

Biochemical effects of urapidil on red cell membrane ion transport systems in a population of elderly essential hypertensives

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Summary: The aim of our work was to verify the effect of urapidil on membrane ion transport systems. This was a randomized, double-blind, cross-over study which evaluated the clinical and biochemical effects of urapidil (30 mg twice daily in comparison with placebo) in a group of 10 elderly hypertensive patients (3 male, 7 female ranging from 68 to 90 years, mean age 79.2 ± 7.6 years). For the evaluation in fresh erythrocytes of principal ion transport systems (cotransport Na^+/K^+ , countertransport Na^+/Li^+ , Na^+/K^+ ATPase pump, intracellular Na^+ and K^+) we used the nystatin technique. We found that urapidil activated the red cell membrane ions cotransport system (basal values: $83.7 \pm 50.3 \mu\text{mol Na}^+ \text{RBC l}^{-1} \cdot \text{h}^{-1}$, after 1 month of urapidil therapy: $181.5 \pm 89.3 \mu\text{mol Na}^+ \text{RBC l}^{-1} \cdot \text{h}^{-1}$) ($P < 0.01$), without significant changes in the other biochemical parameters evaluated.

Our data suggest that one of the mechanisms of the urapidil antihypertensive effect could involve an increase in the membrane sodium cotransport system.

Introduction

Urapidil is a new once-daily antihypertensive drug¹⁻³ whose known mechanism is a balanced action between a peripheral α -1-postsynaptic adrenoceptor block and a reduction of central sympathetic tone.⁴⁻⁶ According to pharmacodynamic studies⁷⁻¹¹ it causes a reduction in total peripheral resistance, has no negative inotropic effects on the heart and does not cause any reflex increase in the sympathetic nervous system activity.

In spite of reduced arterial pressure, renal blood flow is maintained, presumably due to dilatation of renal vessels.^{12,13} Other effects of urapidil can be observed in various biological systems such as platelet aggregation, but to date there is no evidence of its possible action on cellular ion transport systems. Many authors¹⁴⁻¹⁷ consider alteration of membrane ion transport systems, in particular at the kidney tubular cell level,¹⁸ to be involved in the pathogenesis of arterial hypertension (EAH). Since it is very difficult to study these abnormalities in the human kidney cells or vascular smooth muscle, most studies use red blood cells (RBC). Moreover there is important evidence¹⁸ that shows similar physiopathological mechanisms in ion transport systems between RBC and kidney tubular cells.

The aim of our work was to evaluate the effect of this drug on red cell membrane ion transport systems.

Material and methods

We designed a randomized, double-blind, cross-over study in order to evaluate the biochemical and clinical effects of urapidil (30 mg twice daily) vs placebo, in a group of 10 elderly patients (3 male, 7 female ranging from 68 to 90 years, mean age 79.2 ± 7.6 years) affected by mild and moderate EAH (WHO stage I and II). The study design had a run-in period of 1 month; one month of random therapy (placebo or urapidil), a cross over and another month of therapy.

At the beginning of the study, after 1 month of therapy, before the cross over and at the end, we evaluated, in freshly collected peripheral blood erythrocytes, the principal ion transport systems (cotransport Na^+/K^+ , countertransport Na^+/Li^+ , Na^+/K^+ ATPase pump, intracellular Na^+ and K^+) using the nystatin as ionophore to measure ion transports in loaded RBC.¹⁹

Preparation of red cells

Early in the morning 30 ml of venous blood was drawn into lithium-heparin tubes and immediately

processed. Plasma and buffy coat were removed by cold centrifugation (4°C) (3000 × *g* for 15 minutes). The cells were twice rinsed (4°C) with cold washing solution (WS) containing 110 mM MgCl₂, 10 mM Tris-mops, pH 7.4. Later, 1.5 ml of packed red cells were used for each nystatin loading procedure and 1.8 ml for lithium loading. The rest of the packed red cells were washed again and resuspended at 30% haematocrit in WS.

Intracellular cation measurements

Red cell suspension (50 μl) was lysed in three tubes containing 10 ml of distilled water each, to measure haemoglobin and cation content. The same dilution was used for Na⁺, K⁺, and Li⁺ determination by atomic absorption spectrophotometer. Haemoglobin absorbance was stable during the loading procedure which indicates no appreciable swelling of cells.

Cation efflux measurement

Total Na⁺ efflux was measured in a medium containing 6 mM KCl, 73 mM MgCl₂, 75 mM sucrose, 10 mM glucose and 10 mM Tris-mops (pH 7.4). Ouabain and bumetanide-sensitive effluxes were processed in the same medium (without KCl) containing 0.1 mM ouabain and 20 mM bumetanide freshly prepared in DMSO.

Both tubes were incubated at 37°C for 5, 15 and 25 minutes in the first medium; 5, 35 and 65 minutes (at the same temperature) in the second. To stop the reaction the tubes were placed at 4°C and then centrifuged at highest velocity for 1 minute at 4°C in a centrifuge.

The supernatant fluid was transferred in plastic tubes and stored at 4°C until cation content analysis (by a Pn 9000 Philips atomic spectrophotometer).

Nystatin loading procedure

The loading procedure was performed by the Canessa technique¹⁹ utilizing only two point loading. Packed red cells (1.5 ml) were stored briefly at 4°C in a Falcon tube in presence of solution containing 20 mM NaCl, 120 mM KCl, 50 mM sucrose and 90 mM NaCl, 50 mM KCl, 50 mM sucrose, using nystatin as an ionophore twice for 20 and 10 minutes respectively). The cells were washed 4 times with solutions at the same concentration of NaCl, KCl and sucrose for each loaded red cell sample but in the presence of 10 mM glucose and 0.1% albumin (at 37°C) to eliminate the ionophore. The external cations were removed by five washes with WS solution, and cells were resuspended at 30% haematocrit before processing for cation efflux measurement as previously described.

