Vitamin C augments the renal response to L-arginine in smokers

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Abstract
Background. In the coronary and the forearm circulations, endothelium-dependent vasomotion is impaired in smokers, but can be augmented by L-arginine or vitamin C. We examined whether smoking similarly affects the renal circulation.

Methods. In 20 smokers (age 26 ± 4 years) and in 20 non-smokers (age 28 ± 3 years) changes of renal plasma flow (RPF), glomerular filtration rate (GFR), blood pressure and heart rate in response to the subsequent intravenous infusions of NG-monomethyl-L-arginine (L-NMMA), L-arginine and L-arginine plus vitamin C were studied by use of a constant infusion input clearance technique.

Results. Systemic haemodynamic parameters did not differ between smokers and non-smokers during each experimental phase. At baseline, RPF and GFR were similar between the groups. The infusion of L-NMMA led to a similar decrease of RPF, while GFR did not change in either group. During the infusion of L-arginine RPF increased similarly. Finally, the co-infusion of L-arginine plus vitamin C led to a significantly greater increase of RPF (Δ +277 ± 395 vs +79 ± 76 ml/min, P = 0.03) and GFR (Δ +12.1 ± 10.6 vs +3.4 ± 11.2 ml/min, P = 0.02) in smokers as compared to non-smokers.

Conclusions. L-NMMA-induced vasoconstriction of the renal vasculature was similar in smokers compared to non-smokers. L-arginine alone induced a similar increase of RPF. The co-infusion of vitamin C and L-arginine led to a greater increase of RPF and GFR in smokers. This might suggest that oxidative stress is increased in the renal vasculature of smokers.

Keywords: nitric oxide; oxidative stress; renal haemodynamics; smoking; vitamin C

Introduction

Smoking has been established as a risk factor for coronary artery disease and stroke for many years. More recently, the renal consequences of smoking are becoming increasingly recognized. In the large, prospective Multiple Risk Factor Intervention Trial (MRFIT) including 332,554 men, smoking was shown to be an independent risk factor for end-stage renal disease (ESRD), with an increase in relative risk of ESRD up to 69% [1]. Furthermore, smoking has been shown to accelerate the progression of various primary renal diseases such as IgA nephropathy and polycystic kidney disease [2], and secondary renal diseases such as hypertensive renal disease [3] and diabetic nephropathy [4]. The pathogenetic mechanisms of smoking-induced renal damage, however, still remain to be elucidated.

Due to its various anti-atherosclerotic properties, the endothelium has been recognized as a major player in cardiovascular disease. Endothelial dysfunction as reflected by impaired endothelium-dependent, nitric-oxide-mediated vasodilatation is an early step in the process of atherosclerosis, known to precede structural changes in the vessel wall. During recent years various cardiovascular risk factors were found to impair endothelium-dependent vasodilatation. As one of these cardiovascular risk factors, smoking was found to be associated with impaired endothelium-dependent vasodilatation in the coronary and in the forearm circulation [5,6].

In smokers, blunted endothelium-dependent vasodilatation can be ameliorated by the acute systemic administration of L-arginine, the substrate of endothelial nitric oxide synthase (eNOS), pointing at a defect of the L-arginine/nitric oxide (NO) pathway [5,6]. However, due to the complex formation and metabolism of NO, multiple mechanisms can reduce NO bioavailability. Oxidative stress is known to initiate biochemical reactions that lead to the enhanced breakdown of NO. The administration of
vitamin C restores endothelium-dependent vasodila-
tion in the coronary and forearm circulations of
smokers, suggesting that oxidative stress is increased
in these vascular beds [7,8]. Thus, oxidative stress is
another major contributor to decreased NO avail-
ability in this setting.

So far, no data exist on vascular responses of the
renal circulation of smokers. Since the renal vasculature is not as easily accessible as the forearm vasculature, changes of renal haemodynamics in response to the intravenous administration of \(\text{N}^2\)-
monomethyl-L-arginine (L-NMMA), an inhibitor of
endothelial NO synthase, or L-arginine, the substrate of eNOS, have been used to assess endothelial-
dependent responses of the renal circulation [9–13].

To investigate the effects of smoking on vascular
responses of the renal vasculature, this study was
carried out to examine L-NMMA- and L-arginine-
induced changes of renal haemodynamics in smokers.
Furthermore, to elucidate the role of oxidative stress
in the renal circulation of these subjects, the effect
of the antioxidant vitamin C on the renal haemo-
dynamic response to L-arginine was determined. 

