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Inhibition of Vascular Oxidative Stress in Hypercholesterolemia by Eccentric Isosorbide Mononitrate

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OBJECTIVES We sought to determine if the nitric oxide (NO) donor isosorbide mononitrate (ISMN) (200 mg/kg body weight/day) decreases vascular bioavailability of superoxide in atherosclerosis.

BACKGROUND Vascular oxidative stress limits the bioavailability of endothelial NO and promotes atherosclerosis, while NO itself exerts antioxidative effects. It is unknown if therapeutic NO impacts on vascular oxidative stress in atherosclerosis.

METHODS New Zealand white rabbits (n = 10 each group) were fed either normal chow (control), cholesterol chow (CHOL) (0.75 %), or cholesterol chow enriched with ISMN (CHOL-ISMN). Rabbits were fed twice daily. After 16 weeks we used aortic segments to measure vascular superoxide (5-μM lucigenin), intimal lesion formation, and vasoreactivity to acetylcholine (ACH) and ISMN.

RESULTS Plasma cholesterol increased by 40-fold in CHOL and CHOL-ISMN. The plasma concentration of ISMN in CHOL-ISMN was 1,529 ± 447 ng/ml. Superoxide formation (control: 228 ± 20 counts/20 min/mg) was strongly enhanced in CHOL (345 ± 46 counts/20 min/mg, $p = 0.02$) but not in CHOL-ISMN (229 ± 23 counts/20 min/mg) demonstrating antioxidative effects of eccentric ISMN in vivo. In parallel, intima-media thickness of thoracic aorta (159 ± 4 μm in control) was reduced from 645 ± 41 μm (CHOL) to 440 ± 51 μm (CHOL-ISMN, $p < 0.05$). Likewise, eccentric ISMN partially restored vascular responses to the NO donor S-nitroso-N-acetyl-D,L-penicillamine and improved endothelium-dependent vasorelaxation. The maximal ACH relaxation increased from 26.3 ± 9.6% in CHOL to 49.7 ± 8.1% in CHOL-ISMN; ISMN treatment induced a moderate nitrate tolerance as evidenced by diminished ISMN-induced vasodilation.

CONCLUSIONS These data suggest that eccentric ISMN can completely inhibit the increase of vascular bioavailability of superoxide and partially prevent intimal lesion formation and endothelial dysfunction in hypercholesterolemia. (J Am Coll Cardiol 2004;44:624–31) © 2004 by the American College of Cardiology Foundation

Hypercholesterolemia promotes atherosclerosis and is a major risk factor of acute coronary syndromes and cardiovascular death. Its pathogenesis is multifactorial and includes vascular inflammation and increased generation of vascular superoxide and other reactive oxygen species such as peroxynitrite, hydroxyl radicals, and hydrogen peroxide (1,2). One of the major consequences of increased vascular

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bioavailability of superoxide is an impairment of vascular endothelial function. This has been initially demonstrated many years ago (3,4), and evidence for an involvement of superoxide in endothelial dysfunction induced by hypercholesterolemia in rabbits was provided (5). Today, it is very well-established that the so-called vascular oxidative stress is associated with a variety of cardiovascular diseases such as coronary artery disease, hypertension, heart failure, and noninsulin-dependent diabetes mellitus (2).

The mechanism underlying the increased bioavailability of vascular reactive oxygen species is multifactorial and involves an increased expression and activity of enzymes generating oxygen radicals such as NADPH-oxidase, a decreased expression and activity of enzymes such as superoxide dismutase (SOD), which detoxifies reactive oxygen species, and a decreased concentration of cellular antioxidants such as cysteine and glutathione (6). At the same time, oxidative stress reduces the bioavailability of vascular nitric oxide (NO) and initiates endothelial dysfunction (7). These findings lead to the hypothesis that an imbalanced generation of superoxide and NO contributes to the pathogenesis of cardiovascular diseases. Both radicals are important biological mediators that promote pathogenic and protective effects in the vasculature, respectively (6,8).

