Combination oral antioxidant supplementation reduces blood pressure

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1. Hypertension affects 30% of adults and low intakes of antioxidants have been associated with increased risk of hypertension and cardiovascular disease. To investigate the effect of short-term high-dose antioxidant supplementation on blood pressure in hypertensive and normotensive outpatients, we undertook a randomized, double-blind, crossover design placebo-controlled study.

2. Forty subjects were recruited from medical outpatient clinics, of whom 38 completed the study. Twenty-one were attending for treatment of hypertension and 17 were normotensive, attending for minor gastrointestinal complaints. Subjects were randomly assigned to receive either 8 weeks placebo followed by 2 weeks washout then 8 weeks antioxidants or vice versa. The combination of antioxidants consisted of 200 mg of zinc sulphate, 500 mg of ascorbic acid, 600 mg of α-tocopherol (sodium succinate salt) and 30 mg of β-carotene daily.

3. Systolic blood pressure fell at the end of the antioxidant phase compared with the placebo phase both in subjects receiving anti-hypertensive therapy (P < 0.01) and those who were normotensive (P = 0.067). Circulating levels of β-carotene and α-tocopherol increased in all subjects during supplementation (P < 0.01) and urine nitrite increased in hypertensive patients (P < 0.05).

4. Short-term oral high-dose combination antioxidant therapy reduces blood pressure, possibly via increased availability of nitric oxide. This study may have implications for the innovative use of antioxidants as an adjunct to anti-hypertensive therapy.

INTRODUCTION

Hypertension affects 30% of adults resulting in increased mortality from cardiovascular diseases [1]. Epidemiological studies have demonstrated that low dietary intakes and plasma concentrations of antioxidant are associated with enhanced risk of hypertension, atherosclerosis, myocardial infarction and stroke [2–5]. The risk is additive for sub-optimal plasma levels of combinations of antioxidant vitamins [5] and marked synergism between the activity of some antioxidants has been described in vitro and in vivo [6, 7]. Blood pressure is regulated in part by the vasodilator nitric oxide and essential hypertension has been linked to impaired nitric oxide activity [8]. Studies in vitro suggest an inter-relationship between nitric oxide and antioxidants [9].

There have been no previous studies investigating the effect of combination antioxidant therapy on blood pressure in man, and specifically the potential additive effects in patients attending hospital with hypertension. We therefore investigated the effect of short-term oral supplementation with combination dietary antioxidants on blood pressure and nitric oxide metabolism in patients with hypertension and normotensive controls.

MATERIALS AND METHODS

Subjects

The study was approved by the local Clinical Research (Ethics) Committee and written informed consent was obtained from all subjects. Forty subjects were recruited from medical outpatient clinics. Thirty-eight completed both phases of the study and it is those data which are presented here. Subjects were identified at outpatient hospital visits and invited to attend a separate research clinic. Patients aged under 18 or over 80 years, who had diabetes, impaired renal function or malignant hypertension or who refused consent were excluded. Twenty-one subjects were attending hospital because of hypertension and were receiving anti-hypertensive therapy, which was unaltered throughout the study. The remaining 17 subjects were used as a control group of similar ages and were referred to medical outpatient clinics with dyspepsia or other minor gastrointestinal complaints and not for hypertension. Pretreatment biochemical and blood pressure data were also compared with a group of 35 healthy sub-

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jects of a similar age range, who were not attending hospital or taking any medication.

Subjects were computer-randomized by the pharmacy department to receive either 8 weeks placebo followed by 2 weeks washout then 8 weeks antioxidants or vice versa. The combination of antioxidants consisted of 200 mg of zinc sulphate, 500 mg of ascorbic acid, 600 mg of α-tocopherol (sodium succinate salt) and 30 mg of β-carotene daily, as four capsules, two taken with breakfast and two with an evening meal. The placebo was glucose, and antioxidant and placebo capsules were identical in appearance. The study was performed double blind. Subjects were instructed not to take additional dietary supplementation preparations during the study, but to continue with usual dietary habits.

Capsule purity was quality controlled by the pharmacy department. Capsules were stored at −20°C for a maximum of 2 weeks after manufacture and then at 4°C at home by the volunteers. Unused capsules were discarded after a further 2 weeks when subjects received new supplies. Volunteers attended for 18 weeks at fortnightly intervals at the same time of day ±1 h. Compliance was checked by capsule counts and reinforcement at each visit. Blood pressure was taken three times at each visit using a dedicated mercury sphygmomanometer, after subjects had been lying down for 5 min, by the same trained research nurse who was blinded to the trial medication. Diastolic blood pressure was measured using the fifth Korotkoff sound. Smoking habit and alcohol intake was recorded.

