Effects of losartan treatment on T-cell activities and plasma leptin concentrations in primary hypertension

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Abstract

Recent evidence shows that leptin may contribute to elevated blood pressure (BP) and interact with the renin-angiotensin-aldosterone and cellular immune systems. Altered T-cell activities and changes in T-cell subset ratios have also been reported in hypertension. However, little is known about the effects of AT_1-receptor antagonism on T-cell activities and plasma leptin concentrations in primary hypertension. We have, therefore, investigated the relationship between leptin and T-cell activities and the effect of an AT_1-receptor antagonist, losartan, in primary hypertension. Twenty recently-diagnosed and untreated young adults (11 males and 9 females, age; 39.9±7.6 years, range 23–49 years, BMI; 27.6±3.7 kg/m^2) and 20 normotensive healthy, age-, sex- and BMI-matched controls were studied. The [^{3}H]-thymidine uptakes of cultured lymphocytes were determined, both spontaneously and after stimulation with phytohaemagglutinin. The tests were performed before and after three months of treatment with losartan. The results indicate that the blastogenic responses of T-cells to phytohaemagglutinin are significantly higher in the patient group compared with controls (p=0.02). After normalisation of BP, T-cell responses were significantly reduced and were lower than in the controls (p=0.01). Pretreatment plasma leptin levels were significantly higher in hypertensives than in controls (p=0.01). However, losartan treatment had no significant effect on leptin concentrations; moreover, no correlation between leptin levels and T-cell activity was found. Our data show that plasma leptin levels and T-cell activity are markedly enhanced in untreated essential hypertension and that the alteration of T-cell activity is not related to plasma leptin levels. Antihypertensive treatment with losartan decreases T-cell activities but does not influence plasma leptin levels. We conclude that leptin levels are not affected by AT_1-receptor blockade and are not related to T-cell activity.

Introduction

The pathophysiology of primary hypertension is still an enigma. In addition to many well-known factors, recent evidence shows that *ob* gene product, leptin, may contribute to elevated blood pressure (BP). There are adequate data that refer to the elevation of plasma leptin levels in primary hypertension.[1,2] Chronic leptin infusion increases arterial pressure in rats by an unknown mechanism, which may involve both central and peripheral affects.[3] The major contribution of leptin to high BP seems to be related to its sympatho-excitatory action.[3,4]

Recent data suggest a probable interaction between leptin and the renin-angiotensin-aldosterone system (RAAS), which is another major regulator of BP. Both angiotensinogen and the enzymes involved in its conversion to angiotensinogen II are expressed in human adipose tissue[5–7] and it is not surprising that obese people have high angiotensinogen levels.[8,9] Schorr *et al.* reported that leptin, angiotensinogen and BP were positively correlated in normotensive young men. Interestingly, they found that subjects with a positive family history of hypertension tended to have higher leptin and angiotensinogen levels than subjects with a negative family history.[10] Current reports suggest that this correlation between leptin and angiotensinogen levels is also present in primary hypertension.[2,10] We have recently shown that this relationship is present not only in obese, but also in lean hypertensive patients.[11] We have also demonstrated that human leptin deficiency is associated with alterations in the RAAS.[12] Moreover, reduced plasma leptin levels were reported in primary aldosteronism[13] and chronic leptin infusion in rats evoked a clear-cut increase in plasma aldosterone levels.[14] Thus, these findings strongly indicate that there is an interaction between leptin and the RAAS, which may represent an additional mechanism in the elevation of BP by leptin. However, little is known about the effects of AT_1-receptor antagonism on plasma leptin levels.

In addition to its roles in the regulation of neuroendocrine, metabolic, reproductive and haematopoietic functions, a novel function of leptin in the orchestration of cellular immune responses has been demonstrated.[15–17] Recent data has shown that both CD4(+) and CD8(+) T-cells express leptin receptors and that leptin directly enhances the proliferation and activation of circulating human T-cells.[17] Previous studies have also shown that lymphocytes express AT_1-receptors[18] and are activated by angiotensin II (Ang II).[19,20] Blood mononuclear cells, with the help of this 'mobile' RAAS, may join in the pathophysiology of various cardiovascular events, including hypertension and atherosclerosis.[21]

