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Organic Anion Transporter 3 Inhibitors as Potential Novel Antihypertensives

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Abstract

Hypertension is an exceedingly common disease with potentially devastating complications. Unfortunately, existing treatments are often only partially effective, indicating the utility of the development of novel therapeutics. It has recently been discovered that loss of renal organic anion transporter 3 (Oat3) results in decreased blood pressure. This suggests (regardless of the underlying mechanisms, which are, as yet, unclear) that inhibition of Oat3 function might also decrease blood pressure, and, therefore, that inhibitors of Oat3 might serve as novel drugs in the treatment of hypertension. In support of this hypothesis, it has been found that at least some potent Oat3 inhibitors can lower blood pressure. These recent findings are discussed in the context of the alternating access model that has been proposed for organic anion transporter function, with particular attention to the little-appreciated phenomenon of *trans*-inhibition.

Keywords

hypertension; blood pressure; antihypertensive; organic anion transporter; Oat3; Oat3 inhibitor; alternating access; *trans*-inhibition

Organic Anion Transport and Regulation of Blood Pressure

The proximal tubule of the kidney mediates the efficient secretion of a diverse array of organic anionic compounds. The rate-limiting step in this secretion is the uptake of substrates from plasma into tubular cells across their basolateral membranes. This step operates via anion exchange, whereby intracellular dicarboxylates (particularly the Krebs cycle intermediate, α -ketoglutarate) are exchanged for extracellular (plasma) substrates (reviewed in [1,2]). Biochemical as well as knockout studies indicate that organic anion transporters 1 and 3 (Oat1 and Oat3) appear to underlie the basolateral uptake step of this pathway (reviewed in [3,4]). A large number of clinically important drugs are substrates of Oat1 and Oat3, including β -lactam antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), loop and thiazide diuretics, and antivirals, among others. Several organic anions of endogenous origin, including prostanoids, cyclic nucleotides, and various metabolites (e.g., short-chain fatty acids, Krebs cycle intermediates) are known to be substrates as well; however, in general, the physiological functions of the OATs are not as well-understood as their pharmacological roles [5].

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It has recently been discovered that blood pressure is decreased in mice null for Oat3 [4], but not in those null for Oat1 [3] (nor in those null for the other proximal tubular OAT, RST [6]). Similarly knockouts of the related organic cation transporters (OCTs) have not been reported to manifest lowered blood pressure [12-14]. Oat3 knockout mice are otherwise grossly normal and have preserved renal function, other than specific defects in organic anion secretion [7-11]. Collectively, these findings suggest that an endogenous compound specifically transported by Oat3, and not significantly transported by other OATs or OCTs, regulates blood pressure, with loss of its transport leading to decreased blood pressure.

Among the multitude of factors that contribute to the regulation of blood pressure, perhaps the most important, at least with respect to renally mediated mechanisms, are the activity of the renin-angiotensin-aldosterone system and Na⁺ balance (see, for example [15]). However, low blood pressure in the Oat3 knockout mouse is not due to decreased plasma renin or aldosterone, or to decreased Na⁺ retention by the kidney - rather the knockout mice in fact manifest significantly *increased* plasma renin and aldosterone as well as a trend towards decreased fractional excretion (i.e., increased renal retention) of Na⁺. These changes might reflect compensatory adaptations in the knockout directed towards correcting the low blood pressure. However, it is also possible that activation of the renin-aldosterone axis might be a direct consequence of Oat3 loss (that just happens to ameliorate the hypotensive phenotype of the knockout mice), rather than a compensatory response to it: Oat3 (unlike Oat1) is expressed in not just the proximal tubule, but also in the distal nephron [16,17], including in the macula densa, the site of tubular regulation of renin secretion [18]. The latter process is believed to be mediated by prostaglandin E₂ (PGE₂; [19]), which is an Oat3 substrate [17,20]. Thus, it has been hypothesized that Oat3-mediated basolateral uptake in the macula densa might serve to curtail the stimulation of renin secretion by PGE₂ [17]. In that scenario, lack of Oat3 function would lead to augmented renin release, consistent with the findings in the Oat3 knockouts. Importantly, to the extent that Oat3-mediated uptake contributes to the clearance of PGE₂, loss of Oat3 might result in higher systemic PGE₂ levels which, owing to the vasodilatory actions of this compound [21], could account for the hypotension observed in the knockouts.