Subjects and methods

Study cohort

Our study population consisted of 20 non-smoking and
20 smoking, healthy, young, white males who were recruited
from the campus of the University of Erlangen-Nürnberg.
Inclusion criteria were age 18–35 years, average
blood pressure < 140/90 mmHg measured twice on two
different occasions, fasting serum LDL-cholesterol concen-
tration < 130 mg/dl, fasting serum triglyceride concentra-
tion < 200 mg/dl, fasting plasma glucose concentration
< 110 mg/dl, serum creatinine concentration < 1.2 mg/dl,
and absence of any chronic or acute cardiovascular, hepatic
or renal disease. Non-smokers were defined as subjects who
had never smoked. Smokers were included if the number of
cigarettes smoked per day during the last year exceeded 10
cigarettes on average. All subjects were on an ad libitum
diet, i.e. no specific dietary guidelines were followed. All
participants were given a thorough physical examination.
Before enrolment in the study, informed written consent
was obtained from each participant and the study protocol
was approved by the Ethical and Investigation Committee
of the University of Erlangen-Nürnberg.

Study design

Subjects were asked to refrain from smoking on the day of the
examination. L-NMMA-induced vasoconstriction, and L-arginine and L-arginine plus vitamin C-induced vasodila-
tion of the renal vasculature were assessed as part of a clearance protocol. To determine renal plasma flow (RPF)
and glomerular filtration rate (GFR), the constant infusion-
input clearance technique was applied, as previously described [10,11,14]. The advantage of this technique lies in
the avoidance of bladder catheterisation or reliance on spontaneous voiding. In brief, subjects fasted overnight for
at least 12 h and were kept in a supine position in a quiet,
dark and temperature-controlled room (22–25°C) through-
out the study. A catheter was inserted into the left ante
cubital vein for infusion of \(\text{p-aminohippuric acid (PAH)}\), inulin, L-NMMA, L-arginine and vitamin C. A second catheter was placed into the right ante
cubital vein to obtain blood samples. Filtration fraction (FF) was
calculated by dividing GFR by RPF. Renal vascular resistance (RVR) was calculated as MAP \times (1-haematocrit)/RPF.

Infusions were prepared immediately before the admin-
istration. After a priming dose of PAH (Nephrotest\textsuperscript{®},
Merck Sharp & Dohme, Herts., UK) and inulin (Inutest\textsuperscript{®},
Fresenius, Graz, Austria) that was adjusted according to
body-weight, a constant infusion of both tracer substances
was given to achieve steady-state conditions. Since in
previous experiments, PAH and inulin measurements after
105, 120 and 135 min had a coefficient of variation < 5%,
we used a duration of 120 min to establish steady-state
concentrations of PAH and inulin [14]. The infusion of L-
NMMA (Cinalfa AG, Laeufelfingen, Switzerland) fol-
lowed. The rationale of using an infusion duration of
30 min for each infused vasoactive agent is that the constant
infusion technique requires at least 20 min to achieve a new
steady-state condition [14]. After a priming dose of 3 mg/kg
given as a bolus injection over 5 min a constant dose of
3 mg/kg/h over the next 25 min was applied. The safety of this
dose has been shown in various recent clinical trials
[9–11]. During the second infusion phase, L-arginine
(L-arginine hydrochloride, Braun, Melsungen, Germany) at
a dose of 100 mg/kg/30 min was infused. In contrast to
higher doses of L-arginine, this dose has previously been
shown to be most suitable due to the lack of unspecific
renal haemodynamic changes [13]. Finally, L-arginine at the
same dose with the addition of vitamin C at a dose of 3 g/
30 min was infused. At the end of each phase, blood
samples were drawn to measure PAH and inulin
concentrations. Throughout the infusion protocol, blood
pressure and heart rate (HR) were monitored at fixed
intervals (every 5 min) using an oscillographic device
(Dinamap 1846 SX, Criticon, Norderstedt, Germany). The
time interval was changed to one measurement per minute
over 5 min before the collection of blood samples, and mean
values were computed from these five consecutive measure-
ments. All clearance protocols were performed in the
morning, between 8.00 and 12.00 h. Twenty-four-hour urine
samples were collected to estimate the excretion of sodium.

Laboratory measurements

PAH was quantified according to the modification of Smith
et al. [15]. Inulin was measured indirectly by converting
inulin to fructose; fructose was then estimated enzymatically
(Boehringer Mannheim, Mannheim, Germany). Measure-
ments were made in duplicate for each blood sample and
the coefficients of variation were < 5%.

