHA also elicited endothelium-independent vasorelaxation in rat aorta, implying that these compounds may have cellular targets in addition to the arginases. It should be noted, however, that neither arginase activities nor NO production were directly measured in this study.

In addition to their application in numerous studies using isolated tissues or cultured cells, arginase inhibitors have been applied *in vivo* to evaluate their effects on allergen-induced airway hyper-responsiveness and inflammation (Ckless *et al.*, 2008; Maarsingh *et al.*, 2008), liver ischaemia/reperfusion injury (Reid *et al.*, 2007; Jeyabalan *et al.*, 2008), experimental

autoimmune encephalitis (Xu *et al.*, 2003) and erectile function (Bivalacqua *et al.*, 2007). Dosages and duration of treatment are provided in Table 2. In general, administration of the inhibitors resulted in improvement of pathological features, usually ascribed to increased NO production and activity of NO-dependent processes. However, inflammation was unexpectedly increased in one model of allergic inflammation (Ckless *et al.*, 2008) but decreased in another model (Maarsingh *et al.*, 2008). Additional studies are required to determine whether the different outcomes reflect differences in species, the specific arginase inhibitor used or route of administration. Because there are no assays to evaluate arginase activity *in situ*, evaluation of arginase inhibition *in vivo* is indirect. So far, there has been no systematic study to identify possible off-target effects of arginase inhibitors *in vivo*, nor is there published data on pharmacokinetics of the arginase inhibitors. This information is essential for evaluating the therapeutic potential of these compounds. Cost can become a significant factor in experimental studies of the arginase inhibitors *in vivo*. Based on October 2008 catalog prices from a company that sells all the arginase inhibitors, 50 mg of the inhibitors would cost $720 (nor-NOHA), $1300 (BEC) or $2400 (ABH).

Localized effects of arginase inhibitors on vascular function in humans have been carried out using microdialysis. Administration of a combination of BEC and nor-NOHA resulted in augmented reflex vasodilation in skin of hypertensive subjects but not in age-matched normotensive subjects (Holowatz and Kenney, 2007). These authors speculated that arginase activity was elevated in skin of the hypertensive individuals, thus limiting availability of arginine for NO synthesis; it should be noted, however, that arginase activity, urea production or NO production were not measured in this study.

As currently available arginase inhibitors are not isoform-selective, RNA interference has been used to inhibit expression of specific arginase isoforms. Two studies employing this technique to selectively inhibit arginase expression *in vivo* have been reported. In one study plasmids encoding short hairpin RNA (shRNA) directed against arginase I were administered to mice by intratracheal instillation, followed by similar administration of IL-13 to induce airway hyperresponsiveness (Yang *et al.*, 2006b). Administration of shRNA directed against arginase I resulted in greatly reduced expression arginase I in the lung at the mRNA and protein levels, with no effect on expression of arginase II, and also significantly attenuated IL-13-induced airway hyper-responsiveness. Although the impact of shRNA on total arginase activity was not determined, the results clearly indicated a specific role for arginase I underlying the induction of airway hyperresponsiveness in this model. Another group used adeno-associated virus to deliver antisense RNA to arginase I in order to evaluate the role of this arginase in erectile function of mice (Bivalacqua *et al.*, 2007). This group had shown that arginase I expression was elevated in penis of aged mice, compared with young mice, and correlated with erectile dysfunction and inhibition of NO-dependent processes. Virus was injected directly into the corpus cavernosum, and arginase I expression and erectile function were evaluated 30 days later. Arginase I expression was decreased, cGMP concentra-

**Table 2** Studies that have used arginase inhibitors in vivo

| *Species (strain)* | *Inhibitor* | | *Dose and route of administration* | *Duration* | *Equivalent dose/day in 70-kg human* | *References* |
|---|---|---|---|---|---|---|
| Mouse (ApoE$^{-/-}$) | ABH | A | ~200 μg/day; in drinking water | 14 days | ~0.7 g | (Ryoo *et al.*, 2008) |
| Mouse (B6/129) | ABH | B | 3.2 μg/kg; intracavernosal | 20 min | 224 μg | (Bivalacqua *et al.*, 2007) |
| Mouse (C57BL/6) | ABH | B | 0.7 mg/day; i.p. | 23 days | ~2.4 g | (Xu *et al.*, 2003) |
| Mouse (C57BL/6) | nor-NOHA | B | 2 × 100 mg/kg; i.v. | ≤24 h | 14 g | (Jeyabalan *et al.*, 2008) |
| Mouse (C57BL/6) | nor-NOHA | B | 100 μg/day; i.p. | 14 days | ~0.35 g | (Bratt *et al.*, 2009) |
| Rat (Lewis) | nor-NOHA | B | 2 × 100 mg/kg; i.v. | ≤24 h | 14 g | (Reid *et al.*, 2007) |
| Rat (SHR) | nor-NOHA | B | 10 or 40 mg/kg/day; i.p. | 21 days | 0.7 or 2.8 g | (Bagnost *et al.*, 2008) |
| Mouse (BALB/c) | BEC | C | 2.3 μg; single aspiration | 48 h | ~8 mg | (Ckless *et al.*, 2008) |
| Guinea pig (Dunkin Hartley) | ABH | C | 25 mmol·L$^{-1}$ in aerosol; 1–3 × 15 min inhalation | ≤24 h | unknown | (Maarsingh *et al.*, 2008) |

A, oral; ABH, 2(S)-amino-6-boronohexanoic acid; B, injection; BEC, S-(2-boronoethyl)-L-cysteine; C, inhalation; nor-NOHA, N$^{\omega}$-hydroxy-nor-L-arginine;

tion increased and erectile responses improved in mice that received virus encoding antisense sequence to arginase I, whereas a control virus encoding beta-galactosidase had no effect on these parameters.

