Interactions of L-Arginine, Isosorbide Mononitrate, and Angiotensin II Inhibitors on Arterial Pulse Wave

Gordon S. Stokes, Edward S. Barin, Kerry L. Gilfillan, and Wayne H. Kaesemeyer

**Background:** Deficiency of nitric oxide (NO) production has been implicated in the pathogenesis of increased pulse wave reflection associated with systolic hypertension. We investigated the effects on systolic blood pressure (BP) and pulse wave contour of two nitrate donors, isosorbide mononitrate (ISMN) and L-arginine.

**Methods:** The subjects were 14 elderly patients chronically treated with antihypertensive agents. In seven of the subjects, agents causing angiotensin II (AII) inhibition (angiotensin-converting enzyme [ACE] inhibitor or AT₁ receptor antagonist, or both) were used. Study entry required systolic BP of 150 to 200 mm Hg, and aortic pulse wave augmentation more than 15 mm Hg. Pharmacodynamic responses to ISMN, L-arginine, and ISMN plus L-arginine, were assessed in double-blind crossover studies by standard sphygmomanometry and applanation tonometry.

**Results:** Peripheral systolic BP, aortic systolic BP, and the aortic augmentation index were decreased (P < .001) by ISMN, irrespective of AII inhibition. L-Arginine enhanced these effects (P < .001) in the subjects without AII inhibition, but not in those receiving AII inhibitors. Given without ISMN or AII inhibitors, L-arginine decreased peripheral systolic BP, but to a lesser extent than ISMN.

**Conclusions:** L-Arginine has potential value as an adjunct to ISMN in combination with antihypertensive therapy in elderly patients with systolic hypertension. However, when given with single-dose ISMN, its vasodilator activity may overlap with that of AII inhibitors. Future studies of L-arginine in conjunction with chronic continuous ISMN dosing are warranted. Am J Hypertens 2003;16:719–724 © 2003 American Journal of Hypertension, Ltd.

**Key Words:** Hypertension, isosorbide mononitrate, L-arginine, pulse wave.

Deficiency of endothelial nitric oxide (NO) production has been identified as a potential factor in the pathophysiology of increased arterial stiffness, which in turn leads to the exaggerated pulse wave reflection and wide pulse pressure typical of systolic hypertension of the elderly (SHE). Increased systolic blood pressure (BP) is an important cardiovascular risk factor in elderly persons and is often resistant to standard antihypertensive agents, including angiotensin-converting enzyme (ACE) inhibitors. It is an attractive concept that SHE might be controlled best if endothelial NO dysfunction is redressed as well as increased BP. The ACE inhibitors are known to regress structural changes in the endothelium of hypertensive patients, but there is disagreement as to whether they improve NO bioavailability.

We have shown previously that the systemic arterial BP and amplitude of the aortic pulse wave of elderly patients with SHE can be decreased by the use of a NO donor, isosorbide mononitrate (ISMN), given as an adjunct to conventional combined antihypertensive therapy. The decrease in aortic pulse pressure, measured by applanation tonometry, was comprised in part by a decrease in height of the systolic ejection wave (first peak, P₁) and in part by a decrease in the exaggerated wave reflection component (second peak, P₂) seen in SHE. The effect of ISMN on P₂ exceeded that of angiotensin II (AII) inhibitors. We concluded that this marked effect of ISMN might denote correction of endothelial NO deficiency in SHE, and suggested that co-administration of L-arginine with ISMN should be tested in such patients. The rationale was that exogenous L-arginine, shown to ameliorate adverse car-


From the Hypertension Unit, Royal North Shore Hospital (GSS, ESB, KLG), St. Leonards, Australia and Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, Georgia.

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Address correspondence and reprint requests to Prof. G. S. Stokes, Hypertension Unit, Block 1A, Royal North Shore Hospital, St. Leonards, NSW, 2065, Australia; e-mail: gstokes@med.usyd.edu.au

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diovascular consequences of hypertension in aged spontaneously hypertensive rats (SHR), might augment the beneficial effect of ISMN on endothelial dysfunction in SHE by boosting endogenous NO production.

In the present study, we determined the antihypertensive activity and effects on pulse wave profile of single doses of ISMN, with or without oral L-arginine supplements, in two groups of nitrate-naive elderly patients with prevalent systolic hypertension. In one group, one or more AII inhibitors were included in baseline therapy, and in the other group, these agents were excluded.

Methods

Study Population

The study was carried out in 14 elderly hypertensive patients, six men and eight women, aged 56 to 82 years (mean, 70.3 years). Plasma creatinine concentration was <0.14 mmol/L in every case. All patients were fully ambulant without exercise limitation, cardiac disease, or vascular aneurysm. All had long-standing systolic hypertension and had been treated with conventional antihypertensive therapy (which had been stable in type and dosage for >3 weeks, and was continued through the present study). The study was approved by the relevant institutional ethics committee. Written informed consent was obtained from all subjects.