Organic nitrates are a structurally diverse group of compounds that all carry an aliphatic nitrate moiety that is enzymatically reduced by a flow of three electrons to the

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Abbreviations and Acronyms

cGMP = cyclic guanosine monophosphate
ecSOD = extracellular superoxide dismutase
GCMS = gas chromatography mass spectrometry
GTN = glyceryl mononitrate
ISMN = isosorbide mononitrate
NO = nitric oxide
sGC = soluble guanylyl cyclase
SNAP = S-nitroso-N-acetyl-D,L-penicillamine
SOD = superoxide dismutase

nitrate nitrogen to generate NO (9,10). This reduction of the nitrate moiety occurs in many cell types including vascular smooth muscle and endothelial cells, and it has been shown that treatment with organic nitrates increases the NO content of rabbit arteries and veins (11). Although antioxidative effects of NO itself have been well-established in vitro, it is not known if NO donors such as organic nitrates are capable of reducing vascular oxidative stress in hypercholesterolemia.

We sought to determine if the NO donor isosorbide mononitrate (ISMN) decreases vascular bioavailability of superoxide and endothelial dysfunction in hypercholesterolemia and investigated the effects of long-term oral ISMN on changes of vascular reactivity in hypercholesterolemic rabbits. We found that eccentric ISMN completely inhibits the increase of vascular bioavailability of superoxide and partially prevents intimal lesion formation and endothelial dysfunction. These data indicate that eccentric ISMN might provide vasoprotection in hypercholesterolemia.

METHODS

Animals studied. A total of 30 female New Zealand white rabbits (10 to 12 weeks, mean body weight 2,105 ± 47 g) were housed individually as described previously (12). Rabbits were randomly divided in 3 groups of 10 animals and were fed either a standard diet (control), a 0.75 % w/w cholesterol diet (CHOL), or a cholesterol diet supplemented with ISMN to achieve a daily dosage of 200 mg ISMN/kg body weight ISMN (CHOL-ISMN) for 16 weeks. The dosage of ISMN was given in two identical portions in the morning (8:00 AM) and in the early afternoon (3:00 PM). During the study the animals were supervised by a veterinarian. Body weight was determined weekly, and plasma cholesterol concentrations were determined before and eight weeks after initiation of treatment. Animals were sacrificed 3 h after the last application of ISMN in the morning.

Permission for this study was provided by the regional government (AZ 23.05-230-3-77/99, AZ 23.05-230-3-52/99), and the experiments were performed according to the guidelines for the use of experimental animals as given by "Deutsches Tierschutzgesetz" and the "Guide for the Care and Use of Laboratory Animals" of the U.S. National Institutes of Health.

Vasorelaxation studies. Rabbits were anesthetised by injection of a mixture of xylazine (5 mg/kg^{-1}) and ketamine (25 mg/kg^{-1}) into the tibialis muscle. The animals were killed by exsanguination in deep anesthesia, and the entire thoracic and abdominal aorta were dissected free. Preparation of four thoracic ring segments and equilibration was performed in Krebs-Henseleit buffer as described previously (12). Function of endothelium was examined by cumulative addition of acetylcholine (0.01 to 10 μM) after submaximal precontraction with 0.2 μM phenylephrine. This was followed by a washout and a cumulative application of phenylephrine (0.01 to 10 μM). Thereafter the aortic rings were again rinsed with buffer and divided in subgroups, and the vasorelaxations to different types of NO donors such as S-nitroso-N-acetyl-D,L-penicillamine (SNAP) (1 nM to 10 μM) and ISMN (10 nM to 1 mM) were studied by cumulative application after precontraction with phenylephrine (0.2 μM); KCl, acetylcholine, phenylephrine, and each NO donor was studied in endothelium intact, and in endothelium-denuded thoracic rings from each animal.

Determination of aortic superoxide. Aortic superoxide was determined as described previously (13). Briefly, equilibrated segments of thoracic aorta were incubated at 37°C in albumin-buffer (pH 7.4) of the following composition (in mM): Na^+ 144.93, K^+ 7.23, Cl^- 138.77, $H_2PO_4^-$ 4.55, HPO_4^{2-} 8.03, glucose 5.55, and bovine serum albumin (0.1 %, weight/volume). This buffer was enriched with lucigenin (5 μM), and superoxide was calculated from chemiluminescence measurements (Picolite A6112 Luminometer, Packard, Downers Grove, Illinois).

