Journal of Human Hypertension (2008) 22, 141–143

www.nature.com/jhh

RESEARCH LETTER

'Induction of inflammatory gene expression by THP-1 macrophages cultured in normocholesterolaemic hypertensive sera and modulatory effects of green tea polyphenols'

Journal of Human Hypertension (2008) **22,** 141–143;
doi:10.1038/sj.jhh.1002277; published online 30 August 2007

Hypertension is a disorder controlled by multiple genes and inflammation and vascular remodelling of arteries have been implicated in pathogenesis of this disease. Green tea polyphenols (GrTPs) are rich in antioxidants and are known to inhibit inflammatory responses. A significant time-dependent increase in mRNA expression of both IL-6 and MMP-9 were observed in THP-1 macrophages when cultured in normocholesterolaemic hypertensive sera ($P<0.05$). Treatment with GrTPs, significantly abolished the induced expression of IL-6 and MMP-9 in a time-dependent manner ($P<0.05$). Our studies show presence of some humoral factors in hypertensive sera, which may be responsible for changing the phenotype of THP-1 macrophages to express genes with atherosclerotic potential.

Inflammation and vascular remodelling are implicated in the pathogenesis of essential hypertension and may contribute to the development and complications of hypertension.[1] In uninjured arteries and veins, matrix metalloproteinases (MMPs), are constitutively expressed, and are responsible for degradation of extracellular matrix scaffold. GrTPs, rich in antioxidants, have been shown to inhibit inflammatory response and, therefore, may be useful in preventing cardiovascular disorders.[2]

Therefore, in the present study, given the potential roles of inflammatory cytokines (IL-6) and MMPs (MMP-9) in cardiovascular diseases, we sought to determine, whether, serum from normocholesterolaemic hypertensive subjects would induce expression of these genes in THP-1 macrophages *in vitro*. We then used GrTPs *in vitro* as a therapeutic approach to delineate its effects on transcriptional expression of these genes.

The study involved two groups of human subjects as participants after obtaining a prior written informed consent. The blood pressure was recorded as per JNC VI recommendations.[3] Twenty patients, freshly diagnosed as hypertensives and never been on any antihypertensive, anti-inflammatory or antioxidant drugs were recruited as Group I. A total of 20 normal, healthy, age and sex-matched volunteers belonging to the same ethnic and socio-economic background were enrolled as controls (Group II). Serum from each subject was analysed for lipid profiles and for determining the circulating levels of IL-6, MMP-9 and hsCRP using ELISA. Sera from individual subjects were heat inactivated, filtered through 0.22 μm filter and stored at −20 °C till further use for *in vitro* experiments. All *in vitro* experiments were performed with serum from individual subjects. The human leukaemic monocytic cell line (THP-1) was obtained from the National Center for Cell Sciences (NCCS), Pune, India. The cells were maintained in RPMI-1640 media supplemented with 10% heat-inactivated calf serum, 2 mM L-glutamine, 100 μg ml^{-1} streptomycin and 100 U ml^{-1} of penicillin at 37 °C in a humidified 5% CO_2 incubator.

Green tea polyphenols (GrTPs) were isolated and purified through HPLC.[4] A stock of 50 mg ml^{-1} GrTPs dissolved in distilled water was used. THP-1 cells were differentiated to macrophages in the presence of 5 ng ml^{-1} phorbol-12-myristate-13-acetate for 18–24 h.[5] For further experiments with GrTPs, the differentiated macrophages were cultured in media supplemented with 10% control or hypertensive sera instead of 10% calf serum for different time intervals, that is 12, 24 and 48 h. Time- and dose-dependent studies were carried out initially,[4,6] where a dose of 50 μg ml^{-1} GrTPs was found to be effective.

Semi-quantitative RT-PCR was performed for determining mRNA expression of IL-6 and MMP-9 in THP-1 macrophages. mRNA expression levels were calculated by normalizing the band intensities of the test gene (Using Scion Image Analyzer) to that of β-actin and expressed as percentage value. GrTPs in different doses had no effect on either cell count or cell viability of THP-1 cells. Also, no morphological abnormalities were observed. The data were analysed using Student's paired and unpaired '*t*'-test.