Study Design and Protocols

The patients were divided into two groups on the basis of whether they were or were not receiving at study entry an ACE inhibitor or an AT1 receptor antagonist, in addition to their other baseline medications (Table 1). Before entry, the time of dosing for baseline therapy was adjusted so as to fall between 4 and 8 PM daily. Entry criteria were as follows: stable regimen of one to three antihypertensive drugs at baseline; systolic BP (at trough) 150 to 200 mm Hg; diastolic BP (at trough) <100 mm Hg; aortic pulse wave augmentation >15 mm Hg.

Group I had four single-day, double-blind studies, 1 to 2 weeks apart, during a total period of 5 weeks. For the first 2 study days, the subjects were randomized with crossover to 60 mg of ISMN, versus encapsulated single doses of a placebo, each given at 8 AM on a separate day (Fig. 1). For the third and fourth study days, the subjects were randomized (with crossover) to 60 mg of ISMN (at 8 AM) plus 2 g of L-arginine (1 g at 8 AM and 1 g at noon), versus placebo (given at 8 AM) plus 2 g of L-arginine (1 g at 8 AM and noon).

Group II were randomized (with crossover) to two double-blind study phases, each of 2 consecutive days duration and separated by 2 weeks (Fig. 1). On day 1 of

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Table 1. Comparison of characteristics and baseline treatment for groups I and II

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>67</td>
<td>74</td>
</tr>
<tr>
<td>Gender (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Treatment at study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>AT1 receptor blockers</td>
<td>0</td>
<td>5*</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Diuretics</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

* Two patients received both an ACE inhibitor and an AT1 receptor blocker.

ACE = angiotensin-converting enzyme.

FIG. 1. Study design. Single columns denote a 1-day study; double columns denote a 2-day study with active isosorbide mononitrate (ISMN) given on the first day only. Baseline therapy was continued during the study drug washout periods between studies.
one phase, they received 60 mg of active ISMN plus 2 g of active L-arginine (1 g at 8 AM and noon) and on day 1 of the other phase, they received 60 mg of active ISMN plus placebo L-arginine. The second day of each phase was an ISMN recovery study, with placebo ISMN administered at 8 AM and active/placebo L-arginine given as on day 1. The purpose of this recovery study was to assess any effect that L-arginine might have in protracting the activity of ISMN at trough. For this reason, an overnight sustain-

effect that L-arginine might have in protracting the activity quanti-

was determined; P1 and P2 (augmentation pressure) were measured at the same intervals. Brachial BP was recorded by sphygmomanometer, and pulse wave tonometry was performed in duplicate at the same intervals. Brachial BP was recorded by sphygmomanometer, and pulse wave tonometry was performed at the radial artery. The aortic pulse waveform was determined; P1 and P2 (augmentation pressure) were quantified by computer software using a transfer function (SphygmoCor, AtCor Medical, Sydney, Australia). Augmentation index (a measure of wave reflection) was determined from the ratio of P2 height to aortic pulse pressure. Other details of the tonometry procedures have been described previously.11

Statistical Analysis

Statistical analysis was by repeated measures analysis of variance, followed by one-way analysis of pooled post-dose data and post-hoc paired t tests. For graphics and data analysis, GraphPad PRISM (GraphPad Software, Inc., San Diego, CA) was used. Values given are mean and SEM, and the level of significance was taken as P < .01, unless otherwise stated.

**Results**

**Effects of L-Arginine and ISMN in Group I**

Table 2 shows mean values for the postdose period (9 AM to 4 PM) of the 4 study days in group I. There were no significant changes in heart rate. L-Arginine significantly decreased brachial systolic BP (seated and standing) in relation to placebo. The ISMN (given alone) decreased brachial systolic pressure by 22 mm Hg, both seated and standing. With ISMN plus L-arginine, the corresponding decreases were 30 mm Hg and 32 mm Hg, respectively. These changes were each significantly greater than with ISMN alone. Brachial diastolic BP (seated or standing) was decreased by ISMN (P < .005) to a similar extent with or without L-arginine.

Fig. 2 shows for group I the effects of treatment with L-arginine and ISMN, separately and together, on aortic systolic BP. The ISMN lowered aortic systolic pressure significantly within 2 h, and this effect was sustained throughout the remainder of the observation period. The addition of L-arginine was associated with flattening of the morning peak observed after placebo, and also with a greater and more rapid depressor response after ISMN (P < .001). Augmentation index was decreased by ISMN relative to placebo from 37.8% to 25.2% (P < .001); the decrease with ISMN plus L-arginine (from 37.5% to 21.5%) was slightly greater (P < .05).