Other Oat3 substrates implicated in blood pressure regulation include the cyclic nucleotides, cAMP and cGMP [5], which generally promote relaxation of vascular smooth muscle (the latter is among the principal mediators of the vascular effects of nitric oxide [NO]; [22]) and thereby vasodilation and reduction in blood pressure [23]. Moreover, the nucleoside, thymidine, which was identified as a potential endogenous Oat3 substrate via a metabolomic screen of Oat3 knockout mouse plasma and urine (reasoning that endogenous substrates would be relatively increased in concentration in knockout plasma and/or relatively decreased in knockout urine owing to loss of their Oat3-mediated renal secretion), has been found to acutely lower blood pressure following intravenous administration [4]. Intriguingly, there appears to be some early evidence that Oat3 is expressed in vascular smooth muscle [24]. Thus, it is conceivable that altered transport of cyclic nucleotides in this tissue - for instance, loss of Oat3-mediated efflux resulting in intracellular accumulation and prolonged cGMP signaling - explains the low blood pressure in the knockout mice.

Blood pressure effects of Oat3 inhibitors

Although, as discussed above, there are some plausible candidates, the putative blood pressure-regulating Oat3 substrate(s) and the associated mechanisms remain to be identified [4]. Nevertheless, since loss of Oat3 results in lowered blood pressure, inhibition of Oat3 function might also result in lowered blood pressure, raising the possibility that Oat3 inhibitors could be of use in the treatment of hypertension.

In support of this line of reasoning it has been found that inhibition of Oat3 can indeed reduce blood pressure in mice [4]. Specifically, of five tested Oat3 inhibitors (selected from a larger screen of approximately thirty organic anions), the two most potent compounds, eosin-Y and probenecid, produced an acute reduction of blood pressure (following intravenous administration under anesthesia), while the three less potent compounds were without effect (Fig. 1). (Of note, probenecid had previously been shown by other investigators to cause an acute decrease in blood pressure [25]). Overall, the findings are consistent with the hypothesis that inhibition of Oat3 via administration of sufficiently potent inhibitors could result in reduction of blood pressure.

Implications of the alternating access model for Oat3 inhibition

The OATs are members of the major facilitator superfamily of proteins (MFS) postulated, on the basis of biochemical as well as structural data, to function via a single binding site/alternating access model of action [26]. According to this model, the mode of function of Oat1 and Oat3 in the basolateral uptake of substrate (from plasma into the tubular cell) would involve the following steps (Fig. 2): 1) substrate binding from plasma to the outward-facing conformation of the transporter (C_o); 2) switching of the substrate-transporter complex to the inward-facing conformation ($C_o \rightarrow C_i$); 3) substrate dissociation into the intracellular space; 4) binding of an intracellular counterion (e.g., a dicarboxylate) to C_i ; 5) switching of the counterion-transporter complex back to the outward-facing conformation ($C_i \rightarrow C_o$); and 6) counterion dissociation into the plasma, returning the transporter to its original state. There is also the explicit possibility of conformational switch of transporter in the absence of complexed substrate or counterion (7); depending on the transporter and/or the interacting compound involved, this might occur at a greater or lesser rate than the conformational switch of the complexed transporter.

An important consideration arises from this model: Uptake of a substrate, A, from the plasma into the cell will be competitively inhibited by another substrate, B, in plasma (*cis*-inhibition). However, the latter substrate might either increase (*trans*-stimulate) or decrease (*trans*-inhibit) the rate of efflux of an intracellular counterion, C, depending, respectively, on whether the rate of conformational switch of the transporter complexed with B is greater or less than that of the 'empty' transporter. Thus, a given OAT substrate would always be a *cis*-inhibitor of the uptake of other substrates, but might be either a *trans*-stimulator or *trans*-inhibitor of counterion efflux. It should be noted that *trans*-inhibition is not merely a theoretical concept, but has, in fact, been well-documented for some OAT substrates (e.g., indomethacin [27] and estrone sulfate [28]). However, in general *trans*-stimulation would be expected to be the outcome in the case of efficiently transported (efficacious) substrates (those with relatively high V_{max}), since they would be associated with more rapid rates of conformational switch of the substrate-transporter complex; conversely, *trans*-inhibition might be expected for substrates with low V_{max} .

