Vitamin C modifies the cardiovascular and microvascular responses to cigarette smoke inhalation in man

J. GAMBLE*, P. S. GREWAL† and I. B. GARTSIDE‡

*School of Sport and Exercise Sciences, Birmingham University, Birmingham B15 2TT, U.K., †Accident and Emergency Department, John Radcliffe Hospital, Headington, Oxford OX3 9DU, Oxon, U.K., and ‡Department of Physiology, Charing Cross and Westminster Hospital Medical School, Fulham Palace Road, London W6 8RF, U.K.

ABSTRACT

Both neutrophil margination and increases in the non-invasively assessed parameter, isovolumetric venous congestion cuff pressure ($P_v$), are symptomatic of some inflammatory diseases. Neutrophil margination occurs primarily, though not exclusively, at the post-capillary endothelial surface. The local haemodynamic changes resulting from margination may be responsible for the observed increases in $P_v$. Smoke inhalation has been shown in animal studies to cause an increase in post-capillary neutrophil margination by mechanisms that can be blocked by oral vitamin C administration. We looked for indices of a relationship between margination and $P_v$ in man, using cigarette smoke inhalation as a pathophysiological challenge. We also examined the effect of prophylactic vitamin C on the response. Smoke inhalation was associated with highly significant increases in both $P_v$ and heart rate. After vitamin C pre-treatment, no increase in $P_v$ was observed in response to the smoke inhalation; however, whilst heart rate still increased significantly, the duration of this response was attenuated. The results suggest that vitamin C affords protection against some of the cardiovascular and microvascular changes associated with cigarette smoke inhalation in man. They also support the notion that non-invasive assessment of changes in $P_v$ may provide a measurable index of systemic changes in inflammatory conditions.

INTRODUCTION

The role of vitamin C as a natural defence against superoxides and other reactive oxygen species is well documented [1]. Superoxides have been shown to induce P-selectin translocation [2], thereby initiating the formation of platelet aggregates and the adhesion of neutrophils to endothelial cells [3]. These events occur preferentially, though not exclusively, at the venular end of the microvascular bed [4]. Increased neutrophil sticking, rolling and adhesion to the venular endothelium will have profound upstream microvascular haemodynamic consequences [5]. Moreover, the increase in neutrophil rigidity, which has been demonstrated in response to cigarette smoke exposure, may also influence microvascular rheology [6]. Furthermore, these effects would be exacerbated if the resulting reduction in shear rate, at the venular end of the microcirculation, increased red-cell aggregation, which has been shown to have a marked effect on post-capillary resistance [7].

We have used a venous congestion plethysmography (VCP) technique [8] for non-invasive investigation of changes in microvascular parameters in a variety of chronic and acute inflammatory diseases. Two main parameters can be studied with VCP: (1) the microvascular filtration capacity ($K_f$), an index of microvascular permeability to water, and (2) the isovolumetric venous congestion cuff pressure ($P_v$). $P_v$ is the venous con-
gestion pressure that needs to be applied to overcome the balance of hydrostatic and plasma colloid osmotic (onotic) forces at the microvascular interface [9]. Using VCP, we found that $P_v$ was increased in pathologies such as venous ulceration [10], pre-eclampsia [11], adult sepsis [12] and in children with either dengue haemorrhagic fever [13] or meningococcal disease [14]. All of these conditions are also associated with increased adhesion molecule expression. In the present study, we tested the hypothesis that post-capillary margination of neutrophils and platelets will, by increasing post-capillary resistance, increase the value of $P_v$.

Exposure to cigarette smoke evokes the sequence of rolling and sticking of fluorescently labelled leucocytes to the microvascular endothelium of hamsters [15]. Moreover, vitamin C was shown to prevent the smoke-induced changes [16]. The experimental protocol used in these animal model studies offered a possible, albeit indirect, means of testing the relationship between post-capillary neutrophil margination and changes in $P_v$ in man. We have demonstrated previously that cigarette smoke inhalation is associated with an increase in $P_v$ [17]. The present study sought to extend both these and the original observations on hamsters [16] by non-invasively investigating the effect of prophylactic vitamin C on the responses to cigarette smoke inhalation in man.