Statistics

All data were analysed using the PC version of the
Statistical Package for the Social Sciences [16]. Analysis
of variance for repeated measurements with the
Bonferroni correction was performed to compare the renal
haemodynamic responses between smokers and non-smokers. Changes of the various parameters within the groups are given in the tables as changes from the antecedent infusion phase. Paired Student’s t-tests were performed to compare changes within groups. For correlation analysis, Pearson’s correlation coefficients were calculated. Results are given as means ± SD in the text and as mean ± SE in the figures. A two-tailed P-value < 0.05 was considered significant.

Results

Study population and clinical characteristics

Only young and healthy non-smoking and smoking subjects were included according to our study protocol (Table 1). Smokers and non-smokers did not differ with respect to blood pressure, serum creatinine, haematocrit, total cholesterol, LDL cholesterol, triglyceride levels and urinary sodium excretion. Apart from cigarette consumption only the level of HDL cholesterol was significantly different between the two groups.

Systemic haemodynamic parameters

Baseline mean arterial blood pressure (MAP), systolic and diastolic blood pressure (data not shown) and HR were similar at baseline (Table 2). The infusion of L-NMMA caused a significant increase of MAP and a decrease of HR, with no difference between the two groups. During L-arginine-infusion, MAP fell to a similar extent in both groups. In addition, there was no difference with regard to the increase of HR between the groups. Furthermore, during the subsequent co-infusion of L-arginine and vitamin C, heart rate rose further to a similarly small extent in both groups. No further decrease of MAP was detected during the co-infusion of L-arginine and vitamin C.

Renal haemodynamic parameters

At baseline, RPF, RBF, GFR, FF and RVR were similar in both groups (Table 3). The infusion of L-NMMA led to a similar decrease of RPF and RBF and a similar increase of RVR and FF in both groups (Figure 1). GFR did not change significantly in both groups (Figure 2).

During the infusion of L-arginine, RPF and RBF increased similarly in both groups (Figure 1). FF and RVR decreased in both groups, but again no difference between the groups could be detected. GFR increased only in smokers, but the changes of GFR during L-arginine infusion were not different between the groups (Figure 2).

The co-infusion of L-arginine and vitamin C led to a much higher increase of RPF and RBF and a higher decrease of RVR in smokers (range of ΔRPF 29.4–817 ml/min, median 169 ml/min) (Figure 1). GFR increased only in smokers (range of ΔGFR –0.7–46.1 ml/min, median 9.2 ml/min) (Figure 2). FF decreased similarly in both groups. In smokers, the increase of GFR and the decrease of RVR during co-infusion of L-arginine and vitamin C significantly correlated with the quantity of cigarettes smoked, determined as cigarettes/day × years (Figure 3 for GFR and Figure 4 for RVR). The correlations between the increase of RBF and RPF and cigarettes/day × years were only borderline significant (γ = 0.42, P = 0.08 and γ = 0.39, P = 0.09, respectively).