T-cell activation studies, performed in spontaneous hypertensive rats (SHR) and human hypertensives, have demonstrated depressed proliferative responses to specific lectins.[22–25] However, the

contribution of these lowered T-cell activities to increased BP remains uncertain. Both the total T-cell and the non-helper T-cell populations were depressed in the SHR and this was inferred to be a primary mechanism in the development of hypertension.[26] However, in hypertensive humans the total numbers of T-cells and T-helper cells were depressed and the proportion of CD22 cells were increased, compared with controls.[27] This finding was speculated to be the consequence of an undefined antigenic stimulus that may activate both T- and B-cells.[27] In addition to the alteration of activity and ratio of T-cells, elevated levels of immunglobulins[28,29] and increased autoantibodies[22] were also reported in primary hypertension. Alterations in immunological reactivity were suggested to contribute to the initiation and the maintenance of primary hypertension.[30,31] However, these studies were conducted in a heterogeneous group of patients, who were either elderly or had a long duration of hypertension, who might be predicted to have already developed vascular complications. Besides, most of these patients were on antihypertensive medications that may have significantly influenced the observed findings.[32-35] As the current data is inadequate, we aimed to evaluate the T-cell activities and lymphocyte subpopulations of young, uncomplicated and untreated patients with primary hypertension. Our further quest was to investigate the relationship between plasma leptin levels and T-cell activities, both before and after treatment with losartan.

Materials and methods

Twenty hypertensive patients (11 males and 9 females, mean age; 39.9±7.6 years, range 23–49 years, mean BMI; 27.6±3.7 kg/m^2, waist/hip ratio; 0.9±0.07) and 20 age-, sex- and BMI-matched normotensive subjects (10 male and 10 female, mean age 40.0±4.3 years, range 22–46 years, mean BMI; 26.3±3.6 kg/m^2, waist/hip ratio; 0.9±0.09) were enrolled in the study. None of the subjects were on any medication or dietary restriction. They reported no alterations of body weight for at least three months before the study. All patients underwent routine investigations for secondary causes of hypertension and accompanying metabolic, hepatic, renal and psychiatric diseases. No patient had any complications of hypertension, including retinopathy (higher than Keith Wegener classification grade I), nephropathy, or cardiomegaly.

The diagnosis of hypertension was based on the criteria of the Sixth report of Joint National Committee.[36] All patients were given losartan, 50 mg once-daily. Plasma leptin measurements, phenotypic analyses of lymphocytes and lymphocyte cultures were performed, both before and three months after the treatment. Plasma leptin levels were measured by radioimmunoassay (Human Leptin RIA Kit, Linco Research Inc., St. Louis, MO, USA). Fasting blood samples were collected via an intravenous catheter in ethylendiamine tetraacetate-coated venepuncture tubes, promptly centrifuged, separated and stored at –70°C until leptin assays were performed. All plasma samples were run in the same assays in duplicate. The assay sensitivity was 0.5 ng/ml. The intra-assay coefficient of variation (CV) of the assay at 4.9 ng/ml was 8.3% (n=5) and at 25.6 ng/ml was 3.4% (n=5).

Lymphocytes were isolated from heparinised venous blood by Ficoll-isopaque gradient centrifugation, according to Boyum.[37] Cells were washed three times with phosphate-buffered saline, resuspended in Sigma medium-199 and supplemented with streptomycin (100 mg/L), penicillin (100,000 U/L) and $NaHCO_3$ (2.2 g/L). Adding 20% of autolog plasma, 2x10^6/ml of human peripheral blood lymphocytes were cultivated in 96-well microtitre plates in a total volume of 10^5 cells/well. Phytohaemagglutinin (PHA;Seromed), at a final concentration of 10 µg/ml, was added, and cells were incubated for 72 hours at 37°C in 5% CO_2. 0.5 µCi of [^{3}H]-thymidine (Amersham) was then added to cells and incubation continued for a further 4 hours, after which cells were harvested using an automatic cell harvester (PHD cell harvester, Cambridge Technology). The amount of radioactive thymidine was measuerd by a beta-counter (Packard); the results were expressed as cpm of [^{3}H]-thymidine incorporation. In order to compare the relative increase in [^{3}H]-thymidine incorporation into DNA in the presence and absence of mitogen, the stimulation index (SI=cpm of lymphocytes with PHA / cpm of lymphocytes without PHA) was calculated.

