

Short Communication

Hydrochlorothiazide increases interleukin-1 beta (IL-1 β) secretion by peripheral blood mononuclear cells in healthy subjects

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Recent study shows close relationship between hypertension and inflammation. The concentration of inflammatory mediators is increased in patients with essential hypertension. Angiotensin II (Ang II) may contribute to inflammatory process. Previous studies showed that individuals with essential hypertension had increased interleukin-1beta (IL-1 β) secretion by peripheral blood mononuclear cells (PBMCs) and also valsartan and simvastatin reduced this inflammatory marker. In this study, the effect of hydrochlorothiazide on IL-1 β secretion by PBMCs in healthy subjects was investigated. PBMCs in healthy subjects were isolated by gradient centrifugation. After incubation with Ang II and hydrochlorothiazide, IL-1 β concentrations in supernatant from PBMCs were measured by enzyme-linked immunosorbent assay (ELISA). When compared with the control group, hydrochlorothiazide (10^{-8} , 10^{-9} M) increased secretion of IL-1 β from PBMCs after stimulation by Ang II. Hydrochlorothiazide may increase inflammatory mediator secretion from PBMCs.

Key words: Hydrochlorothiazide, hypertension, interleukin-1 beta, peripheral blood mononuclear cells (PBMCs).

INTRODUCTION

Inflammation plays a pivotal role in the genesis of hypertension and its complications (Li et al., 2007). Peripheral blood monocytes (PBMCs) were preactivated in patients with essential hypertension (EH) and produced inflammatory mediator like interleukin-1 beta (IL-1 β) (Dorffel et al., 1999). IL-1 β is one of the main regulators of the immune and inflammatory response with a lot of proinflammatory properties (Andrzejczak et al., 2007). It was shown that individuals with essential hypertension had increased IL-beta secretion in human peripheral blood mononuclear cells (PBMCs) after been stimulated by angiotensin II (Ang II) (Zhao et al., 2004). Ang II, the key effector of the rennin-angiotensin system, is also capable of inducing inflammatory response in the vascular wall. Ang II enhances the production of reactive

oxygen species through stimulation of NAD(P)H oxidase. Increased oxidative stress act as signal transduction messengers for several important transcription factors including nuclear factor-kappaB (NF-kappaB), which is a pivotal transcription factor in chronic inflammatory diseases. NF-kappaB regulates the transcription of genes for pro-inflammatory cytokines, adhesion molecules and chemokines (Cheng et al., 2005).

It has been previously shown that some drugs used in cardiovascular diseases, such as valsartan (Li et al., 2005) and simvastatin (Zhao et al., 2004), could reduce IL-1 β secretion in PBMCs of patients with essential hypertension. Thiazides are also used to treat hypertension. In this study, we investigated the effect of hydrochlorothiazide (HCT) on the secretion of IL-1 β by PBMCs in healthy subject.

MATERIALS AND METHODS

Blood samples were taken from fasting healthy volunteers in the

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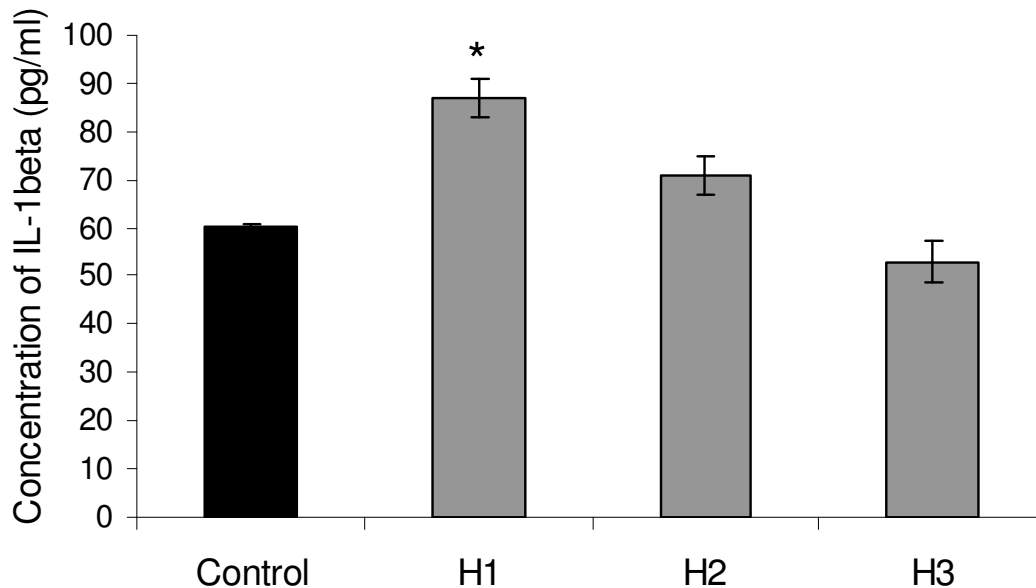