Biochemical analyses

Reagents were purchased from Sigma Chemical Co. Ltd, Poole, Dorset, U.K. Serum α-tocopherol (vitamin E) and β-carotene were measured simultaneously using a modification of the method described for retinol and tocopherol [10]. Briefly, serum was deproteinized with ethanol, and the lipid layer extracted with hexane. This was then evaporated under nitrogen and redissolved in retinol palmitate as internal standard before injection onto a 5 μm C18 HPLC column. Identically treated standard solutions of α-tocopherol and β-carotene, spectrophotometrically assessed and corrected for purity, were used for calibration. The mobile phase consisted of methanol/acetonitrile/chloroform (47:42:11) run isocratically at 1.5 ml/min. Tocopherol and retinol palmitate were detected at 285 nm and β-carotene at 450 nm using a diode array detector coupled to a data management system.

Blood pressure

The hypertensive subjects had a median basal systolic blood pressure of 165 (123–207) mmHg. The median duration of treatment for hypertension was 4 (1–15) years. Nine patients were taking nifedipine, four were taking captopril, three were taking lisinopril, two were treated with atenolol and one was taking metoprolol. Two patients were taking combinations of these anti-hypertensive drugs. Treatment remained unaltered throughout the study. Systolic blood pressure was 132 (107–160) mmHg in the subjects who were normotensive, which was not significantly different from that in healthy subjects [124 (107–140) mmHg]. All basal blood pressures did not differ from those at the end of the placebo phase of the study confirming stability in the hypertensive patients.

In the hypertensive subjects systolic blood pressure was 159.7 (119.0–183.0) mmHg after antioxidant administration, which was significantly lower than at the end of the placebo phase [168.7 (120.0–206.5) mmHg, P < 0.01]. Blood pressure decreased in all subjects at the end of the antioxidant supplementation phase (Fig. 1). In normotensive subjects systolic blood pressure was 131.0 (105.3–153.5) mmHg at the end of the antioxidant phase, compared with 135.2 (107.5–159.0) mmHg at the end of the placebo phase (P = 0.067). Diastolic blood pressure also tended to decrease with antioxidant supplementation. In hypertensive subjects diastolic blood pressure decreased from 89.3 (67.0–110.0) mmHg to 85.5 (64.5–106.5) mmHg.
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FIG. 1. Effect of antioxidant administration on systolic blood pressure in 21 subjects attending hospital for hypertension. Data were compared using Wilcoxon signed ranks test for paired data.

after antioxidant supplementation (not significant). In normotensive subjects diastolic blood pressure was 80.0 (68.5–90.0) mmHg at the end of the placebo phase and 79.3 (71.0–87.0) mmHg after antioxidant supplementation (not significant).

Biochemical measurements

Pretreatment median serum vitamin E concentration was higher in hypertensive patients than in healthy controls ($P < 0.01$, Table 1) but was not different from that in normotensive subjects ($P = 0.2$). Pretreatment β-carotene was similar in all subjects (Table 1). Vitamin E and β-carotene rose in all patients after antioxidant supplementation (Fig. 2).

Pretreatment urine nitrite excretion was 188 (19.9–911.0) nmol/h in subjects treated with antihypertensive agents and 67.3 (4.6–692.0) nmol/h in normotensive subjects ($P = 0.05$). Nitrite was significantly increased at the end of the antioxidant treatment phase compared with the placebo phase in the hypertensive subjects only ($P < 0.05$, Fig. 3).

Blood pressure at the end of the treatment phases was not related to whether subjects received antioxidants or placebo first. In addition, no association between alcohol intake or smoking habit and blood pressure or biochemical indices was noted.

DISCUSSION

It is accepted that in essential hypertension there is an impaired endothelial vasodilator response, probably through reduced generation of nitric oxide [8]. Nitric oxide is inactivated in vivo by superoxide anion thereby decreasing its vasodilator action [12]. Since dietary antioxidants are important in the removal of excess superoxide, antioxidants may be expected to potentiate the action of nitric oxide and reduce blood pressure. This study has shown that short-term oral antioxidant supplementation reduces blood pressure and this effect is most marked in

Table 1. Pretreatment biochemical variables [median (range)]

<table>
<thead>
<tr>
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<th>Vitamin E (mg/l)</th>
<th>β-Carotene (µg/dl)</th>
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<tbody>
<tr>
<td>Healthy subjects</td>
<td>11.0 (6.5–20.5)</td>
<td>14.6 (0.2–34.1)</td>
</tr>
<tr>
<td>Normotensive subjects</td>
<td>14.1 (2.3–27.8)</td>
<td>14.0 (0.2–14)</td>
</tr>
<tr>
<td>Hypertensive patients</td>
<td>15.0* (7.5–24.9)</td>
<td>20.0 (0.2–78.2)</td>
</tr>
</tbody>
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*Significantly higher than in healthy subjects ($P < 0.01$).
patients with hypertension and already receiving anti-hypertensive treatment. Moreover, antioxidant therapy increased urine nitrite in these patients, suggesting increased nitric oxide generation or availability as a mechanism for the reduction in blood pressure.