Discussion

In this study, we examined the effects of chronic smoking on L-NMMA and L-arginine-induced changes of renal haemodynamics. L-NMMA-induced renal vasoconstriction, as reflected by a decrease of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 20)</th>
<th>Smokers (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28 ± 3</td>
<td>26 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79 ± 9</td>
<td>77 ± 8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.83 ± 0.06</td>
<td>1.80 ± 0.07</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 ± 2.0</td>
<td>23.7 ± 2.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Casual systolic blood pressure (mmHg)</td>
<td>124 ± 8</td>
<td>130 ± 13</td>
<td>n.s.</td>
</tr>
<tr>
<td>Casual diastolic blood pressure (mmHg)</td>
<td>78 ± 9</td>
<td>77 ± 9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>44 ± 3</td>
<td>45 ± 3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>171 ± 32</td>
<td>160 ± 28</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>83 ± 23</td>
<td>89 ± 24</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 9</td>
<td>46 ± 10</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>106 ± 83</td>
<td>122 ± 79</td>
<td>n.s.</td>
</tr>
<tr>
<td>24-h-urinary sodium excretion (mmol/day)</td>
<td>195 ± 79</td>
<td>189 ± 86</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cigarettes/day × years</td>
<td>0</td>
<td>192 ± 134</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Changes of renal haemodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=20)</th>
<th>Smokers (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline RPF (ml/min)</td>
<td>712±131</td>
<td>718±143</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRPF after L-NMMA (3 mg/kg/h)</td>
<td>-123±96</td>
<td>-112±99</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRPF after L-arginine (100 mg/kg/30 min)</td>
<td>+70±68</td>
<td>+89±126</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRPF after L-arginine + vitamin C (3 g/30 min)</td>
<td>+79±76</td>
<td>+277±395</td>
<td>0.03</td>
</tr>
<tr>
<td>Baseline GFR (ml/min)</td>
<td>127±16</td>
<td>132±19</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔGFR after L-NMMA (3 mg/kg/h)</td>
<td>+1.5±8.0</td>
<td>-1.7±12.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔGFR after L-arginine (100 mg/kg/30 min)</td>
<td>+2.3±11.6</td>
<td>+5.5±8.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔGFR after L-arginine + vitamin C (3 g/30 min)</td>
<td>+3.4±11.2</td>
<td>+12.1±10.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Baseline FF (%)</td>
<td>18±3</td>
<td>19±2</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔFF after L-NMMA (3 mg/kg/h)</td>
<td>+3.9±2.4</td>
<td>+3.3±2.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔFF after L-arginine (100 mg/kg/30 min)</td>
<td>-2.0±1.6</td>
<td>-2.2±2.6</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔFF after L-arginine + vitamin C (3 g/30 min)</td>
<td>-1.5±1.8</td>
<td>-2.7±4.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Baseline RBF (ml/min)</td>
<td>1268±251</td>
<td>1329±260</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRBF after L-NMMA (3 mg/kg/h)</td>
<td>-220±173</td>
<td>-206±178</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRBF after L-arginine (100 mg/kg/30 min)</td>
<td>+124±120</td>
<td>+164±244</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRBF after L-arginine + vitamin C (3 g/30 min)</td>
<td>+139±134</td>
<td>+522±761</td>
<td>0.04</td>
</tr>
<tr>
<td>Baseline RVR (U)</td>
<td>72±15</td>
<td>70±14</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRVR after L-NMMA (3 mg/kg/h)</td>
<td>+19±13</td>
<td>+17±12</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRVR after L-arginine (100 mg/kg/30 min)</td>
<td>-15±10</td>
<td>-18±15</td>
<td>n.s.</td>
</tr>
<tr>
<td>ΔRVR after L-arginine + vitamin C (3 g/30 min)</td>
<td>-6±9</td>
<td>-14±17</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Means ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.
RPF, was similar between smokers and non-smokers. In addition, L-arginine induced renal vasodilatation was similar. Most interestingly, L-arginine-induced renal vasodilatation was significantly enhanced by vitamin C in smokers but not in non-smokers.

Baseline renal haemodynamics were similar in smokers and in non-smokers in our young study population. This is in agreement with a previous study [17], also examining young subjects with a mean age of 38 years. In older smokers with a mean age of 68 years there has been a report documenting a reduction of RPF determined by MAG3 clearance, whereas GFR was similar to age- and sex-matched non-smokers [18].

The infusion of L-NMMA induced a similar decrease of RPF and RBF and a similar increase of RVR and FF, while GFR did not change in either group. Blood pressure rose similarly, which is most important with respect to the correct interpretation of the changes in renal haemodynamics. Since no difference in the parameters of systemic haemodynamics was observed, it is possible to assume that basal nitric oxide production in the renal vasculature was not altered in smokers. Although it may be argued that the young age of our subjects explains why no alteration in L-NMMA-induced vasoconstriction of the renal vasculature was seen, a significantly reduced L-NMMA response of the forearm vasculature has previously been found in smokers of similar age [7]. Thus, the response to L-NMMA may in fact differ in various vascular beds of smokers. The dose that we applied was chosen since it was shown to be safe in several previous clinical trials and has been shown to affect both systemic and renal circulation to a significant extent [9–11]. However, we cannot completely rule out that a different response might have been detected if higher doses of L-NMMA were used.

The infusion of L-arginine increased RPF to a similar extent in both groups. GFR significantly increased in smokers only; however, the responses of GFR were not different between groups. With regard to GFR, previous studies either reported no change [12] or a small increase in GFR [13] in response to L-arginine in healthy volunteers.

Due to the profound differences in methodology in the assessment of endothelium-dependent vasodilatation in the renal versus the forearm circulation, we are not sure what kind of response could have been anticipated in smokers. Impaired endothelial function in the coronary circulation of smokers as determined by the increase of myocardial blood flow in response to the cold pressor test can be enhanced by the administration of L-arginine [6]. In addition, flow-mediated vasodilatation and acetylcholine-induced vasodilatation of the forearm vasculature can be enhanced by the intravenous administration of L-arginine in smokers [5].