Determination of plasma and tissue concentrations of ISMN. Plasma concentrations of ISMN were determined with support of ACC GmbH, Leidersbach, Germany. Briefly, blood samples were taken by punctation of the middle ear artery with heparin (15 U/ml) containing syringes 3 h after the last morning dose of ISMN. Blood samples were rapidly centrifuged at 4°C and 1,000 × *g* for 10 min. The supernatant was frozen at −20°C and stored until use. Vascular concentrations of ISMN were determined in frozen aortic rings; ISMN was determined by gas chromatography mass spectrometry (GCMS) (HP6890, Hewlett Packard, Waldbronn, Germany) after liquid-liquid extraction with ethyl acetate.

Histologic and histochemical procedures. One aortic ring (1-cm length) of each rabbit of the three groups was fixed in buffered 4% formalin and embedded ethanol-free in the water-soluble plastic resin Technovit 7100 (Heraeus-Kulzer, Hanau, Germany) to prevent shrinkage and lipid extraction; 4-μm sections were stained with 1% toluidine blue, and intima as well as intima-media thickness were measured using the image-analyzing system CUE-3 (Olympus Ltd., version 4.5, 1993, Hamburg, Germany). For a better determination of plaque formation, aorta sections of all groups were also stained lipid histochemically with sudan black B (saturated in 70% ethanol at 4°C). The measurements were routinely performed in a double-blind experi-

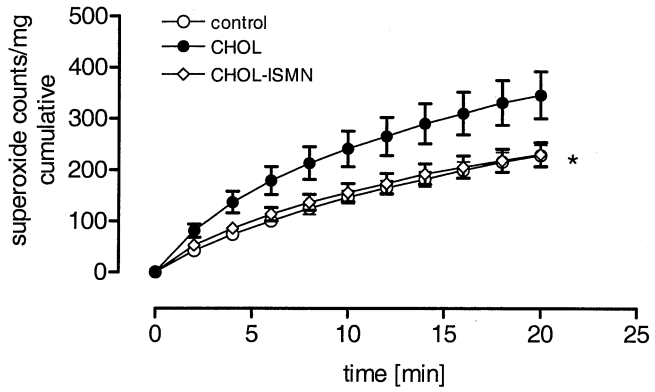

Figure 1. Effect of a four months' lasting oral treatment with isosorbide mononitrate (ISMN) on changes of aortic superoxide induced by hypercholesterolemia. Given are the cumulative counts/mg tissue measured during incubation with 5 μM lucigenin. The significant increase of superoxide in cholesterol chow group (CHOL) was completely abolished by treatment with ISMN (*p < 0.01 CHOL vs. control and CHOL-ISMN).

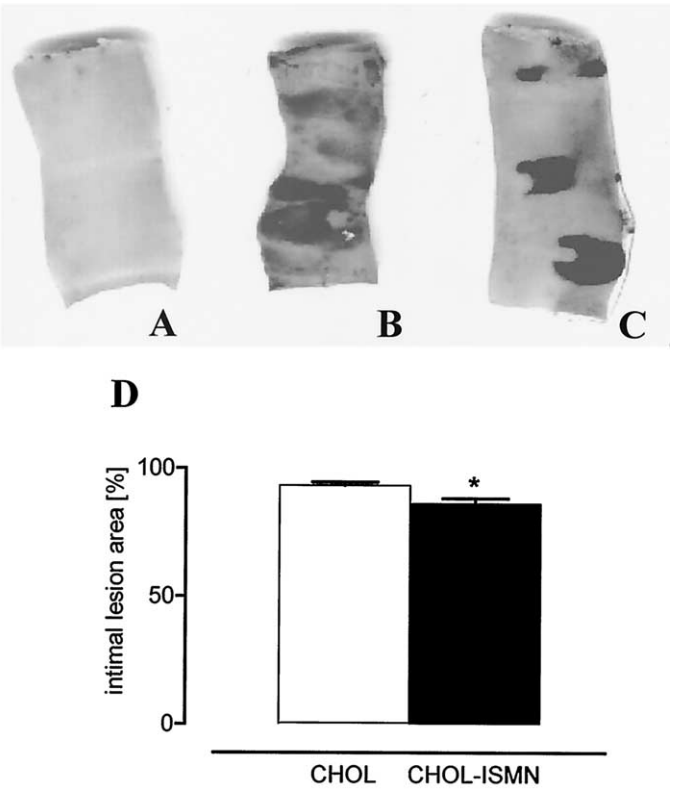

Figure 2. Area of intimal lesions in the aorta stained with sudan IV **(black areas, panels B and C)**. Given are representative examples of aortic segments of **(A)** control, **(B)** cholesterol chow (CHOL), and **(C)** cholesterol chow-isosorbide mononitrate (CHOL-ISMN), and **(D)** the mean values of aortic arch segments of CHOL and CHOL-ISMN (*p = 0.0094).

mental approach by a technical assistant that had no knowledge of the specific scientific background of the study.