## Mouse strains with genetic ablation of arginase expression

Mouse strains carrying genetic knockouts of each of the arginases have been developed. Mice with homozygous deletion of arginase I expression (Arg $I^{-/-}$) die in the perinatal period, apparently as a consequence of a nonfunctional urea cycle (Iyer *et al.*, 2002). In contrast, mice with homozygous deletion of arginase II expression (Arg $II^{-/-}$) are viable and exhibit mild phenotypic changes, such as elevated plasma levels of arginine (Shi *et al.*, 2001). The elevated plasma levels of arginine are consistent with earlier work indicating that arginine catabolism plays a significant role in regulating arginine homeostasis (Castillo *et al.*, 1993). Studies using the arginase deletion strains have documented changes in tissue levels of polyamines, amino acids and guanidino compounds (Deignan *et al.*, 2006; 2007; 2008). The arginase deletion strains also are being utilized to evaluate physiological functions of the arginases. For example, pressurized carotid arteries from Arg $II^{-/-}$ mice exhibited an enhanced vasorelaxation response to acetylcholine and a reduced response to phenylephrine, compared with carotid arteries from wild-type mice, indicating that endothelial arginase II normally limits arginine availability to endothelial NOS (Lim *et al.*, 2007). This same group also found that when the Arg $II^{-/-}$ strain was crossed into the ApoE deletion strain ($ApoE^{-/-}$), there was a reduction in development of atherosclerotic plaque in the double deletion strain, relative to the $ApoE^{-/-}$ strain when fed a high cholesterol diet (Ryoo *et al.*, 2008). The diet-induced decreases in vascular NO synthesis and increases in endothelial production of reactive oxygen species also were less pronounced in the double deletion strain.

Studies also have been conducted using cells or tissue isolated from the arginase knockout mice. For example, ablation of arginase I expression was found to result in increased proliferation of neural stem cells isolated from embryonic and newborn mice (Becker-Catania *et al.*, 2006). This result was unexpected in view of earlier work supporting the notion that arginase expression can be limiting for polyamine synthesis and cell proliferation (Holtta and Pohjanpelto, 1982; Singh *et al.*, 2000; Li *et al.*, 2001; 2002; Wei *et al.*, 2001). Microarray and real-time polymerase chain reaction analyses of RNA from neural stem cells isolated from the arginase I knockout mice demonstrated altered expression of several genes, some of which may be involved in an alternate pathway of polyamine synthesis (Becker-Catania *et al.*, 2006). Importantly, these changes in expression of other genes illustrate the difficulties in interpreting the metabolic and physiological consequences of ablating expression of specific genes. Without additional experimental data, it is unclear whether the increased proliferation rate of the neural stem cells from the arginase I knockout mice is due to the polyamine synthesis via an alternate biosynthetic pathway, to increased uptake of polyamines from the culture medium, or by the altered expression of other genes involved in cell proliferation. Similarly, ablation of arginase II expression in a mouse model of prostate cancer resulted in more aggressive tumour growth and development, again suggesting that the alternate pathways of polyamine biosynthesis or uptake may have become more active in the cancer cells in the absence of arginase II expression (Mumenthaler *et al.*, 2008).

Given the lack of isoform-specific arginase inhibitors and the limitations of studying mice with global knockout of arginase expression, more precise elucidation of the function of the arginases *in vivo* will require mouse strains with conditional knockout of arginase isoform expression in specific cell types. This is particularly important in the case of arginase I because mice homozygous for deletion of arginase I die in the perinatal period (Iyer *et al.*, 2002). Thus, mouse strains with ablation of arginase I expression in macrophages have recently been developed to demonstrate the role of this enzyme in modulating host defense against intracellular pathogens (El Kasmi *et al.*, 2008). We anticipate that additional strains will be developed to address the role of arginase I in other cell types.

## Conclusions

It is now well-appreciated that expression of the arginases is highly regulated in many tissues and cell types and that changes in their activity can have a significant impact on a variety of critical physiological and pathophysiological processes. The application of potent new arginase inhibitors and recombinant DNA tools to selectively inhibit arginase expression is beginning to yield new insights into the roles of the arginases in isolated cells and tissues and in animal models. As indicated by this review, much attention is being devoted to evaluating the roles and regulation of the arginases in the function of vascular cells. However, elucidation of their roles in human health and disease is complicated by the fact that the patterns of arginase expression in animal cells do not always accurately represent the patterns of expression in corresponding human cell types. As discussed in more detail elsewhere (Morris, 2007), we also should not overlook the fact that a more complete understanding of arginine metabolism and the roles of the arginases will require greater characterization of the roles and regulation of the many other enzymes and transporters involved in determining the metabolic fates of arginine. Thus, there are many important questions yet to be addressed by talented investigators.

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## Conflict of interest

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