Probenecid and eosin-Y as *trans*- as well as *cis*-inhibitors

Interestingly, in addition to being potent *cis*-inhibitors (Fig. 1), both probenecid and eosin-Y are likely potent *trans*-inhibitors as well. Probenecid has previously been demonstrated to inhibit OAT function in *trans* (see, e.g., [29]). In the case of eosin-Y, earlier measurements of V_{max} of fluorescent organic anions [30] revealed it to be far and away the least efficacious of the compounds in Fig. 1, with a V_{max} value in mOat3-expressing oocytes almost a thousand-fold less than that of most efficacious substrate, 5-carboxyfluorescein (Fig. 3A). Of note, the data reveal a clear negative correlation between *cis*-inhibitory potency, pK_i , and efficacy, $\log(V_{max})$ (Fig. 3A). Thus, for at least this set of structurally homologous fluorescent substrates (see structures in Fig. 1), high *cis*-inhibitory potency is associated with low V_{max} , and therefore, likely with high *trans*-inhibitory potency. The underlying biophysical explanation for this

phenomenon might be that if the complex between transporter and a particular substrate is highly stable, any alteration in that complex, whether dissociation of the substrate or conformational switch of the transporter-substrate complex, becomes relatively unlikely, resulting both in high substrate affinity (which would correlate with high *cis*-inhibitory potency) and low turnover rate of the substrate-transporter complex (which would correlate with low efficacy) (Fig. 3B).

If the putative blood pressure-regulating compound that interacts with Oat3 is a substrate for proximal tubular organic anion secretion, then the direction of its Oat3-mediated transport would be from plasma into the tubular cell. The converse possibility is that the direction of transport of the blood pressure-regulating compound is from the *tubular cell into plasma* (i.e., that this compound functions as a counterion for the basolateral uptake of organic anions by Oat3) (this might be considered less likely given the limited number of physiologically relevant counterions compared with the large number of biochemically diverse compounds that are substrates for organic anion secretion in the proximal tubule [2]). In the former scenario, inhibition of its transport, and thereby reduction in blood pressure, would result from the introduction of *cis*-inhibitors into the bloodstream; in the latter scenario, blood pressure reduction would result from administration of a *trans*-inhibitor. Thus, the blood pressure-lowering effects of eosin-Y and probenecid might be due to either their *cis*-inhibitory activity or to their *trans*-inhibitory activity. For example, their lowering of blood pressure might be due to *cis*-inhibition of the tubular uptake (and therefore urinary secretion) of a blood pressure-lowering compound (e.g., a vasodilator), or to the *trans*-inhibition of the counter-transport (reabsorption) of a blood pressure-raising compound (e.g., a vasoconstrictor).

Conclusions and future directions

Development of the approach of targeting Oat3 as a means to treat hypertension will require elucidation of the mechanism whereby Oat3 regulates blood pressure. As noted in this review, it is not yet clear whether hypotension due to lack of Oat3 function arises from loss of renal clearance of a blood pressure-reducing compound, loss of renal reabsorption of a blood pressure-increasing compound, or loss of transport at an extra-renal site of action such as the smooth muscle of the vasculature. A first step in addressing this issue might be to determine the levels of blood pressure-regulating Oat3 substrates in urine, plasma, and select intracellular compartments (renal parenchyma, vasculature) of the hypotensive Oat3 knockout mouse in comparison to those in the wild-type mouse. These assays should include novel endogenous Oat3 substrates (as identified, for example, by metabolomic screens for organic anions that are altered in concentration in the knockouts) as well as known Oat3 substrates implicated in the regulation of blood pressure (such as prostaglandins and cyclic nucleotides). Such studies would be complemented by determination of whether the physiologic basis for lowered blood pressure with loss of Oat3 function is decrease in intravascular volume or in vascular tone.