Substances and solutions. S-nitroso-N-acetyl-D,L-penicillamine was synthesized in our laboratory as described previously (14). Isosorbide mononitrate was provided by Schwarz Pharma, Monheim, Germany. All other chemicals were obtained from Merck, Darmstadt, Germany, or from Sigma, Deisenhofen, Germany, in analytical grade. The stock solutions of acetylcholine (10 mM), phenylephrine (10 mM), and ISMN (100 mM) were prepared in distilled water. Solutions of SNAP (200 mM) were prepared in dimethylsulfoxide. All stock solutions were prepared daily, diluted with Krebs-buffer as required, kept on ice, and protected from daylight until use. All concentrations indicated in the text and figures are expressed as final bath concentrations.

Statistics. All data were analyzed by standard computer programs (GraphPad Prism PC Software, San Diego, California, version 3.0, analysis of variance [ANOVA]) and are expressed as mean values and standard error of the mean (SEM). Significant differences were evaluated using either unpaired two-tailed Student *t* test or ANOVA with subsequent Newman-Keuls multiple comparison test when appropriate. A p value <0.05 was considered significant.

RESULTS

All animals completed the study. Animals of CHOL and CHOL-ISMN showed some hair loss at their feet, neck, and belly and had an enlarged liver with easily visible yellow fat deposits. Such deposits also occurred in the skin and the iris. The increase in body weight observed during the study was slightly greater in controls (from 2,162 ± 20 g to 3,752 ± 99 g; p < 0.003 for comparison of final body weights) compared with CHOL (from 2,122 ± 34 g to 3,314 ± 76 g) and CHOL-ISMN (from 1,953 ± 60 to 3,255 ± 67). During the study the plasma concentration of total cholesterol increased in CHOL (from 75 ± 11 mg/dl to 2,133 ± 95 mg/dl) and in CHOL-ISMN (from 72 ± 10 to 2,030 ± 135) but not in controls (from 52 ± 4 mg/dl to 53 ± 4 mg/dl). The plasmatic concentration of ISMN in CHOL-ISMN was 1,529 ± 447 ng/ml (corresponds to 8 μM), and the ISMN concentration in washed aortic segments used for superoxide and vasorelaxation measurements was 53.5 ± 8.4 pmol/g aorta (corresponds to 53.4 nM). Control experiments in rat aorta showed that 65 nM ISMN had no effect on the detection of superoxide with lucigenin as indicated by identical accumulation at rates (in counts/mg/min, n = 3 each) of 10.4 ± 0.55 (control) and 9.7 ± 0.83 (ISMN).

Vascular superoxide. Superoxide in aortic rings was significantly increased in CHOL compared with controls (Fig. 1). The mean maximal values were 228 ± 20 counts/20 min/mg in controls and 345 ± 46 counts/20 min/mg in CHOL (p = 0.02) confirming that hypercholesterolemia induces vascular oxidative stress. In striking contrast, there was no increase of superoxide in CHOL-ISMN as evidenced by the maximal mean value of 229 ± 23 counts/20 min/mg. These data suggest that eccentric ISMN can completely prevent the increase of vascular superoxide in hypercholesterolemia.