Several studies suggest an inverse association between dietary intake or plasma concentrations of the antioxidants vitamin E, vitamin C and β-carotene, and hypertension, atherosclerosis and stroke [2-4, 13, 14]. Sub-optimal levels of vitamin C, vitamin E and β-carotene are additive for risk of cardiovascular disease [5], and have been suggested to be stronger predictors of cardiovascular disease than classic risk factors such as hypertension and hypercholesterolaemia [15]. Supplementary intakes of these vitamins are linked to reduced risk of cardiovascular disease, and antioxidants therefore appear to have an important preventative role in cardiovascular disease.

We have shown that combination oral antioxidant therapy reduced blood pressure in patients attending hospital for hypertension and also tended to decrease blood pressure in normotensive controls. The fall in blood pressure in hypertensive subjects seems to be more pronounced in those patients with the highest blood pressures. Further larger studies are needed to confirm this finding. The combination of antioxidants used in this study was chosen because of the known synergistic action between vitamin E and vitamin C [7], β-carotene and vitamin E [6] and zinc and vitamin E [16]. This is the first study examining the effect of such short-term combination therapy on blood pressure. Animal [17] and human [18, 19] studies have shown that longer-term oral supplementation with vitamin C reduces blood pressure. In another study acute intravenous administration of the antioxidants vitamin C, glutathione or thiopronine to hypertensive diabetic patients reduced blood pressure [20]. There have been no short-term studies investigating the influence of vitamin E or β-carotene on blood pressure, although long-term supplementation (greater than 2 years) is associated with decreased risk of cardiovascular events [14].

Blood pressure is regulated in part by the vasodilatory action of nitric oxide produced in the endothelium. Superoxide rapidly inactivates nitric oxide and antioxidants might be expected to potentiate nitric oxide activity through removal of superoxide. We have previously shown that a vitamin E analogue (Trolox), vitamin C and other non-dietary antioxidants regulate the activity of nitric oxide synthase in endothelial cells, probably through removal of superoxide [9]. There is impaired endothelium-dependent relaxation in patients with untreated essential hypertension, thought to be mediated via impaired release or action of nitric oxide [8]. Reduced urinary nitrite excretion has been demonstrated in untreated essential hypertension [21], although normal responses to exogenous nitric oxide donors have been demonstrated [22]. There is evidence to suggest that the impaired release or action of nitric oxide is not genetically predetermined [23] or localized to a single signal-transduction pathway abnormality [8].

Impaired responses to nitric oxide inhibitors lend further support for the role of nitric oxide in essential hypertension [24]. However, these responses are normalized after anti-hypertensive treatment with a variety of agents [25]. These findings suggest that there is a relative deficiency of nitric oxide in essential hypertension and that anti-hypertensive agents exert a common effect in augmenting the nitric oxide pathway. This explains the increase in urinary nitrite in our treated hypertensive patients in contrast to findings in untreated essential hypertension [21]. Supplemental antioxidants enhance further the nitric-oxide-mediated reduction in blood pressure by removal of superoxide anion. This hypothesis is supported by our findings of increased nitrite, an index of nitric oxide production, as a result of antioxidant therapy. However, it must be noted that nitrite/nitrate excretion is not specific solely to nitric oxide metabolism and dietary sources may confuse interpretation, although we have no reason to suppose that there was any alteration in subjects' dietary habits during the study.

Epidemiological studies have shown a detrimental additive influence of sub-optimal levels of vitamin E, vitamin C and β-carotene on risk of cardiovascular disease. Antioxidants decreased blood pressure in all our hypertensive patients. Although the changes in blood pressure were small, further lowering of blood pressure in treated hypertensive subjects must be beneficial and could be achieved by relatively minor adjustments in dietary habit. We suggest that hypertensive patients have a relative dietary inadequacy of...
antioxidants and may require higher than normal concentrations of circulating antioxidant. Since these subjects have a relatively high risk of cardiovascular disease the changes in blood pressure observed in this study may be associated with improved morbidity. Reinforcement of dietary advice and possibly revision of dietary recommendations for such patients may therefore be warranted. Longer-term studies will be required to confirm clinical benefit. Not only do antioxidants reduce the complications of atherosclerosis but supplementation therapy with dietary antioxidants may provide a novel adjunct to conventional treatment for hypertension.

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REFERENCES