METHODS

Subjects

The studies were performed on two groups of young, healthy subjects. The first group comprised 15 smokers (four female) aged $20 \pm 1.3$ years (mean $\pm$ S.D.). The median cigarette consumption of this group was $7 (\times 1.0–15)$ (median range) cigarettes per day. The other group comprised 12 non-smoking controls (five female) aged $21.6 \pm 0.5$ years. All subjects were asked to refrain from smoking, taking supplementary vitamins and anti-inflammatory drugs for 24–48 h prior to each study. The subjects were also asked to refrain from either eating or taking caffeine-containing beverages for at least 2 h prior to the studies.

Protocol

The investigations were made in a temperature-controlled environment at $24 \pm 1.0$ °C. The subjects were supine on a tiltable bed, positioned so that the mid-calf was at right atrial level, which was assumed to be one third of the distance down from the sternal angle to the posterior surface when supine.

Alveolar CO

The subject’s previous smoke exposure was assessed by measuring alveolar CO concentration in p.p.m., using a Micro CO Meter (type MC01; Micro Medical Ltd, Rochester, Kent, U.K.). The measurements were made at the start of each study and repeated after finishing the cigarette. CO measurements were also made on the ten, age-matched, non-smoking students who assisted in the studies and, therefore, experienced passive smoke exposure. The CO meter was not available for the studies on the control group.

Heart rate

All subjects were wired for continuous heart rate recording. The signal was derived from the R–R interval of a standard chest lead electrocardiogram recording. The values, averaged over 1 min, were studied 2 min before, at 2, 5 and 10 min, and then at 10 min intervals after starting the smoke inhalation. Heart rate signals could only be analysed in nine of the fifteen non-vitamin C subjects and six of the ten subjects treated with vitamin C.

Arterial blood pressure

Three measurements of arterial blood pressure were made on the contralateral calf at the start of the study and then again, 5 min after completing the smoke inhalation. We used a Critikon Dinamap Vital Signs Monitor (type 8100; Critikon, Tampa, Florida, U.S.A.) for these measurements and the averaged values are used in the study.

VCP study and analysis

All microvascular parameters were derived from the changes in tissue volume that occurred in response to increases in venous congestion pressure. The protocol involved application of a series of five to seven small (8–10 mmHg) cumulative congestion pressure steps, each of 5 min duration. The maximum pressure used never exceeded the subject’s own diastolic pressure. This protocol does not interfere with calf arterial inflow [18]. Since illustrated descriptions of the analysis procedure have been given previously [8,19], a brief summary will suffice. Venous congestion pressures in excess of the ambient venous pressure cause a change in limb volume, attributable to venous filling ($V_s$). At higher congestion cuff pressures, a slow, steady-state volume change also occurs reflecting fluid filtration ($J_v$). The analysis procedure used enables differentiation between venous filling and filtration responses [8]. The microvascular filtration capacity ($K_v$ units ($K_vU$) = $ml \cdot min^{-1} \cdot 100 mL^{-1} \cdot mmHg^{-1}$) is the slope of the linear relationship between the congestion cuff pressure ($P_{cuff}$) and $J_v$ co-ordinates obtained at $P_{cuff}$ values above those causing a measurable increase in fluid filtration [8]. The intercept of the slope, on the abscissa, represents the isovolumetric congestion cuff pressure. $P_v$ is the congestion cuff pressure that has to be exceeded to induce net fluid filtration at the level of the strain gauge. $P_v$ has been shown to reflect the effective
colloid osmotic (oncotic) pressure at the microvascular interface [9]. However, the measured value of $PV_i$ may differ from the pressure at the microvascular interface, the discrepancy depending upon the values of post-capillary resistance and blood flow.

The relationship between $V_i$ and $P_{cuff}$ is curvilinear and is a function of the vascular and surrounding tissue compliance. The intercept on the $P_{cuff}$ axis, at $V_i = 0$, gives a non-invasive estimate of the venous pressure at the level of the strain gauge [20].

