Review

PST 2238: A New Antihypertensive Compound that Modulates the Na-K Pump ‘in Vivo’ and ‘in Vitro’

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A primary renal alteration due to a genetic polymorphism of the cytoskeletal protein adducin associated with an up-regulation of the renal Na-K pump and increased levels of ouabainlike factor (OLF) has been identified as a possible causes of hypertension in Milan rats (MHS). This adducin polymorphism has also been found to be associated with hypertension and the blood pressure changes related to renal Na handling in humans and increased OLF levels have been found in a relevant portion of hypertensive patients. Increased activity and expression of the Na-K pump has also been observed under the following ‘in vitro’ and ‘in vivo’ conditions: rat renal cells transfected with the ‘hypertensive’ variant of adducin, as compared with normal cells; normal rat renal cells incubated for 5 days with 10^-9 M ouabain and normal rats made hypertensive by a chronic infusion of low doses of ouabain (OS rats). An up-regulation of the Na-K pump seems therefore to be a common biochemical alteration induced both by an adducin polymorphism and/or chronic exposure to low concentrations of ouabain (or OLF). A new antihypertensive compound, PST 2238, that selectively antagonizes the pressor effect and the alteration of the renal Na-K pump induced both by an adducin polymorphism and OLF, is described. The ability of PST 2238 to lower blood pressure and normalize the Na-K pump both in MHS and OS rats suggests that this compound could be useful in the treatment of those forms of essential hypertension in which renal Na-handling alterations are associated with either adducin polymorphisms and/or increased OLF levels.

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Introduction

Essential hypertension is a complex disease in which many genetic and environmental factors interact to result in a final blood pressure increase and the risk to develop specific organ complications (mainly at the cardiac, renal, and brain levels) (1, 2). Although the antihypertensive efficacy of different classes of drugs is widely recognized and is similar when large populations are compared, there is much individual variability in the response to a given therapeutic regimen (3). Population surveys have demonstrated that less than 30% of patients are adequately treated. These problems arise primarily from the lack of a complete understanding of the mechanisms that underlay the development of primary hypertension and its organ complications. Therefore, the success of a future new therapy for hypertension will depend upon our understanding of the molecular-genetic mechanisms operating in a subset of patients and the ability of the new drug to correct such a mechanism. A pharmacogenomic approach (4, 5) has been adopted by Prassis sigma-tau to develop a new class of antihypertensive compounds able to lower blood pressure by correcting a specific genetic-molecular mechanism previously demonstrated to be involved both in rat and human hypertension. As a consequence, the
Background

For many years our group has tried to identify at least one of the genetic mechanism that causes hypertension both in the rat and in humans and to use the results obtained to identify not only new targets for innovative treatment, but also genetic markers to characterize essential hypertensive (EH) patients likely to respond successfully to treatment with such compounds. The strategy has included studies of renal function and cellular, biochemical and molecular characteristics of Milan hypertensive rats (MHS) (7) which are considered to be a suitable animal model to compare with human hypertensive subjects. Indeed, we have been able to identify common genetic-molecular mechanisms underlying the disease in both species (7). In particular, the primary involvement of the kidney in the development of some forms of human essential and animal genetic hypertension has been clearly demonstrated (7, 8). Studies in MHS rats and their normotensive controls (MNS) (9) and in essential hypertensive patients (10, 11) have shown that mutations in the genes coding for the cytoskeletal protein, adducin, are genetically associated with hypertension and salt sensitivity in both species.

Rat Model

In MHS rats, adducin mutations are linked to increased expression and maximal activity of the renal Na-K pump (12), which is the most important mechanism regulating constitutive tubular sodium reabsorption in the kidney. Transfection of ‘hypertensive’ and ‘normotensive’ α-adducin variants in rat kidney cells have shown that only the former increases the surface expression and maximal rate of the Na-K pump (13). Moreover, we have demonstrated in a cell-free system that adducin directly and specifically interacts with the Na-K pump and that the ‘hypertensive’ variant of the α-adducin stimulates Na-K pump activity at significantly lower concentrations than the ‘normotensive’ one (14). This finding therefore provides the genetic-molecular basis for a constitutive increase in tubular sodium reabsorption in MHS rats. Moreover, elevated levels of an endogenous inhibitor of the Na-K pump, the so-called ouabainlike factor (OLF), have been observed in adult MHS rats (15, 16).