Endothelium-dependent vasodilatation in the forearm vasculature is usually determined by the infusion of substances that stimulate the activity of eNOS, such as acetylcholine, or by induction of shear stress during reactive hyperaemia, which results in flow-mediated vasodilatation. L-arginine is then usually administered 'on top' of acetylcholine or reactive hyperaemia to additionally stimulate the L-arginine/NO pathway by providing substrate for eNOS. An increased response after the administration of exogenous L-arginine is then interpreted as a defect in the L-arginine/NO pathway. The precise mechanisms why exogenously administered L-arginine enhances the response of the NO pathway despite sufficient intracellular L-arginine concentrations, markedly above the Km value of the eNOS enzyme, still remain unsolved. In smokers, one favoured theory is that the deficiency of essential co-factors of the eNOS, such as the deficiency of tetrahydrobiopterin (BH4), may render the eNOS particularly susceptible to supraphysiological L-arginine levels.

Since acetylcholine cannot be given systematically due to the well-known side-effects and direct administration of acetylcholine into the renal artery is a very invasive procedure, the systemic administration of L-arginine has been used to assess endothelium-dependent vasodilatation in the renal vasculature. Interestingly, the renal circulation seems to be particular sensitive to the effects of systemic L-arginine administration, since even healthy subjects respond with an increase of RPF despite a decrease in systemic blood pressure. Therefore, this method has been widely used by several investigators [12,13]. Due to unspecific, NO-unrelated effects of L-arginine at higher doses, partially caused by an increase in osmotic pressure, we could recently demonstrate that a dose of 100 mg/kg/30 min is most specific for stimulation of the NO system and thus suitable for studies on renal vascular responses [13]. However, with respect to the comparison of data obtained in various vascular beds, the profound differences in the methods applied should always be kept in mind.

Finally, the co-infusion of vitamin C and L-arginine led to a significantly higher increase of RPF, RBF and GFR and a higher decrease of RVR in smokers compared to non-smokers. Moreover, the greater the number of cigarettes smoked, the more marked was the change of GFR and RVR. In parallel, vitamin C has previously been shown to enhance endothelium-dependent vasodilatation in the forearm vasculature and in the coronary circulation of smokers [7,8]. Thus, our results further suggest that oxidative stress seems to be increased in the renal circulation of smokers. The fact that L-arginine-induced renal vasodilatation was not different in smokers as compared to non-smokers despite higher oxidative stress in the renal circulation deserves further discussion. As mentioned above, higher oxidative stress in the renal circulation may deplete BH4, thus rendering eNOS partly a superoxide-producing enzyme, which may counteract the potentially beneficial effect of L-arginine supplementation on NO production. When vitamin C is administered additionally, superoxide production by eNOS may decrease through enhanced co-factor availability and thus net NO production increases [10,19]. However,
at present this remains speculative. In accordance with this notion, an enhanced response to vitamin C has been shown solely under conditions that stimulate endothelium-dependent vasodilatation so far. In the forearm vasculature of smokers, reduced basal, nitric-oxide-mediated vascular tone as determined by the administration of L-NMMA is not enhanced by vitamin C [7]. It should be noted that vitamin C alone does not exert any effect on renal haemodynamics, either in healthy control subjects or in smokers [20].

As mentioned earlier, the major limitation of our study derives from the fact that we cannot precisely determine to what extent the nitric oxide synthase was blocked by the L-NMMA dose applied. The dose that we applied has been used in several previous clinical trials and has been shown to affect both systemic and renal circulation to a significant extent without producing serious side-effects [9–11]. Dose-ranging studies with L-NMMA have shown that by using a dose of 3 mg/kg/h systemically, a blood concentration of 40 μg/ml can be achieved, which is higher than the 20 μg/ml achieved in brachial-artery studies using an intra-arterial dose of 4 μmol/min [9]. Nevertheless, we cannot rule out that the dose used in this study may have been too low to detect a true difference in basal renal nitric oxide bioavailability between smokers and non-smokers.

In conclusion, L-NMMA-induced vasoconstriction was similar in smokers and non-smokers. In addition, L-arginine-induced renal vasodilatation was similar. Vitamin C enhanced the renal response to L-arginine in smokers, suggesting that oxidative stress is increased in the renal circulation of smokers.

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