We used our own strain gauge plethysmography system [8] rather than a commercial one since, at the time of the studies, no commercial instruments offered the versatility of protocol and analysis that our micro-circulation studies required.

All measurements were made before and then 20 min after either, completion of the inhalation of smoke from one medium-strength cigarette (advertised values: 9 mg of tar and 0.7 mg of nicotine per cigarette) in the smoking group, or 30 min supine rest in the control group. The full study protocol was repeated on ten of the smoking group one month later. The remaining five subjects withdrew for either personal reasons or reasons of ill health. On the second occasion the subjects took oral vitamin C (500 mg slow release, twice a day; Redoxon, Roche, Welwyn Garden City, Herts., U.K.) for 36 h before the study. Each subject also took one 500 mg soluble chewable vitamin C tablet (Redoxon, Roche) at the start of the 120-min protocol.

**Statistics and ethical clearance**

Paired assessments were made using Student’s $t$ test or, for non-normally distributed data, Wilcoxon’s signed rank test. One-way analysis of variance (ANOVA) was used for multiple comparisons or, if the data were not normally distributed, the Kruskal–Wallace ANOVA on ranks. When all pair-wise multiple comparisons were made either Dunn’s method for non-normally distributed or Dunnett’s for normally distributed data were used. Data comparisons against a single control were performed using the Bonferroni t-test. Significance was assumed when $P < 0.05$. Ethical clearance was obtained from the Ethical Committee of the Charing Cross and Westminster Medical School. The procedures, which were wholly non-invasive, were explained to the subjects, who gave verbal informed consent.

**RESULTS**

**Alveolar CO**

The initial value of CO of the students who assisted in the study, $4.0 \pm 1.1$ p.p.m. (mean $\pm$ S.D.), was uninfluenced by the passive smoke exposure, $3.9 \pm 0.8$ p.p.m. Neither of these values differed significantly from the pre-smoke values of the smoking group. In this group, the post-smoke values were not normally distributed, reflecting differences in the smoke inhalation. Their alveolar CO rose from the resting value of 3.7 $\pm$ 1.8 to 11.0 (6.5–25.0) p.p.m. [median (range)], the difference was highly significant ($P \ll 0.001$, ANOVA with All Pair-wise Multiple Comparison Procedure; Dunn’s Method). Pre-treatment with oral vitamin C affected neither the initial, 4.4 (2–16), nor the post-smoke, 13.1 (7.3–25), values of alveolar CO. The difference between these values was also highly significant ($P \ll 0.001$).

**Calf arterial and venous pressures**

All subjects were normotensive. The pre- and post-smoke values of systolic and diastolic arterial pressure, which were 128 $\pm$ 13.9 and 55.4 $\pm$ 9.1, and 132.5 $\pm$ 16.4 and 57.1 $\pm$ 10.7 mmHg (mean $\pm$ S.D.) respectively, were not significantly different from one another. Neither of these groups of values differed from those obtained in the non-smoking controls, which were 132.6 $\pm$ 14.4 and 50.5 $\pm$ 5.6 mmHg respectively. Neither the arterial nor venous pressure values were influenced by pre-treatment with vitamin C.

$K_i$

Consecutive studies on the non-smoking controls gave normally distributed $K_i$ values of 3.38 $\pm$ 0.9 (mean $\pm$ S.D.) and 3.39 $\pm$ 1.26 $K_i$ U (ml min$^{-1}$ 100 ml$^{-1}$ mmHg$^{-1}$ $\times 10^{-3}$) respectively, that were not significantly different from one another (paired $t$ test). The values of $K_i$ obtained in the smoking group were not normally distributed. $K_i$ before smoking, 3.52 (1.21–9.43) $K_i$ U [median (range)], was uninfluenced by the smoke exposure, 4.38 (1.63–8.52) $K_i$ U. After vitamin C pre-treatment the differences between the pre- and post-smoke median values of $K_i$, 3.64 (1.03–6.17) and 2.82 (1.83–6.46) $K_i$ U respectively, were not significantly different from each other or the non-vitamin C pre-treatment study values.