Figure 1. The effect of hydrochlorothiazide (H1 = 10^{-9} , H2 = 10^{-8} , H3 = 10^{-7} mol/L) on secretion of IL-1 β (pg/ml) by PBMCs after stimulation by Ang II (10^{-10} mol/L). PBMCs were obtained from healthy volunteers. After 24 h of incubation, IL-1 β concentrations in supernatant from PBMCs were measured by ELISA. *P < 0.05 vs. control group.

morning. None of the participants had diabetes mellitus, macroproteinuria, creatinemia, hypothyroidism, abnormal liver and muscle enzymes, acute and/or chronic infections, autoimmune or neoplastic diseases. None had a history of cardiovascular disease or were taking medication or other agents known to affect inflammatory response. None of them were smokers. Informed consent was obtained from all participants. The protocol was approved by the local ethical committee.

Isolation of peripheral blood mononuclear cells

Peripheral blood was immediately drawn into sterile 15 ml containing sodium heparin, layered onto an equal volume of Ficoll and centrifuged at $1500 \times g$ for 20 min. Cells were then harvested from the Ficoll-plasma interface and washed three times in RPMI1640 medium (Gibco-BRL) containing 2 mmol/L glutamine (Sigma), 10% heat-inactivated fetal calf serum (Gibco-BRL), 100 U/ml penicillin and 100 μ g/ml streptomycin. Cells were suspended at 1×10^6 /ml in RPMI1640 supplemented as above. Cell viability was always >95%, as estimated by trypan blue exclusion. The cell suspension was plated at 1 ml per well in 24-well flat-bottomed tissue culture plates. Then, they were incubated with or without a physiologically relevant concentration of Ang II (10^{-10} ml/l) at 37°C in 95% humidified air and 5% CO₂. In additional experiments, cells were preincubated with hydrochlorothiazide (HCT, 10^{-7} , 10^{-8} , 10^{-9} mol/L). After 24 h, cell supernatants were harvested and stored at -70°C for cytokine analysis (Li et al., 2005).

Statistical analysis

Results were presented as mean \pm SEM. Differences between control and test values were determined by Student's *t* test and were accepted as significant when $p < 0.05$.

RESULTS AND DISCUSSION

Figure 1 demonstrates the effect of HCT (H1 = 10^{-9} , H2 = 10^{-8} , H3 = 10^{-7} mol/L) on the secretion of IL-1 β (pg/ml) by PBMCs after stimulation by Ang II (10^{-10} mol/L). The results show that HCT, at concentration of 10^{-9} mol/L, significantly increased IL-1 β secretion when compared with the control group. Although another concentration (10^{-8} mol/L) also increased IL-1 β secretion, the difference was not significant when compared with the control group.

In this study, we investigated the effects of HCT on IL-1 β secretion by PBMCs obtained from healthy subjects. The results show that HCT at concentration of 10^{-9} mol/L significantly increased IL-1 β secretion stimulated by Ang II (10^{-10} mol/L) to 43%. Although the level of increment was about 17% for the concentration of 10^{-8} mol/L, the difference was not statistically significant when compared with the control. The concentration, 10^{-7} mol/L of HCT not only did not increase IL-1 β secretion but also a rather non-significant decrease was observed. The latter concentration was not clinically relevant because the peak achievable concentration of HCT was about 10^{-8} mol/L (Brunton et al., 2006).

Prior reports have emphasized the importance of inflammation in hypertension. The data raises a possible role of anti-inflammatory therapy as an experimental means to reduce risk in patients with hypertension. On the contrary, any agent capable of stimulating inflammatory responses might worsen the condition. The findings

of this preliminary study indicate that clinically relevant concentrations of HCT could increase IL-1 β secretion by PBMCs. Future studies may clarify administration in patients with essential hypertension. HCT might result in aggravation of inflammatory processes in vascular wall and worsen the condition in long term.

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