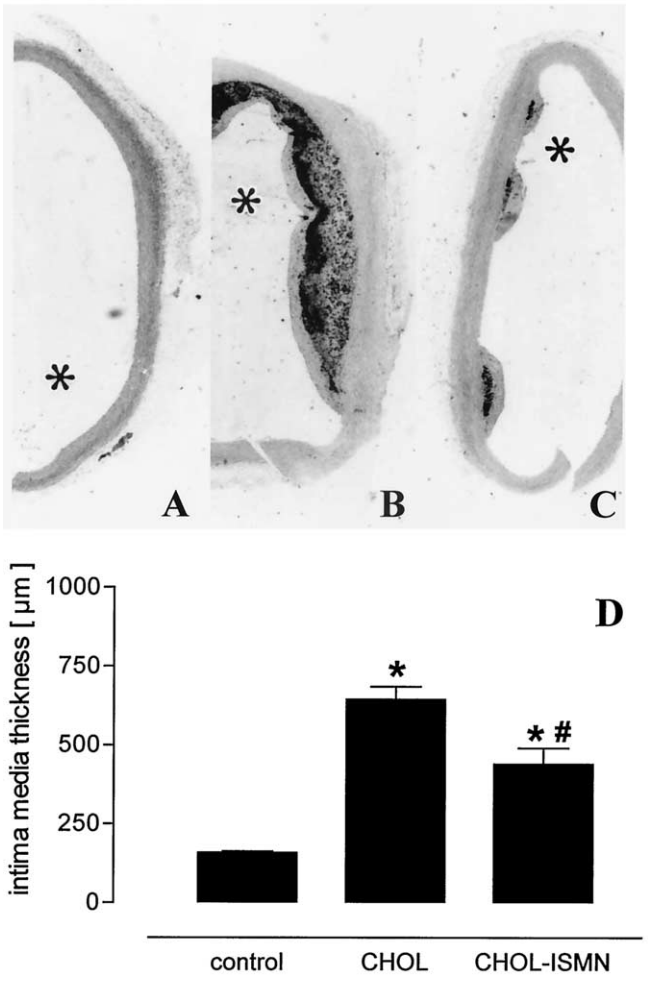

Figure 3. Cross-sections of thoracic aortic rings stained with sudan black B to visualize atherosclerotic areas **(black areas)**. Given are representative examples of aortic cross-sections (* = lumen) of **(A)** control, **(B)** cholesterol chow (CHOL), and **(C)** cholesterol chow-isosorbide mononitrate (CHOL-ISMN), and **(D)** the mean values of the intima-media thickness (*$p < 0.001$, vs. control, #$p < 0.001$ CHOL-ISMN vs. CHOL).