$PV_i$

The data from the two consecutive assessments on non-smoking controls, 15.9 $\pm$ 6.6 (mean $\pm$ S.D.) and 16.3 $\pm$ 6.3 mmHg respectively, were not significantly different from each other (paired $t$ test; Figure 1a). The value of $PV_i$, following smoke inhalation, increased in 14 of the 15 subjects. The median (range) rose from 19.9 (3.8–29.5) to 26.0 (11.4–32.4) mmHg (Figure 1b). After prophylactic vitamin C treatment the values of $PV_i$ before and after smoke exposure were normally distributed. The pre-smoke value of $PV_i$ (22.7 $\pm$ 5.3) was not significantly different from the post-inhalation value of 20.0 $\pm$ 6.8 mmHg (Figure 1c). All values of $PV_i$, that is those from the control group and the smoking group, both with and without vitamin C pre-treatment, were compared using Student–Newman–Keuls All Pair-wise Multiple Comparison Procedure. This analysis showed
Figure 1 The effect of cigarette smoke exposure on the value of $P_v$, before and after specific procedures

The open circles joined by lines represent the responses of individual subjects. (a) Non-smoking control data. (b) The responses in the smokers without vitamin C pre-treatment. (c) The responses in the smokers obtained after vitamin C pre-treatment. The filled circles and error bars in each figure are the means $\pm$ S.D. for these data; *, $P < 0.001$. The values in these studies were compared using a one-way ANOVA and the Student–Newman–Keuls All Pair-wise Multiple Comparison Procedure.

Figure 2 The effect of cigarette smoke exposure on heart rate

The open and closed circles are the means $\pm$ S.E.M. obtained with and without prophylactic vitamin C respectively. All the data were analysed using ANOVA with Dunnett’s All Pair-wise Multiple Comparison Procedure. The symbols * and † indicate that the values of heart rate differed significantly from their corresponding pre-smoke value ($P < 0.001$).

That the value of $P_v$, after exposure to cigarette smoke, but in the absence of vitamin C pre-treatment, was significantly greater than the pre-smoke value of this group, from the post-smoke vitamin C study value and also from the values obtained from the control group ($P < 0.001$).

Heart rate

After the start of smoke inhalation, heart rate increased to its maximum, an increase of $46 \pm 10\%$ (mean $\pm$ S.E.M.) relative to the pre-smoke value, 5 min after the initial smoke inhalation (Figure 2). The increase was highly significant ($P < 0.001$). Whilst heart rate declined after the 5 min peak, it was still significantly elevated 40 min after the initial smoke inhalation (Dunnett’s All Pair-wise Multiple Comparison Procedure). After vitamin C pre-treatment, the maximum increase in heart rate, which was $45 \pm 9\%$ of the pre-smoke value, was achieved 2 min after the initial smoke inhalation (Figure 2). At 5 min, heart rate had decreased from the 2 min peak, but was still significantly elevated ($P < 0.001$). In this group, however, the significant elevation of heart rate was sustained for less than 10 min from the start of smoke inhalation.

DISCUSSION

In the present study, we tested the hypothesis that the haemodynamic changes resulting from the post-capillary neutrophil margination and platelet clumping, which occur in inflammatory diseases, might be associated with local increases in post-capillary resistance and therefore $P_v$. We had previously used a cigarette smoke challenge, which has been shown to increase neutrophil margination [15], to show that exposure to the inflammatory agonists in cigarette smoke resulted in an increased value of $P_v$ [17]. The present study repeated and then extended this protocol to test the hypothesis that pre-treatment with vitamin C, since it inhibits smoke-induced neutrophil margination in an animal model [16], might also block the smoke-induced increase in $P_v$ in man. The results we obtained, which reiterated those from the preliminary study [17], provided support for this hypothesis.

That the initial values of $P_v$ in both smokers and non-smokers were not significantly different, suggests that the
smokers had indeed refrained from smoking, an observation that was supported by their comparable alveolar CO values. These observations also imply that the effect of smoking on $P_{vi}$ may be of relatively short duration.

