# Hydrogen sulfide: in the aftermath of argininosuccinate lyase and nitric oxide deficiency

**To the Editor**: It is with interest that we read the the article titled "Argininosuccinate Lyase Deficiency" by Nagamani et al.,[1] a GeneTest Review that dealt with argininosuccinate lyase deficiency (ASLD) and its metabolic and clinical implications. The deranged nitric oxide (NO) milieu is a likely predecessor for several of the systemic manifestations and long-term presentations of this inherited urea cycle disorder. Although this calls for a therapeutic intervention with NO donors in the symptomatic management of endogenous nitric oxide depletion,[2] there are some potential drawbacks. Classical NO donors have been in the market for more than five decades.[3] In addition to known contraindications and adverse profiles, their prolonged use has attendant drug tolerance and clinical inefficacy. The variability of median effective dose for different therapeutic benefits also makes it difficult to customize the dosage regimen when wider systemic effects are intended—even newer NO donors designed with tissue specificity have not surpassed the conventional agents in their pharmacokinetic or dynamic characteristics.[3] Obviously, the cellular production of NO through NOS is dependent on the availability of the precursor, L-arginine, which is compromised in ASLD. An impromptu alternative is a non-enzymatic mode of NO generation from endogenous nitrite, which is activated or sustained under specific disease states with reduced (acidic) physiological pH.[4] Although there is limited utilization of exogenous arginine for the NO pathway in ASL,[2] it may open up another window of indirect physiological activity by increasing the systemic levels of hydrogen sulfide ($H_2S$).[4]

Studies of reciprocity between the two signaling systems (NO and $H_2S$) have shown over 10-fold accentuation of $H_2S$-mediated effects by NO donors.[5] Recently, scientific evidence has unfolded a multitude of regulatory roles for $H_2S$ (similar to NO), thereby accounting for a shift in our earlier perspective of this "toxic pollutant."[6] Coexistent with NO and sharing functions, $H_2S$ is endogenously generated in tissues that are targets of its physiological actions as a direct vasorelaxant or as a neuromodulator. Reports on the delicate nature of cross-talk also include $H_2S$-mediated NO production by an Akt-dependent mechanism[7] and formation of sulfinyl nitrite (an NO donor) as a reaction byproduct with peroxinitrite.[8] Hence, the endogenous $H_2S$ pathway can be a likely contender for the manifestations of the arginine–NO–cGMP system.[9]

While the efforts to understand the biological relevance of having two parallel systems (NO and $H_2S$) are under way, a number of $H_2S$-donating drugs are in various stages of clinical development. In view of its ability to target multiple (NO-independent) mechanistic pathways,[4,6] $H_2S$ has the potential to be effective in patients with inherent arginine and/or NO deficiency or accompanying endothelial (eNOS) and neurological (nNOS) impairments. In conclusion, the downstream NO insufficiency in patients suffering from ASLD warrants additional strategies to activate alternative effector systems. Future studies are therefore needed to unravel L-arginine- and/or NO-independent and self-reliant treatment modalities, which will improve overall clinical outcome and prevent the long-term complications of this genetic disorder.

## DISCLOSURE

The authors declare no conflict of interest.

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# Response to Srilatha *et al.*

**To the Editor:** We thank Drs Srilatha, Meng, and Adaikan for their letter "Hydrogen sulfide: in the aftermath of argininosuccinate lyase and nitric oxide deficiency,"[1] regarding therapeutic strategies aimed at treating the nitric oxide (NO) deficiency in subjects with argininosuccinate lyase deficiency (ASLD). The authors suggest the interesting possibility of providing $H_2S$ donors as a therapeutic modality for ASLD instead of NO donors and discuss the potential drawbacks with NO donor therapy.

We agree that long-term NO supplementation for the treatment of conditions such as hypertension in the general

population have met with limited success.[2] However, the systemic deficiency of NO in ASLD makes NO supplementation an attractive treatment option for this condition. Whereas the usage of organic nitrates is compromised by tachyphylaxis, nitrite, as an active metabolite of nitrate, can be used therapeutically to bypass enzymatic tolerance.[3] We have investigated the utility of NOS-independent NO supplementation in a mouse model and in human subjects with ASLD.[4] Human subjects with ASLD have decreased NO production and impaired NOS-dependent vascular relaxation that responds to NO donors.[4] Mice that are hypomorphic for ASLD have global NO deficiency and multiorgan involvement including systemic hypertension.[4] Treatment of these mice with the NO donor sodium nitrite led to improved weight gain, increased survival, and correction of hypertension. To determine whether the systemic phenotype, in this case hypertension, is independent of the hepatic urea cycle disorder, we treated ASLD mice with liver-directed helper-dependent adenoviral gene therapy.[5] Not surprisingly, despite correction of the metabolic defect in hepatic ureagenesis, mice continued to have hypertension.[5] In ASLD mice treated with gene therapy, aortic ring studies continued to show impaired vascular relaxation because of the tissue autonomous NO deficiency. The short-term infusion of sodium nitrite indeed resulted in correction of hypertension. We translated these findings in a proof-of-principle study by testing the potential efficacy of organic nitrates and sodium nitrite in treating long-term resistant hypertension in a subject with ASLD.[5] In this ASLD subject, hypertension was diagnosed at 5 years of age and for a period of over 10 years, the blood pressure of the subject was significantly elevated despite therapy with four different classes of first-line antihypertensive medications (beta blocker, calcium channel blocker, diuretic, and angiotensin converting enzyme inhibitor). In contrast, NO supplementation with organic nitrates in this subject resulted in rapid and sustained normalization of blood pressure and allowed for withdrawal of all other antihypertensive medications for 9 months. However, because of the concern that tachyphylaxis could lead to a resistance to organic nitrates, we substituted therapy with a custom formulation containing sodium nitrite. The subject now continues to be normotensive on nitrite monotherapy for an additional period of over 12 months. These data suggest that long-term complications in ASLD, such as hypertension, specifically result from the tissue-specific lack of NO, and that NO donors can provide long-term therapeutic benefit.

The nitrate–nitrite–NO conversion pathway has been extensively studied to show that nitrite can serve as a pool for NO.[6,7] Although it would be valuable to investigate the role of $H_2S$ donors in the treatment for ASLD, we believe that in patients with ASLD who may have congenital deficiency of NO, treatment with NO supplementation would be more efficacious. We agree that systematic long-term clinical studies in randomized controlled settings would be necessary to assess the efficacy of NO supplementation in the prevention and treatment of ASLD complications such as hypertension.

## DISCLOSURE

The authors declare no conflict of interest.

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