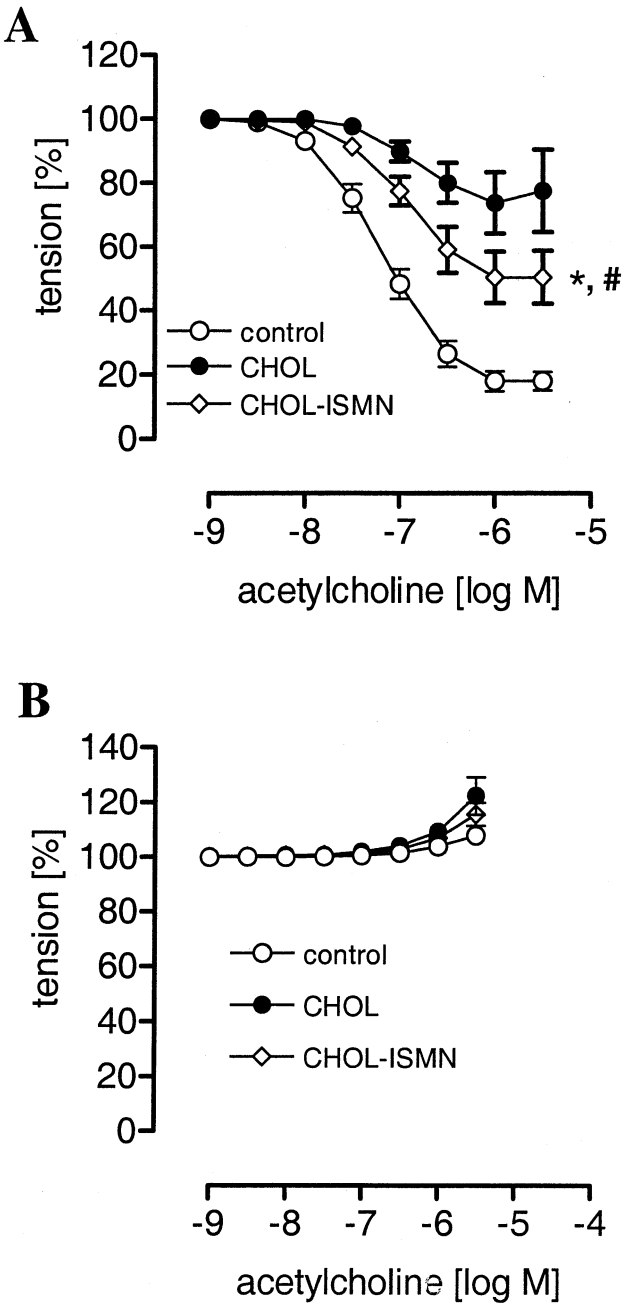

Figure 4. Effect of a four months' lasting oral treatment with isosorbide mononitrate (ISMN) on changes of endothelium-dependent vasorelaxation induced by hypercholesterolemia in **(A)** endothelium-intact aortic rings. The significant impairment of endothelium-dependent vasodilation in cholesterol chow (CHOL) as compared with controls was partially reversed by ISMN (*$p < 0.001$ CHOL vs. CHOL-ISMN; #$p < 0.001$ CHOL-ISMN vs. control). In **(B)** endothelium-denuded rings, acetylcholine induced vasoconstrictions.

Changes of vascular morphology. Hypercholesterolemia induced typical atherosclerotic lesions in the aorta. Macroscopic inspection (Figs. 2A, 2B, and 2C) showed a strong staining in CHOL, which was lower in CHOL-ISMN. A more detailed microscopic evaluation of cross-sections showed a strong accumulation of lipid-laden cells in the plaques of CHOL that was lower in CHOL-ISMN (data not shown). Measurement of the intima-media thickness in cross-sections of the aorta (Fig. 3) showed a strong increase in CHOL that was significantly diminished in CHOL-ISMN suggesting that eccentric ISMN can reduce the formation of intimal lesions in experimental hypercholesterolemia.

Endothelium-dependent vasodilation. In CHOL, relaxation of aortic rings induced by 1 μM acetylcholine showed a maximum of only 26.3 ± 9.6% (Fig. 4) indicating severe impairment of normal endothelial function (controls: 82 ± 2.8%). In striking contrast, endothelium-dependent vasorelaxation in CHOL-ISMN showed a maximum of 49.7 ± 8.1% ($p < 0.05$ vs. CHOL) indicating a reduced endothelial dysfunction in hypercholesterolemic rabbits treated with ISMN. In addition, there was a significant linear relationship between the maximal relaxation to acetylcholine and the severity of aortic intimal lesion area in both CHOL ($r = 0.839$, $p < 0.0001$) and CHOL-ISMN ($r = 0.708$, $p =$

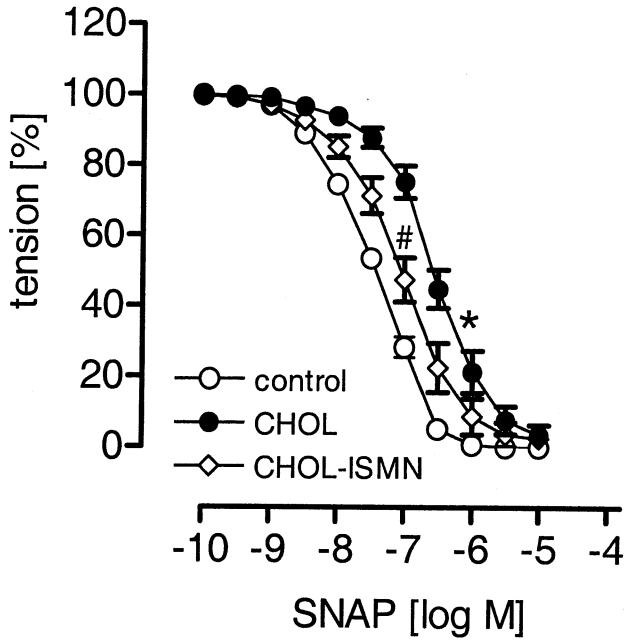

Figure 5. Effect of a four months' lasting oral treatment with isosorbide mononitrate (ISMN) on changes of vasorelaxations to the nitric oxide (NO) donor S-nitroso-N-acetyl-D,L-penicillamine (SNAP) induced by hypercholesterolemia. The significant reduction of the vascular sensitivity against NO-dependent vasodilation induced by SNAP (rightward-shift, *$p < 0.0001$, control vs. cholesterol chow [CHOL]) was partially reversed by ISMN (CHOL vs. CHOL-ISMN: #$p < 0.001$).