Mean systolic and diastolic blood pressure was found to be significantly higher in Group I as

compared to Group II ($P<0.05$). No significant difference in lipid or lipoprotein profile was observed between the two groups confirming that, both groups were normolipidaemic. Significantly augmented circulating levels of IL-6 (14.9 ± 3.8 vs $8.29\pm2.29\,pg\,ml^{-1}$; $P<0.05$) and hsCRP (3.84 ± 4.93 vs $0.15\pm0.21\,\mu g\,ml^{-1}$; $P<0.05$) and higher levels of MMP-9 (489 ± 151.99 vs $301.0\pm123.57\,ng\,ml^{-1}$; $P>0.05$) were found in hypertensives as compared to normotensives.

A time-dependent increase in IL-6 and MMP-9 gene expression was observed in macrophages cultured in hypertensive sera, the maximum being at 48 h, whereas, no significant effects of normotensive sera were observed (Figure 1). GrTPs in a dose of $50\,\mu g\,ml^{-1}$, significantly attenuated mRNA expression of these genes ($P<0.05$) (Figure 1). No significant alteration in expression of these genes was observed on cells cultured in control sera and treated with GrTPs. Comparison between the two groups showed that the results were significant ($P<0.05$).

Hypertension may promote endothelial expression of cytokines and stimulate inflammation,[7] conversely, inflammation may play a role in the pathogenesis of hypertension or it may characterize a functional state of the vessel wall as a consequence of high blood pressure. It remains to be determined whether there exists any causal relationship between the two conditions. Considering the potentially important roles of proinflammatory molecule IL-6 and MMPs, our findings provide *in vitro* evidence that predisposition to hypertension may be related to activation of inflammatory pathways and enzymes *in vivo*. Our observations demonstrated significant induction of IL-6 and MMP-9 expression in THP-1 macrophages by normocholesterolaemic hypertensive sera. The induction of these genes by lipids can be ruled out as all the subjects were normocholesterolaemic. We can, therefore, hypothesize that increased IL-6 may be a stimulus for inflammation in early hypertension as it stimulates hepatic production of C-reactive protein.[8] Corroborating this statement, we also observed high-hsCRP levels in hypertensives. It is possible that CRP in sera of hypertensive patients acted as an induction factor for these molecules. The exact mechanisms by which normocholesterolaemic hypertensive sera induces expression of IL-6 and MMP-9 in THP-1 macrophages may require further investigations. Further, GrTPs significantly attenuated the induced expression of proinflammatory genes *in vitro*. Our study clearly demonstrates that THP-1 macrophages responded to certain humoral factors present in the hypertensive sera for induction of genes for IL-6 and vascular remodelling.