The haemodynamic effects of the increase in post-capillary resistance, following adhesion molecule upregulation [5] may lead to measurable alterations in microvascular parameters in the tissues under study. One consequence of raised post-capillary resistance would be increased hydrostatic pressure at the microvascular interface and therefore $P_{vi}$ secondary to enhanced fluid extraction across the exchange vessels. The value $P_{vi}$ has been shown to change in parallel with increases in venous pressure in dependent limbs following a passive tilt [21]. The change is of the same order as that of the oncotic pressure of venous blood, sampled at the dorsum of the foot, during a similar postural challenge [22]. Moreover, that the oncotic pressure of venous blood from non-dependent tissues remained unchanged [22], implies that the altered $P_{vi}$ reflected a local haemodynamic event.

Besides the effect of vitamin C on the value $P_{vi}$, the attenuation of the smoke-induced tachycardia is of interest. It has been suggested that increases in heart rate, in response to cigarette smoke inhalation, result from the sympathomimetic actions of the nicotine component of the inhaled smoke [23]. However, our preliminary investigations into the effects of nicotine ingestion, showed that twice the advertised cigarette nicotine content, delivered orally, had no effect on heart rate [24]. These results suggest that the inhaled nicotine may not be the major active principle in the tachycardic responses to cigarette smoke inhalation.

Some of the effects of smoke inhalation differed from those reported by other workers. Whilst smoke inhalation challenges have been shown to result in a heart rate increase that was, temporally, similar to that found in the present study, modest but significant increases in diastolic and mean arterial blood pressure were also seen [25]. In our studies, arterial blood pressure remained unchanged. These differences may well relate to differences in protocol. Our measurements were made 30 min rather than 10 min after the initial smoke inhalation. Moreover, the cigarettes used contained less nicotine. Clearly, the use of different cigarette brands will provide different pharmacological challenges.

This study did not investigate the mechanisms by which the anti-oxidant vitamin C was acting. However, it is known that plasma vitamin C levels are depressed in smokers, and that this relates to smoking itself rather than to depressed dietary intake [26]. Vitamin C is known to block the effect of inflammatory platelet activating factor mimetics, which are activated within minutes of exposure to cigarette smoke in hamsters [27]. Besides the considerable evidence of the protective effect of vitamin C against the pro-oxidant effects of agents in cigarette smoke, there is burgeoning information on its protective action in other pathologies [1]. In a recent study on patients at high risk of pre-eclampsia, a combination of vitamins C and E were found to be beneficial in the prevention of this condition [28]. Whilst supportive evidence for the benefit of vitamin E in countering the effects of smoking is not overwhelming [16], the study by Chappell et al. [28] does support the notion that vitamin C is of benefit in pathologies associated with oxidative stress. We believe that these observations, together with those made on man in the present study, reinforce our primary hypothesis. In the light of these results, we propose that increased values of $P_{vi}$ may well be used as an indicator of the haemodynamic changes resulting from up-regulation of adhesion molecule expression.

It is clear that the response to cigarette smoke exposure is multifaceted. It is also apparent that vitamin C might ameliorate some of the responses to cigarette smoke inhalation in man. Whilst it might be considered inappropriate to offer a palliative to tobacco companies, there are clear financial advantages in reducing the long term morbidity and mortality associated with the addictive smoking habit [29]. Whilst we feel that the results we have obtained justify our speculation, we also recognise the need to provide firm evidence of a link between changes in $P_{vi}$ and adhesion molecule up-regulation. However, whilst vitamin C might offer protection against some of the detrimental effects of occasional acute cigarette smoke exposure, the possibility that it might benefit chronic smokers, awaits investigation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the enthusiastic help of the second year pre-clinical and intercalated B.Sc. students at the former Charing Cross and Westminster Medical School, who acted as subjects and assisted in gathering the data as part of their course work.

REFERENCES

the microcirculation of cat mesentery. Circ. Res. 43, 738–749

Received 7 December 1999/21 January 2000; accepted 31 January 2000