Hydrochlorothiazide exerts no direct vasoactivity in the human forearm

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Background: Recently, hydrochlorothiazide has been shown to relax vascular smooth muscle in vitro in clinically relevant concentrations by the opening of calcium-activated potassium channels, leading to hyperpolarization and consequent closing of voltage-operated calcium channels. Long-term administration of hydrochlorothiazide reduces peripheral vascular resistance in vivo in man. These results indicate that hydrochlorothiazide has hemodynamic activity, and we therefore examined the direct vascular action of this drug in vivo.

Subjects and methods: Forearm vasodilator responses to the infusion of a placebo and five increasing doses of hydrochlorothiazide into the brachial artery were recorded by venous occlusion strain-gauge plethysmography (perfused forearm technique) in eight normotensive male volunteers. Venous samples were taken from an ipsilateral antecubital vein at the end of each infusion period to measure the hydrochlorothiazide concentration.

Results and discussion: Plasma concentrations of hydrochlorothiazide averaged 3.5±0.3 µg/ml at the highest infusion rate. This concentration leads to a 60±10% relaxation in vitro and is more than 10 times the therapeutic plasma concentration. Despite these supratherapeutic levels, we were unable to demonstrate a change in forearm blood flow and vascular resistance. Also, no significant changes were observed in blood pressure and heart rate.

Conclusion: In contrast to in vitro results, hydrochlorothiazide does not exert any direct vasoactivity in the forearm vascular bed of healthy normotensive male volunteers at (supra)therapeutic plasma concentrations.


Keywords: Hydrochlorothiazide, vasodilation, hypertension, forearm blood flow, plethysmography, human

Introduction

Thiazide diuretics are widely used and considered treatment of first choice in most patients with essential hypertension as they reduce the risk of stroke and other cardiovascular events [1]. Evidence is accumulating that their efficacy is at least partly due to a direct hemodynamic action. During chronic thiazide administration, the plasma volume returns to baseline and peripheral resistance decreases [2], suggesting a vascular action besides the diuretic effects. Chronic treatment with thiazides has also been reported to reduce the vasoconstrictor action of norepinephrine in both normotensives and hypertensive patients [3,4]. An acute diuretic-induced fall in blood pressure is incidentally observed, dissociated from changes in plasma volume in patients with renal failure [5], although there are no consistent data.

In animals, parenterally administered hydrochlorothiazide causes an immediate fall in blood pressure associated with inhibition of the vasoconstrictor response to stimulation of adrenergic vasomotor fibers, while prolonged treatment with hydrochlorothiazide in rabbits and dogs...
has been reported to inhibit norepinephrine-induced vasoconstriction both in vivo and in vitro [6].

In the past few years concentration-dependent relaxant effects of clinically relevant concentrations of hydrochlorothiazide have been demonstrated in human and guinea-pig isolated resistance arteries [7]. This vasodilator activity is associated with a fall in intracellular Ca$^{2+}$ [8]. Its action is inhibited by charybdotoxin, a blocker of large-conductance Ca$^{2+}$-activated K$^+$ channels but not by glibenclamide, a blocker of ATP-sensitive K$^+$ channels [7]. In summary, it seems that hydrochlorothiazide increases the permeability of smooth muscle membranes to K$^+$ [9], probably by opening Ca$^{2+}$-activated K$^+$ channels, thereby leading to K$^+$ efflux [10] and membrane hyperpolarization, and consequent closing of voltage-operated Ca$^{2+}$ channels and smooth muscle relaxation.

The present study was aimed at investigating the putative direct vascular effects of hydrochlorothiazide in humans in vivo.

**Subjects and methods**

The study protocol was approved by the Ethics Committee of our hospital and informed written consent was obtained from each subject before participation. Eight healthy male volunteers were submitted to clinical examination before admission to the study. None of the subjects smoked or was on any kind of medication. The age, weight, height, body mass index, forearm volume and systolic/diastolic blood pressure of these subjects were, respectively, 24.8±5.0 years, 78.5±4.7 kg, 186.0±5.0 cm, 22.7±1.6 kg/m$^2$, 1088±133 ml and 127±9/73±9 mmHg. The subjects were instructed to follow their usual diets, but to abstain from chocolate and alcohol and caffeine-containing beverages 24 h preceding the experiment.

The experiments were performed during a 3-h immobilization period in the supine position in a quiet and temperature-controlled laboratory as described previously [11]. Briefly, the left brachial artery was cannulated for drug infusion and blood pressure monitoring. After occlusion of the hand circulation, forearm blood flow was measured in both arms with venous occlusion plethysmography using mercury-in-silastic strain gauges. The forearm blood flow of the contralateral arm was used as a time-control value so that systemic effects could be observed and vasoactive effects could be expressed as a quotient of the left and right arm [12]. Each infusion period lasted 10 min and was alternated with a 10-min pause during which the wrist cuff was deflated to allow recovery of the hand circulation. In four subjects the highest dose of hydrochlorothiazide was infused for 20 min instead of 10 min. On the ipsilateral side an antecubital vein was cannulated for blood sampling.