The lymphocyte subpopulations and the phenotypes of total T-cells were determined by a direct immunofluorescence method, using monoclonal antibodies (Simultest IMK-Lymphocyte, Becton Dickinson, 340182, Belgium). Whole blood samples were studied immediately after venepuncture. The stained samples were then run on the flow-cytometer (FACSCalibur, Becton Dickinson) and analysed with Simultest IMK-Lymphocyte software.

Statistical analysis

All results are reported as the mean±SD. Differences between groups were tested for significance by the Mann Whitney-U test. To analyse the relationship between variables, Pearson's correlation was used. The criterion of significance was $p<0.05$ for both differences and correlations.

Results

All the patients tolerated the drug well and no severe side-effects were reported during the treatment period. Although both the systolic and diastolic BPs were significantly reduced after treatment, BMI and waist-hip ratios did not change significantly. Plasma leptin levels in patients with primary hypertension were significantly higher than in controls (Table 1).

The spontaneous [^{3}H]-thymidine uptake and SI of the lymphocytes of patients were not statistically different in comparison with controls. Stimulation with PHA significantly enhanced the [^{3}H]-thymidine uptake of lymphocytes of patients compared with controls. After BP-lowering, both the T-cell responses to PHA and the SI were significantly

Table 1 The clinical and laboratory features of the patient and control groups.

Parameters	Patients (n=20)		Controls (n=20)
	Before treatment	After treatment	
Age (year)	39.9±7.6		40.0±4.3
M/F	11/9		10/10
Waist/hip ratio	0.9±0.07	0.9±0.08	0.9±0.09
BMI (kg/m^2)	27.6±3.7	27.0±2.8	26.3±3.6
Systolic BP (mmHg)	159.6±15.7[a]	129.6±11.8[b]	110.2±15.2[c]
Diastolic BP (mmHg)	108.2±10.3[d]	87.5±5[e]	85.7±11.2[f]
Leptin (ng/ml)	15.30±4.75[g]	14.73±10.77[h]	9.33±2.20[i]

BP = blood pressure; BMI = body mass index; M = male; F = female; p>0.05 for BMI and waist/hip ratios of the groups; p=0.008 for [a] *vs.* [b]; p=0.0001 for [a] *vs.* [c]; p=0.05 for [b] *vs.* [c]; p=0.002 for [d] *vs.* [e], p=0.0001 for [d] *vs.* [f]; p>0.05 for [e] *vs.* [f] and [g] *vs.* [h]; p=0.01 for [g] *vs.* [i] and [h] *vs.* [i]

Table 2 [^{3}H]-thymidine uptake of T-cells in patient and control groups.

[^{3}H]-thymidine uptake (cpm)	Patients (n=20)		Controls (n=20)
	Before treatment	After treatment	
Spontaneous	501.1±108.0	534.5±169.2	496±53.4
Phytohaemagglutinin	54886±8155[a]	43933±2206[b]	47045±5471[c]
Stimulation index	109.5±6.3[d]	73.899±21.6[e]	95.8±15.4[f]

p>0.05 for spontaneous T-cell activities of the groups; p=0.002 for [a] *vs.* [b]; p=0.02 for [a] *vs.* [c] and [d] *vs.* [e]; p=0.01 for [b] *vs.* [c] and [e] *vs.* [f]; p>0.05 for [d] *vs.* [f]

Table 3 The comparison of the lymphocyte subgroups of the patient and control groups.

Lymphocyte subgroups	Patients (n=20)		Controls (n=20)
	Before treatment	After treatment	
T-cell (CD3$^+$)	72.66%	73.47%	76.45%
T-helper (CD4$^+$)	39.62%	40.63%	37.12%
T-suppressor (CD8$^+$)	30.74%	28.36%	28.02%
Helper/suppressor (CD4$^+$/CD8$^+$)	1.41	1.57	1.23
B-cell (CD19$^+$)	10.07%	10.94%	11.45%
Natural killer (CD16$^+$+CD56$^+$)	15.40%	13.84%	12.25%

*p>0.05 for all groups

suppressed and no significant change was observed in the spontaneous [^{3}H]-thymidine uptake after treatment. The final activated T-cell responses to PHA and SI in the patients were significantly lower than in the control group (Table 2).

Regarding the lymphocyte subgroups, no significant difference was present between patients and controls, both before and after treatment (Table 3).