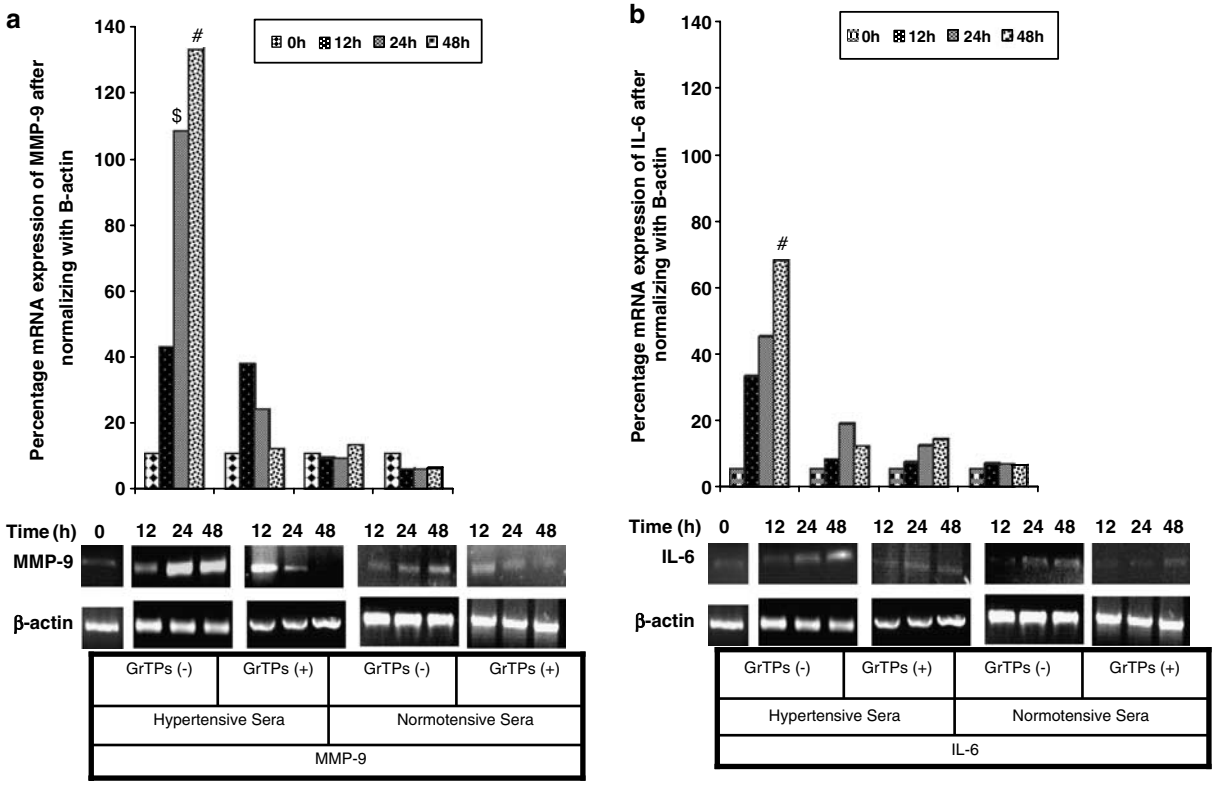

Figure 1 Depicts the mRNA expression of matrix metalloproteinases-9 (**a**) and inflammatory cytokines-6 (**b**) by the human leukaemic monocytic cell line-1 macrophages cultured in normocholesterolaemic hypertensive and normotensive serum in absence (−) and presence (+) of green tea polyphenols at 0, 12, 24 and 48 h, as determined by semi-quantitative RT-PCR (the mean of at least three independent experiments). $^{\$}P<0.05$ (12 vs 24 h), $^{\#}P<0.05$ (12 vs 48 h).

Besides other yet unidentified humoral factors present in the serum, the role of CRP cannot be ruled out.

We, therefore, hypothesize that normocholesterolaemic hypertensive sera induces proinflammatory cytokines and MMP-9 gene expression in THP-1 macrophages and GrTPs successfully inhibited this expression. Our study, points to a potential causal relationship existing between hypertension, inflammation and vascular remodelling in a cohesive and integrated manner. Understanding this correlation would provide novel opportunities to prevent and treat the disease. However, this study warrants further investigations *in vivo*.

What is known about this topic:

- Inflammation may play role in the pathogenesis of hypertension
- Proinflammatory cytokines and matrix metalloproteinases are implicated hypertension
- GrTPs are rich in antioxidants and inhibit inflammatory response

What this study adds:

- Significant induction of IL-6 and MMP-9 expression in THP-1 macrophages *in vitro* by serum of normocholesterolaemic hypertensive patients
- Demonstrated that THP-1 macrophages respond to certain humoral factors in the hypertensive sera for induction of genes involved in inflammation and vascular remodeling
- GrTPs significantly and successfully attenuated the induced expression of these inflammatory genes

Abbreviation: GrTPs, green tea polyphenols.

Acknowledgements

We acknowledge ICMR, New Delhi for providing funding for our research work.

N Mahajan[1], V Dhawan[1], G Sharma[1], S Jain[2] and D Kaul[1]

[1]*Department of Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India and*

[2]*Department of Internal Medicine, Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India*

E-mail: veenad2001@yahoo.com

Published online 30 August 2007

References

1 Intengan HD, Schiffrin EL. Vascular remodeling in hypertension, role of apoptosis, inflammation and fibrosis. *Hypertension* 2001; **38**: 581–587.

2 Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavanoids and risk of coronary heart disease: The Zutphen Elderly study. *Lancet* 1993; **342**: 1007–1011.

3 JNC-VI. The sixth report of Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure. *Arch Intern Med* 1997; **157**: 2413–2446.

4 Kaul D, Shukla AR, Sikand K, Dhawan V. Effect of herbal polyphenols on atherosclerotic transcriptome. *Mol Cell Biochem* 2005; **278**: 177–184.

5 Worley JR, Baugh MD, Hughes III DA, Edwards DR, Hogan A, Sampson MJ *et al.* Metalloproteinase expression in PMA stimulated THP-1 cells: effects of PPARγ agonists and 9-cis-retinoic acid. *J Biol Chem* 2003; **278**: 51340–51346.

6 Kaul D, Sikand K, Shukla AR. Effect of green tea polyphenols on genes with atherosclerotic potential. *Phytother Res* 2004; **18**: 177–179.

7 Liu Y, Liu T, McCarron RM, Spatz M, Feuerstein G, Hallenbeck JM *et al.* Evidence for activation of endothelium and monocytes in hypertensive rats. *Am J Physiol* 1996; **270**: H2125–H2131.

8 Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; **265**: 621–636.