Procedure for Na⁺/Li⁺ countertransport

Packed red cells were incubated at 37°C for 3 hours in a medium containing 150 mM LiCl, 10 mM glucose and 10 mM Tris-mops (at pH 7.4). After incubation cells were washed with WS and incubated for 5, 20 and 35 minutes with and without high Na⁺ concentration (150 mM) for lithium effluxes and countertransport measurement. Samples were performed as previously described (using the same spectrophotometer).

Statistical analysis

Statistical analysis of the results was performed by use of repeated measure analysis of variance (MANOVA) evaluated under the different treatment groups.

The statistical package SPSS following the MANOVA procedure was used, a probability value of less than 0.05 was considered significant for the analysis statistical F test.

Results

As regards the effect of urapidil on red cell membrane ion transports (Table I), we found a significant increase ($P < 0.01$) in the cotransport system.

The intracellular Na⁺ and K⁺ did not show significant modifications: Na⁺ basal values 10.2 ± 1.1 mmol/l, after urapidil therapy: 10.8 ± 1.5, and after placebo 10.2 ± 1.5 mmol/l. K⁺ basal levels 103.7 ± 1.3 mmol/l, after one month of active therapy 110.6 ± 5.8 mmol/l, and after placebo 113.1 ± 3.7 mmol/l. Also Na⁺/Li⁺ countertransport and Na⁺/K⁺ ATPase pump do not show any significant modifications during urapidil therapy.

During the study we did not find any significant changes in the other biochemical parameters evaluated.

ANOVA for repeated measures indicated no significant overall carry-over effect for any parameter and allowed for an analysis of treatment effect.

There were no significant side effects or changes on haematological or biochemical values.

Discussion

Although excess Na⁺ intake has been identified as one of the major pathogenetic factors in many patients with EAH, its real role still remains obscure. Many observations^{15,16,18} of different sodium transport abnormalities in erythrocytes from patients with EAH suggest that the link between

Table I Biochemical effects of urapidil on red blood cell main ion transport systems

	Before treatment Baseline	After treatment Urapidil	Placebo
Na ⁺ /K ⁺ CT ($\mu\text{mol Na}^+$ RBC $1^{-1} \cdot \text{h}^{-1}$)	83.7 \pm 50.3	181.5 \pm 89.3*	50.0 \pm 29.4
Na ⁺ /Li ⁺ CNT ($\mu\text{mol Na}^+$ RBC $1^{-1} \cdot \text{h}^{-1}$)	301.6 \pm 123.0	251.3 \pm 135.6	280.8 \pm 97.9
Na ⁺ (i) (mmol/l)	10.2 \pm 1.0	10.8 \pm 1.5	10.2 \pm 1.5
K ⁺ (i) (mmol/l)	103.7 \pm 1.3	110.6 \pm 5.9	113.0 \pm 3.7

* $P < 0.01$; CT: cotransport; CNT: countertransport; (i): intracellular.

sodium and hypertension could be a disorder of Na⁺ handling by the kidney, by vascular smooth muscle cells and by other possible cells involved in hypertension pathogenesis.

In relation to EAH the most studied membrane ion transport systems are Na⁺/Li⁺ countertransport, Na⁺/K⁺ ATPase pump and Na⁺/K⁺ cotransport. The alterations of these membrane ion transport systems may play an important role in Na⁺ handling, in particular Na⁺/Li⁺ countertransport abnormalities which could reflect an increased renal sodium reabsorption.¹⁸ Conversely, the decrease of apparent affinity of the Na⁺/K⁺ pump for internal Na⁺²⁰ may reflect an abnormal regulation of the increase in cell Na⁺ content. Finally, recent observations underline the possibly important role of the cotransport system as a silent cellular 'second pump'. This process, in fact, is almost non-existent under physiological conditions, but can be unmasked by an increase in cell Na⁺ content.²¹

In particular, the cotransport system (of vascular cells and/or noradrenergic endings) may play two different roles in EAH: as 'defective second pump' in some patients with decreased cotransport affinity for internal Na⁺, or as 'compensatory second pump' in others, where some abnormalities in the ion transport system may predispose the cell to a defective extrusion of excess cell Na⁺ content. The cotransport system helps the Na⁺/K⁺ ATPase pump in extruding any excess cell Na⁺ content.²¹

On the other hand, an increased 'second pump'

activity of the cotransport system may 'compensate' other Na⁺ transport abnormalities which predispose the cell to an abnormal handling of any excess cell Na⁺ content. Recent studies show that in patients with essential hypertension the values of cotransport has a characteristic bimodal distribution with low or high values.²²

Our hypertensive patients are characterized by low cotransport. In such cases we believe that the cotransport can be a 'defective second pump' and that, in particular under a sodium load, the system cannot 'compensate' this abnormal sodium handling. One of the possible consequences is a transient excess in Na⁺ cell content, with a consequent increase of Ca⁺⁺ and a final vascular resistance increase.²³ We think that urapidil activates, in these subjects, the cotransport which becomes more effective when handling excess Na⁺ cell content, with a consequent decrease of vascular resistance. In other words we hypothesize that the peripheral antihypertensive effects of urapidil could not only be an alpha-1-postsynaptic block, but also direct action on the cotransport system, at least in those hypertensives with low cotransport values. We conclude that one of the mechanisms of the urapidil antihypertensive effect could involve an increase in the membrane Na⁺/K⁺ cotransport system.

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