Humans

The involvement of adducin genetic polymorphism in human essential hypertension can be summarized as follows: 1) as in both MHS rats and humans, two missense point mutations in the alpha adducin subunit have been detected (11); 2) either these mutations, or repetitive DNA sequences mapping close to the adducin locus, are associated with hypertension, as shown in a case-control study (10) and the adducin locus linked with hypertension, as observed in a sib-pairs study (11); 3) for the Gly460Tpr alpha adducin variant, an association with alterations of the relationship between blood pressure and renal Na handling has also been shown in humans (11, 17). Some groups have found a similar positive association between the 460Tpr adducin allele and hypertension in different populations (18, 19), although others have failed to confirm these findings (20, 21). However, a recent study (22) comparing two Italian populations, one from Milan and the other from Sardinia, has shown that basal plasma renin activity is lower and blood pressure falls more after diuretic therapy in hypertensives carrying at least one 460 Trp allele than in Gly460Gly homozygotes, irrespective of the presence (Milan population) or absence (Sardinian population) of an association between the 460Tpr variant and hypertension (22). Therefore, the α-adducin polymorphism may be used in humans to identify patients in whom a genetic abnormality in renal sodium handling underlies a salt-sensitive form of essential hypertension. Increased levels of OLF have also been demonstrated in EH patients (15) and may vary according to changes in the sodium balance. Moreover, it has been observed that approximately 50% of patients with uncomplicated essential hypertension have elevated OLF levels that correlate positively with left ventricular mass and stroke volume and negatively with heart rate (23), suggesting that OLF may affect cardiovascular function and structure and could contribute to the risk of morbidity events.

The link between the individual steps connecting the adducin polymorphism to the increased Na-K pump activity, tubular reabsorption and hypertension has not been fully elucidated; however, the available findings indicate that a common molecular mechanism, supported by adducin, is at work both in rats and in a subgroup of hypertensive subjects. Moreover, a link between increased levels of OLF and further stimulation of the renal Na-K pump expression, has been observed when studying rat models:

1. The hyperactivation of the renal Na-K pump observed in young prehypertensive MHS rats also persists in adult animals (12), with the animals observed already being hypertensive and with the above relation also being present when OLF levels were increased to maximum compared with normotensive MNS (24); second, chronic infusions of low doses of ouabain in normal Sprague Dawley rats not only induces a sustained form of hypertension (25) but are also associated with a stimulation of the renal Na-K pump, with these effects mimicking those observed in the genetic MHS model (26). Therefore, an up-regulation of renal Na-K pump activity and expression seems to be a common molecular alteration of forms of
hypertension sustained by adducin polymorphism and/or high circulating levels of endogenous ouabain. As a consequence, any therapeutical maneuver able to interfere with the sequence of events leading to an up-regulation of the renal Na-K pump, such as adducin polymorphisms and/or increased OLF levels, might be able to lower blood pressure. The increase in renal Na-K pump activity and OLF levels could therefore represent a new pharmacological target for the treatment of specific forms of hypertension.

The intent of the present research was to synthesize and select an antihypertensive compound endowed with a unique mechanism of action: to antagonize the pressor effect and the alteration of renal Na-K ATPase expression induced both by the adducin polymorphism and OLF (or ouabain), without interfering with other known physiological pressor mechanisms.

Following this approach, among more than 800 original molecules, compound PST 2238 has been selected (26-28) for its ability to selectively displace the ouabain binding from the Na-K ATPase receptor and to be antihypertensive in experimental and genetic models of hypertension in which the above-mentioned alterations are operating.

Methods and Results

PST 2238

PST 2238 (17β-(3-furyl)-5β-androstan-3β, 14β, 17α-triol) is a digitoxigenin derivative able to displace ‘in vitro’ ouabain from the Na-K ATPase receptor with IC50 of 2 μM (26). It does not interact ‘in vitro’ with other receptors involved in blood pressure regulation or hormonal steroid control (26) and does not cause any cardiac effect when tested both on isolated cardiac preparations and ‘in vivo’ (28). PST 2238, is a potent antihypertensive compound active both in genetic (27) and ouabain-dependent (26) experimental models of hypertension. In MHS rats in which the adducin polymorphism (9) and increased OLF levels are present (16), PST 2238 administered orally for 6 weeks, starting from a prehypertensive age, reduces in a dose-dependent way the development of hypertension with an EC50 of 4 μg/kg os (27). In normal Sprague Dawley rats, made hypertensive by a chronic infusion of 50 μg/kg/day of ouabain (OS rats) (26), PST 2238 completely abolishes the ouabain-dependent blood pressure increase in a dose range between 0.1 and 10 μg/kg os (26). The ability of PST 2238 to antagonize the effects of adducin mutations and ouabain (or OLF) on renal Na-K ATPase activity and expression has been demonstrated both in cultured renal cells and ‘in vivo.’