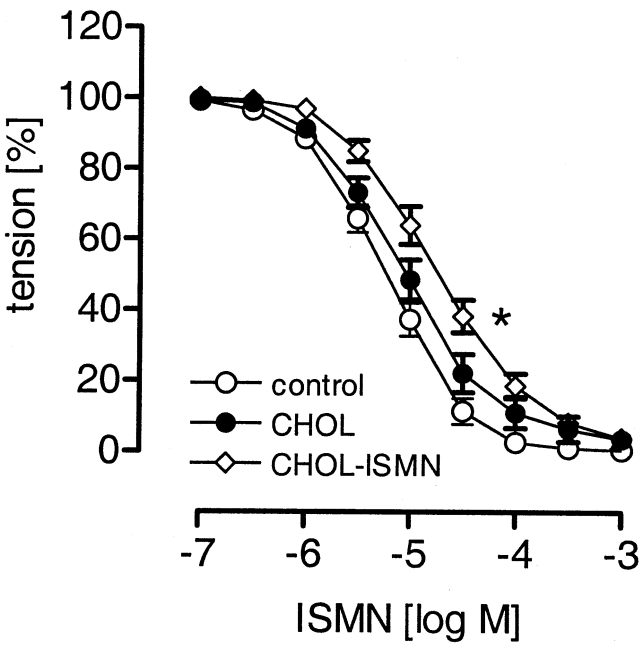

Figure 6. Effect of a four months' lasting oral treatment with isosorbide mononitrate (ISMN) on vasorelaxations to ISMN. There was a rightward shift of the ISMN concentration response curve in cholesterol chow (CHOL)-ISMN as compared with controls and with CHOL (*$p < 0.0001$, each). This significantly less vasodilator response to ISMN indicates the development of nitrate tolerance in CHOL-ISMN.

0.0005). However, acetylcholine-induced vasodilation in CHOL-ISMN was still significantly lower as compared with controls indicating that ISMN did not completely inhibit the development of endothelial dysfunction. In endothelium-denuded aortic rings, acetylcholine evoked vasoconstrictions only.

NO-dependent vasodilation. Hypercholesterolemia induced a decrease of the vasodilator response to the NO donor SNAP (Fig. 5) as evidenced by a significant rightward shift of the concentration-reponse curve. This desensitization against NO-induced vasodilation in hypercholesterolemic rabbits was almost completely reversed by ISMN. The halfmaximal vasodilator concentrations in (−logM) were 7.43 ± 0.03 in controls, 6.56 ± 0.09 in CHOL, and 7.05 ± 0.09 in ISMN-CHOL.

Occurrence of nitrate tolerance. Treatment with eccentric ISMN induced the development of a moderate nitrate tolerance as evidenced by a rightward shift of the concentration response curve for ISMN (Fig. 6). In contrast, hypercholesterolemia itself did not reduce vasodilation by ISMN. The halfmaximal vasodilator concentrations in (−logM) were 5.23 ± 0.03 in controls, 5.08 ± 0.05 in CHOL, and 4.76 ± 0.05 in ISMN-CHOL.

DISCUSSION

The aim of this study was to determine the influence of NO donors, such as organic nitrates, on vascular oxidative stress in hypercholesterolemia. Our new finding is that the NO donor ISMN can completely prevent the increase of vascular superoxide induced by hypercholesterolemia. Furthermore, eccentric ISMN exerted these antioxidative effects despite the development of moderate nitrate tolerance. Isosorbide mononitrate treatment was also associated with an improvement of endothelial function and a reduction of vascular lesion formation. These results suggest that long-term treatment with eccentric ISMN may offer vasoprotection in hypercholesterolemia.

There is substantial evidence that the vascular effects of ISMN are mediated by activation of the NO/cyclic guanosine monophosphate (cGMP) pathway. Nitrates are prodrugs that release NO as their pharmacologically active principle (15). This has been demonstrated by measurements of NO release, accumulation of cGMP, and activation of protein kinase G, and holds true for a variety of structurally very diverse compounds including ISMN, which all carry one or more aliphatic nitrate moiety (16). Nitric oxide can exert a variety of vasoprotective effects such as vasodilation, antioxidative effects, and inhibition of platelet aggregation, leucocyte adhesion, and smooth muscle proliferation (8). Of these, the antioxidative effects of NO appear to be particularly important in our study.