**Effects of L-Arginine and ISMN in Group II**

In group II, brachial systolic BP (seated) was decreased after ISMN from 160 ± 6 mm Hg (at baseline) to 136 ± 2 mm Hg (at nadir), and after ISMN plus L-arginine from 163 ± 3 mm Hg to 139 ± 2 mm Hg. Corresponding decreases in standing brachial BP were from 153 ± 6 mm Hg to 134 ± 2 mm Hg, and from 165 ± 6 mm Hg to 135 ± 1 mm Hg. There were no changes in diastolic BP or heart rate.

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**Table 2.** Group I treated on different days with placebo, L-arginine (2g), ISMN (60 mg), and ISMN combined with L-arginine

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate (beats/min)</th>
<th>Brachial Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seated</td>
<td>Systolic</td>
</tr>
<tr>
<td>Placebo</td>
<td>56 ± 0.6</td>
<td>168 ± 1.4</td>
</tr>
<tr>
<td>L-arginine</td>
<td>57 ± 1</td>
<td>161 ± 0.6</td>
</tr>
<tr>
<td>ISMN</td>
<td>57 ± 0.8</td>
<td>146 ± 1.6</td>
</tr>
<tr>
<td>L-arginine and ISMN</td>
<td>58 ± 0.3</td>
<td>130 ± 1.3</td>
</tr>
</tbody>
</table>

* P < .01 v placebo; † P < .001 v placebo; ‡ P < .001 v ISMN.

ISMN = isosorbide mononitrate.
Table 3 allows comparison of mean values for aortic systolic BP and augmentation index in group II with those in group I. Aortic systolic BP after ISMN was lower in group II than in group I, and was not lowered further by ISMN plus L-arginine. Values for augmentation index at nadir were lower for group II than for group I. However, decreases in this index produced by ISMN were not significantly different between groups, and in group II were not altered when L-arginine was combined with ISMN.

In the recovery study (group II, day 2), brachial systolic BP with continued L-arginine was 160 ± 1 mm Hg (seated) and 157 ± 2 mm Hg (standing). Corresponding values with placebo were 160 ± 1 mm Hg and 158 ± 2 mm Hg, respectively. Aortic systolic BP was 145 ± 1 mm Hg with L-arginine and 146 ± 1 mm Hg with placebo. Augmentation index was 27% ± 1% with L-arginine and 29.5% ± 1% with placebo. There were no significant differences between the recovery values with continued L-arginine and those with placebo.

**Discussion**

The AII inhibition, which has been reported to decrease pulse wave reflection and to increase endothelial NO bioavailability is a potential confounding factor in attempting to characterize effects on the pulse wave of ISMN and L-arginine. Thus, in group I the adjunctive actions of ISMN and L-arginine were studied in patients not treated with AII inhibitors. In this group, L-arginine produced a small decrease in systolic BP. The ISMN had stronger depressor effects, associated with a decrease in augmentation index. The combination of L-arginine with ISMN enhanced these effects. Decrease in augmentation index (which is indicative of a decrease in pulse wave reflection) may signify improvement in NO-dependent relaxation of conduit arteries. Thus, our findings suggest that ISMN induced NO-dependent vasorelaxation, and that L-arginine enhanced this effect in patients not taking AII inhibitors. L-Arginine has been shown before to

**Table 3.** Tonometry values at baseline (8 AM) and nadir (9 AM to 4 PM) with ISMN and ISMN plus L-arginine

<table>
<thead>
<tr>
<th></th>
<th>Aortic Systolic Blood Pressure (mm Hg)</th>
<th>Augmentation Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>Nadir</td>
</tr>
<tr>
<td>ISMN</td>
<td>152 ± 7</td>
<td>131 ± 2</td>
</tr>
<tr>
<td>ISMN with L-arginine</td>
<td>149 ± 2</td>
<td>122 ± 1</td>
</tr>
</tbody>
</table>

* P < .001 v corresponding value in group I (unpaired t test). Abbreviations as in Table 1.
reverse endothelial dysfunction in essential hypertension.\textsuperscript{23} Our finding that L-arginine also may potentiate the response to ISMN suggests that the combination of L-arginine with other NO donors such as glyceryl trinitrate or sodium nitroprusside warrants investigation. A preliminary study of L-arginine combined with continuous glyceryl trinitrate dosing in the treatment of chronic stable angina has suggested prevention of nitrate tolerance by L-arginine;\textsuperscript{24} however, it is not known whether this finding can be extended to the treatment of systolic hypertension.