No correlation was found between plasma leptin levels and T-cell activities, BP, or lymphocyte subgroups in both groups. Similarly, no association between BP and T-cell activities could be demonstrated.

Discussion

Leptin, the product of the *ob* gene, has recently been suggested to be implicated in primary hypertension, though its role remains elusive. In addition to well-established effects on the sympathetic nervous system,[3,4] there may be several other ways by which leptin may increase BP. Since there is evidence of a local renin-angiotensin system in human adipose tissue,[5,6] and a correlation between leptin and angiotensinogen has been shown in patients with primary hypertension,[2,10,11] it is likely that leptin and the RAAS may be associated in the development of high BP. However, according to the results of the present study, plasma leptin levels, that were significantly higher in the study group, did not change despite the significant reduction of BP after treatment with losartan, suggesting that AT_1-receptor blockade does not have a direct effect on plasma leptin levels. From a different point of view, it may also be inferred that BP-lowering may not affect plasma leptin levels in primary hypertension.

It has already been demonstrated that primary hypertension is associated with alterations of lymphocyte subtypes and T-cell activity.[22–25] Also, in patients with primary hypertension, delayed-type hypersensitivity to vascular antigens has been

shown.[38] However, reports concerning the distribution of the lymphocyte subtypes and T-cell activity are conflicting. In SHR, both the total T-cell and the non-helper T-cell populations were found to be depressed, and this was inferred to be a primary mechanism in the development of hypertension.[26] However, in human hypertension, it has been shown that total T-cells and T-helper cells were depressed and the proportion of CD22 cells were increased compared with controls.[27] This finding was speculated to be the consequence of an undefined antigenic stimulus that may activate both T- and B-cells.[27] However, in another study it was reported that the lymphocyte subgroups of the patients with primary hypertension were within the normal limits of the healthy population.[25] Some hypotheses have been suggested regarding the involvement of the cellular immune system in the pathogenesis of primary hypertension. Our data show that the lymphocyte subgroups were not different between the patient and control groups, and also that there was no correlation between lymphocyte subgroups and either plasma leptin levels or BP, either before or after treatment with losartan. From the above data, it is not likely that primary hypertension is related to the alterations of lymphocyte subgroups. Additionally, it was established that AT_1-receptor antagonism has no effect on the distribution of lymphocytes. On the other hand, like plasma leptin levels, T-cell activity was also significantly higher in primary hypertension when compared with controls, though there was no correlation between them. From these data, it is hard to state that plasma leptin levels are the main determinant of T-cell activity in patients with primary hypertension. Moreover, neither plasma leptin levels nor T-cell activities were correlated with BP in either group. Despite the significant elevation of T-cell activities and plasma leptin concentrations, the lack of their association with BP suggests that these factors may affect BP by indirect ways, though the mechanisms are not clear.

The main result of this study is the reduction of activated T-cell responses after treatment with losartan. Regarding the former reports, this result from the present study is not surprising. Kunert *et al.*, in an *in vitro* study, reported that Ang II activates mouse lymphocytes and that losartan inhibits this effect.[20] Hahn *et al.* also stated that human peripheral monocytes are activated by Ang II and that this effect was blocked by the addition of an AT_1-receptor antagonist, ZD 7155, to the culture medium.[19] Previous studies regarding the inhibitory effect of losartan on T-cell activity were performed in healthy subjects and, to our knowledge, our study is the only one performed in primary hypertension. Interestingly, the evidence implies that AT_1-receptor antagonists and ACE inhibitors may have different effects on T-cell activity. Captopril was reported to enhance PHA-induced lymphocyte thymidine uptake in healthy subjects.[39] In another study, the T-cell activity of six hypertensive patients was increased as a result of treatment with captopril.[35] With regard to the evidence about the role of activated mononuclear cells in the pathogenesis of hypertension and atherosclerosis,[31,40] the inhibitory effect of losartan on enhanced T-cell activity may offer favourable outcomes in the management of primary hypertension and the prevention of vascular outcomes.

Our data show that plasma leptin levels and T-cell activity are markedly enhanced in untreated primary hypertension, and that the alteration of T-cell activity is not related to plasma leptin levels. Antihypertensive treatment with losartan decreases T-cell activity, but does not influence plasma leptin levels. We conclude that leptin levels are not affected by AT_1-receptor blockade and are not related to T-cell activity.

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