On the day of each experiment hydrochlorothiazide was reconstituted from a sterile powder, diluted in 1.4% sodium bicarbonate (pH 7.4) to a concentration of 0.1 ng/ml and passed through a 0.22-μm Millipore (Milford, Massachusetts, USA) filter. Further dilutions were prepared immediately before the experiment. Consequently, 1.4% sodium bicarbonate was used during the placebo infusion period.

The experiment started with a single-blind infusion of placebo, and then the diuretic was infused for 10 min at each of five increasing rates (0.1, 0.3, 1.0, 3.0 and 10.0 μg/min per 100 ml forearm tissue). During the last minute of each infusion period of 10 min, an ipsilateral venous blood sample was taken and plasma diuretic concentrations were measured by chromatography [13].

**Statistics**

All data are expressed as means±SEM. Forearm blood flows were averaged from the last 8 min of each infusion period. Ratios of forearm blood flow were calculated by dividing the ipsilateral by the contralateral forearm blood flow.

Analysis of variance for repeated measures was used to test for differences in forearm blood flow during the increasing drug concentrations. P<0.05 (two-sided) was considered significant.

**Results**

Ratios of infused to control forearm blood flow and ipsilateral venous plasma concentrations of hydrochlorothiazide are shown in Fig. 1. During five increasing doses of hydrochlorothiazide there was no significant effect on forearm blood flow compared to the placebo infusion. Using the data from the last 2 min of infusion (instead of the last 8 min) did not change these results. The vascular resistance (quotient of blood pressure and forearm blood flow) of the hydrochlorothiazide-infused forearm averaged 68.7±6.5 AU during placebo, rising to 86.6±9.2 AU at the highest dose (NS). Regional hydrochlorothiazide plasma concentrations ranged from 0.04±0.003 at the lowest dose to 3.5±0.3 μg/ml (comparable to 10 μmol/l) at the highest dose. The hydrochlorothiazide infusion of 20 min in four subjects showed no significant effects on forearm blood flow or vascular resistance.

Intra-arterial blood pressure and heart rate (respectively 124±4/64±2 mmHg and 58±2 beats/min during the placebo and 128±4/67±2 and 58±3 during the highest hydrochlorothiazide dose) did not change significantly during the experiments.
Discussion

In contrast to in vitro experiments by our group [8] and others [7,9,10], and studies on vasoactivity after long-term systemic administration [2-4], the present study showed that the intra-arterial administration of hydrochlorothiazide exerted no direct effects on the forearm vascular bed of male normotensive volunteers. As expected with this technique, there were no blood pressure or heart rate changes during the experiments to suggest any acute effect. So, despite considerable evidence that hydrochlorothiazide exerts direct vasoactivity, our in vivo findings suggest that in pharmacologically relevant concentrations hydrochlorothiazide is not directly vasoactive in the forearm vascular bed.

At the highest infusion rate, the mean plasma concentration reached $3.5 \pm 0.3 \mu g/ml \approx 10 \mu mol/l$. This concentration resulted in a $60 \pm 10\%$ vasodilation in vitro of human subcutaneous resistance vessels [7] and was $10-30$ times the therapeutic plasma concentration [8]. The protein-bound fraction was not measured, but was expected to be in the normal range of 22% [15] to 55% [16]. Thus, even after correction for the protein-bound fraction, the drug concentration remained well above therapeutic levels.

There are a number of potential explanations for this discrepancy between our in vitro and in vivo results. First, the in vitro experiments were performed on resistance arteries from subcutaneous fat or the mesenteric vascular bed. In contrast, the current in vivo experiments predominantly refer to resistance arteries of skeletal muscle. Second, during in vitro experiments, hydrochlorothiazide can gain access to the outside of the vessel, allowing direct exposure of the drug to the vascular smooth muscle cell, instead of the physiological condition of exposure from the inside of the vessel to the endothelium as in the perfused forearm technique. Apart from being an anatomical barrier, the endothelium is important in modulating vascular tone, which could be of significant importance. The possibility that hydrochlorothiazide may influence Ca$^{2+}$-activated K$^+$ channels in endothelial cells as well as vascular smooth muscle cells and so affect endothelial modulation of tone cannot be excluded.

Third, it has been established previously [3,4] that in vivo chronic chlorothiazide administration attenuated the vasoconstrictor response of forearm vessels to noradrenaline. Therefore, the mechanism of thiazide-induced vasodilation in vivo might be a drug-induced reduction in the effect of endogenous vasoconstrictor influences, such as norepinephrine, angiotensin II or sympathetic nervous tone. In patients with essential hypertension, who are known to have an increased vascular reactivity, the vascular effects of hydrochlorothiazide might be more pronounced than in normotensive subjects. This suggestion is in accordance with observations that the antihypertensive action of hydrochlorothiazide is related to the severity of the hypertension. This implies that hydrochlorothiazide has no hypotensive action in normotensive subjects [17] and may therefore exert no vascular action in these subjects.

In conclusion, in contrast to our in vitro observations, hydrochlorothiazide did not have a direct vasorelaxant effect in skeletal muscles in vivo in normotensive subjects.

References