Clarification of the role of Oat3 in regulation of blood pressure relates to the question of what therapeutic advantage Oat3 inhibitors might confer in comparison to existing antihypertensives. In general, the availability of antihypertensives that function via a variety of different mechanisms would allow for targeting of different drugs to different patient populations (as, for example, beta-blockers are targeted to patients with coronary artery disease and ACE-inhibitors to those with proteinuria (www.clinicalpharmacologyip.com)). Thus, depending on the mechanism of action that is eventually elucidated for Oat3, specific 'niches' might exist where the use of Oat3 inhibitors for the treatment of hypertension would be the most appropriate choice. For instance, if Oat3 were shown to modulate blood pressure by acting in vascular smooth muscle to regulate the content of cyclic nucleotides, then Oat3 inhibitors might be particularly useful as antihypertensives in the setting of coexisting peripheral arterial disease. In this regard, it is worth noting that the apparent physiological normalcy of the Oat3

knockout mice (other than lowered blood pressure and altered renal clearance of OAT-substrate drugs; [4,7-11]) suggests that the pharmacological targeting of Oat3 might prove relatively 'safe'.

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Fig. 1. Blood pressure effects of Oat3 inhibitors

A, Tested Oat3 inhibitors listed in order of increasing potency. B, Reduced blood pressure following administration of Eosin-Y and Probenecid. Mice were anesthetized and blood pressures recorded via arterial catheter before and after intravenous administration of ~100 mg/kg of the Oat3 inhibitors listed in panel A. Whereas the three lower potency compounds did not cause any consistent hemodynamic changes, administration of eosin-Y and probenecid resulted in significant decreases in blood pressure; *p<0.05. Data are from ref. [4].

Fig. 2. Alternating access model of Oat1 and Oat3 function

C_o , outward-facing conformation of transporter; C_i , inward-facing conformation; blue circle, substrate; red circle, counterion. Please refer to the text for details.

A

Fig. 3A. Relationship between potency and efficacy

There is a striking correlation between pK_i (potency) and $\log(V_{\max})$ (efficacy) ($r^2 = 0.97$) for the interaction of the fluorescent substrates, fluorescein, 5-carboxyfluorescein, 6-carboxyfluorescein, 4-carboxyfluorescein-dimethylester, and eosin-Y, with Oat3. The position of eosin-Y, the highest potency, lowest efficacy compound is indicated. Please refer to the text for details. The data are from ref. [30].

Fig. 3B. B Potential explanation for the observed potency/efficacy relationship

The graphs illustrate hypothetical free energy changes associated with the process of transport of two different substrates. This process encompasses steps of substrate association with the transporter (1), conformational switch of the substrate-transporter complex (2), and substrate dissociation from the transporter (3) (the steps are numbered in the same manner as in Fig. 2). C_o , outward-facing conformation of transporter; C_i , inward-facing conformation; C_oS_1 & C_iS_1 , outward- and inward-facing conformations, respectively, of the complex of substrate #1 (S_1) with the transporter; C_oS_2 & C_iS_2 , conformations of the complex of substrate #2 (S_2) with the transporter; blue-filled circle, S_1 ; gold-filled circle, S_2 . In the scenario depicted, the free energy of C_oS_2 is much lower than that of C_oS_1 (i.e., the complex of S_2 with the transporter [in its out-facing conformation] is much more stable than that of S_1 with the transporter), with the energy profiles for transport of the two substrates being otherwise similar (including the free energies of the transition states between the transporter conformations). This circumstance would result not only in greater affinity of S_2 for the transporter (affinity being a function of the free energy difference between the free and substrate-bound states of the transporter; green arrows), and therefore in greater inhibitory potency, but also in a lesser rate of turnover of the substrate-transporter complex (this rate being inversely related to the 'height' of the energetic barrier between the conformational states; red arrows), and therefore in lesser transport efficacy of S_2 in comparison with S_1 . Please also see the discussion in the text.