The lack of change in heart rate despite decreases in BP with ISMN, or with ISMN plus L-arginine, is of interest. This constancy was fortuitous in that it permitted direct comparison of the pulse wave before and after these agents, without correction for change in heart rate.\textsuperscript{25} The presence of β blockade in 10 of the subjects (71%), together with the protocol requirement for patients to be kept at minimal physical activity (and at rest before each measurement was made) may have helped to prevent heart rate changes consequent on baroreceptor-mediated adjustments.

The ISMN decreased systolic BP in group II to a lower level than observed in group I. Also, values for augmentation index after ISMN were lower in group II than in group I. The drug classes used for baseline therapy (apart from AII inhibitors) were comparable in distribution in the two groups, as was gender. However, between-group differences may have arisen from selection bias, because our subject groups were small in number and were not matched for degree of vasculopathy. Alternatively, the lower values at nadir in group II are compatible with findings that chronic AII inhibitor therapy can decrease pulse wave reflection\textsuperscript{14–18} and can produce an effect on NO-dependent vasodilatation to which that of ISMN may be additive.\textsuperscript{11}

L-Arginine is thought to have an action on cardiac function through baroreceptor-mediated vagal tone,\textsuperscript{26} as well as one causing vasorelaxation through enhancement of eNOS-mediated NO production\textsuperscript{27} at the conduit artery level. This may explain how L-arginine (given alone) in group I significantly decreased brachial systolic BP without a significant effect on aortic systolic BP or augmentation index, yet given together with ISMN enhanced the strong effect of ISMN on the augmentation index. The lack of such an ancillary action in group II may have arisen because the effect of L-arginine on NO-dependent vasorelaxation was pre-empted by AII inhibition, and therefore, could not be expressed. Again, this possibility is supported by the finding (Table 3) of lower nadir values after ISMN (without L-arginine) in group II than in group I.

Our findings are at variance with those of Komers at al,\textsuperscript{28} who showed that L-arginine had no hypotensive effect in human subjects before treatment with AII inhibitors, but decreased mean arterial pressure and glomerular filtration rate after 3 weeks of ramipril or losartan. They observed no changes in indicators of NO activity, and suggested that the effect of L-arginine may have been NO independent.\textsuperscript{28} Their results were obtained with methods and a study population quite different from ours, as they used 30-min infusions of L-arginine in healthy young subjects. Such subjects would not have had the exaggerated wave reflection amplitude\textsuperscript{29} (and presumed vascular NO deficiency) of our elderly hypertensive patients.

We have observed previously that the depressor effect of ISMN lasts only for 12 h (despite some minor elevation of plasma nitrate concentration after that time).\textsuperscript{10} Results from the ISMN recovery period in group II, which showed that arterial BP and pulse wave components reverted to baseline levels with placebo on day 2, are compatible with this finding. The L-arginine phase was also extended to a second day in group II so as to determine whether as a NO donor, known to diminish nitrate tolerance,\textsuperscript{24,27} L-arginine could protract the activity of ISMN at trough. No such effect was seen on day 2. This finding does not exclude the possibility that L-arginine might prolong the effect of a given ISMN dose in the absence of AII inhibitor therapy, or during nitrate tachyphylaxis induced by chronic continuous ISMN dosing.

The effects of nitrates on pulse waveform\textsuperscript{30} and central hemodynamics\textsuperscript{31,32} have been known for many years. Nevertheless, our findings have topical importance for the treatment of systolic hypertension. First, they are consistent with our previous reports\textsuperscript{9–11} that ISMN decreased systolic BP in elderly patients with high pulse wave reflectance. There were some quantitative differences between the present findings and our study of patients that had received previous nitrate therapy.\textsuperscript{10} The peak decrease in aortic systolic BP with ISMN (>30 mm Hg in both groups) was 50% greater than in the earlier study. Also, the change in augmentation index was greater. These differences could have resulted from the lack of previous exposure to nitrates of the present subjects. Their nitrate-naïve state might have led to lesser effects from L-arginine than if nitrate tolerance had been present.

The second implication for treatment of SHE is that L-arginine, previously shown to lower BP in healthy volunteers,\textsuperscript{33} has potential use as an adjunct to some combination antihypertensive therapeutic regimens. Such regimens may not produce an optimal decrease in systolic BP because of the persistence of an exaggerated aortic pulse wave reflection (P2) like that seen at baseline in the present patient population. Given together with ISMN, which lowered arterial BP and aortic pulse wave reflection irrespective of AII inhibition, L-arginine produced an adjunctive effect in group I but not in group II. Thus, L-arginine supplementation could have a particular role with those regimens that include a slow-release nitrate and do not include an AII inhibitor. However, the apparent overlap in action between L-arginine and baseline AII inhibition observed in group II of this pilot study requires substantiation in larger groups of subjects treated with nitrate and L-arginine on a chronic basis.
References