Cell Studies

In rat renal cells (NRK) transfected with the ‘hypertensive’ variant of adducin (NRK-1), the Na-K pump activity at Vmax is increased as compared with NRK cells transfected with the ‘normotensive’ adducin variant (13). Incubation of NRK-1 cells with PST 2238 at 10^{-10}, 10^{-9} M for 5 days results in a significant reduction of pump activity to the level of normal cells which are not affected by a similar treatment (27). In wild-type NRK cells (expressing the non-mutated adducin variant), 5 days of incubation with 10^{-9} M ouabain induces a significant increase in Na-K pump activity at Vmax (+35%, p<0.01) (26). The simultaneous presence of PST 2238 (10^{-14} to 10^{-9} M) in the ouabain medium normalizes the ouabain-dependent increase of the Na-K pump at Vmax, while the compound does not have any apparent effect on the Na-K pump rate at concentrations up to 10^{-5} M (26).

‘In Vivo’ Studies

In MHS rats, PST 2238 (from 1 to 100 μg/kg os) induces not only a hypotensive effect, but in parallel it normalizes the renal Na-K ATPase activity to the levels of normotensive MNS controls (27). Similarly, in OS rats, the ouabain-dependent blood pressure increase is associated with a significant increase in the renal Na-K ATPase activity at Vmax (26). PST 2238 (from 0.1 to 100 μg/kg os), in parallel with its hypotensive effect, antagonizes the ouabain-dependent increase in renal Na-K ATPase activity and normalizes it to the level of normotensive saline-treated rats (26). Moreover, PST 2238 is also active as antihypertensive agent in other rat experimental models of volume-dependent hypertension, such as the DOCA+salt, DOCA+salt+ouabain and reduced renal mass models (28).

The selectivity of action of PST 2238 is suggested by the following data: PST 2238 does not affect either blood pressure or renal Na-K ATPase activity in normotensive MNS and normal Sprague Dawley rats (26, 27) and does not affect heart rate in any of the animal models studied (26, 27). Moreover, this compound has not been effective in lowering blood pressure in another genetic model of hypertension, the SHR rats (27). This lack of activity is likely due to SHR rats, unlike MHS rats, exhibiting a lower expression of the Na-K ATPase α1 subunit in the kidney than WKY normotensive controls, both before and after the development of hypertension (30). Circulating OLF levels seem to be little involved in SHR hypertension, as they have been found to be reduced in SHR as compared to WKY rats (31). Finally, since both normotensive WKY and hypertensive SHR rats carry the same genetic variant of adducin as MHS (31) and genetic hypertension develops as a polygenic disease in which specific epistatic interactions lead to the final blood pressure rise, it is likely that the genetic background responsible for SHR hypertension is different from that operating in MHS.
General and safety-related pharmacological studies indicate that PST 2238 does not affect cardiac and vessel contractility either 'in vitro,' 'in vivo' or 'ex vivo' (28).

In addition, PST 2238 does not alter the urinary composition and creatinine clearance either after acute or chronic administration at both therapeutic and high doses. Therefore, PST 2238 does not display any 'diuretic' activity (28).

It is devoid of androgenic, estrogenic and corticometric effects 'in vivo,' does not alter ACTH, prolactin secretion and steroidogenesis 'in vitro' and does not affect gastrointestinal motility 'in vivo' (28).

Studies of acute and chronic toxicity have been carried out with the results indicating that PST 2238 is well-tolerated both in the rat and monkey up to doses 25,000 times higher than the therapeutic dose (28). PST 2238 does not show any mutagenic activity (28); moreover, it is devoid of any adverse effects on reproduction and embryonic development up to an oral dose of 135 mg/kg in rats and 400 mg/kg in rabbits (28).

Phase 1 clinical studies on healthy volunteers have been performed showing that PST 2238 has an excellent tolerability both after single and repeated oral administrations (1-2.5-5 and 10 mg/day) (28). Early Phase 2 clinical studies are currently in progress with essential hypertensive patients selected according to the adducin genotype and OLF plasma levels.

Discussion

According to its pharmacological profile, PST 2238 may represent a prototype of a new class of antihypertensive agents that control blood pressure, as it specifically corrects a biochemical alteration (up-regulation of renal Na-K pump) primarily determined by a genetic defect (adducin polymorphism) and further sustained by an hormonal deregulation (OLF increase) triggered in turn by the same generic cause.

The relevance of these findings for human essential hypertension stems from the following considerations: adducin gene mutations have also been demonstrated in humans to be associated with hypertension (10, 11) and with a greater change in blood pressure after variations in body sodium levels (11, 17, 22). Moreover, increased circulating OLF levels have been verified to accompany the development of high blood pressure in a subgroup of essential hypertensive patients (15). When these findings are considered in view of a 'causal' approach to the therapy of essential hypertension, it appears that compounds like PST 2238 are candidates for treating patients in whom, as in MHS rats, alterations in renal Na handling and OLF levels can be associated with specific genetic molecular mechanisms such as an adducin polymorphism or any other protein alterations that affect renal Na-K ATPase activity.

References