The mechanism by which NO exerts antioxidative effects is multifactorial. Nitric oxide has been shown to inhibit peroxidation of lipids such as free fatty acids, phosphatidylcholine, and low-density protein particles (17). In a previous investigation, we have treated hypercholesterolemic rabbits

with another organic nitrate (pentaerythritol tetranitrate) and found a significant reduction of low-density lipoprotein oxidation that was associated with a preservation of endothelial function (18). Another mechanism by which NO exerts antioxidative effects is stimulation of the expression and activity of extracellular superoxide dismutase (ecSOD). We have recently shown that this effect is initiated by both exogenous and endogenous NO and that it occurs in vitro and in vivo (19). Furthermore, preliminary studies in human umbilical arteries and veins and in human vascular smooth muscle cells have indicated that ISMN increases ecSOD expression (T. Fukai, G. Kojda, personal communication, September 2004). Antioxidative effects of NO may also include the induction of ferritin and heme oxygenase (20), and there is evidence that organic nitrates activate this pathway (21). Thus, the complete reduction of increased vascular superoxide formation in hypercholesterolemia by eccentric ISMN is most likely mediated by activation of different pathways including inhibition of lipid peroxidation and induction of ecSOD, ferritin, and heme oxygenase.

Many previous investigations have shown that a variety of cardiovascular diseases including hypercholesterolemia are associated with both increased vascular superoxide and decreased bioavailability of endogenous NO (2). Although controversial findings exist on the direct effects of superoxide on the atherosclerotic disease process in various transgenic animal models (22), it has been well-established that a reduction of vascular superoxide will improve endothelial function (6). This coherency has been demonstrated even before it was known that the endothelium-derived relaxing factor is NO (3,4). Thus, the association between reduced vascular superoxide and improved endothelial function shown in this study is consistent with previous experimental and clinical studies.

Hypercholesterolemia alone inhibited vasorelaxations to the NO donor SNAP, while additional treatment with ISMN resulted in an improvement of SNAP-induced vasorelaxation. Both effects are likely related to the activity of soluble guanylyl cyclase (sGC). This key enzyme of the NO/cGMP pathway is extremely sensitive to oxidative stress and is markedly inhibited by oxidants such as superoxide and peroxynitrite (23). Previous investigations in hypercholesterolemic rabbits have shown a reversible increase of expression of a dysfunctional sGC that was associated with inhibition of endothelium-dependent and SNAP-induced vasodilation (24). Thus, the improvement of SNAP-induced vasodilation in CHOL-ISMN provides further evidence for the antioxidative efficacy of the eccentric ISMN treatment. Our observation of an almost unchanged vasodilatory response of atherosclerotic aortic rings to ISMN might be due to the fact that endothelial NO production could impact on the generation of NO from nitrates. We have reported that NO inhibits the bioactivation of glyceryl trinitrate (GTN) by measuring 1,2- and 1,3-glyceryl trinitrate concentrations as well as vasodilation in aortic rings that were subjected to continuous NO before incubation with GTN (25).

We found that complete inhibition of increased vascular superoxide only partially reduced morphologic and functional sequels of hypercholesterolemia. These results confirm that vascular superoxide plays a role in the atherosclerotic disease process but suggests at the same time that its contribution to the pathogenesis of atherosclerosis is limited. However, it is likely that vascular superoxide generation increases when plasma concentrations of ISMN decrease in the course of the treatment. For example, in nonatherosclerotic rabbits the plasma concentration of ISMN 3 h after dosing was 1,768 ng/ml but dropped to 14.5 ng/ml 16 h after dosing (26). In addition, there are other radicals and/or oxidants such as the hydroxyl radical, hydrogen peroxide, and peroxynitrite, which are likely involved in the pathogenesis of atherosclerosis (2,27). Thus, we cannot exclude intermittent increases of vascular superoxide or the formation of peroxynitrite when ISMN plasma levels drop. Such a phenomenon might have reduced the beneficial effect of ISMN in our study.

Previous investigations have shown that the total vascular SOD activity is increased in hypercholesterolemic rabbits (28). Although this might decrease the likelihood of peroxynitrite formation (29) and the toxic effects of its reaction products such as nitrogen dioxide, thiyl, sulfinyl, and disulfide radicals (8), it will almost certainly increase the steady-state concentration of vascular hydrogen peroxide. Furthermore, uncoupled eNOS produces superoxide and NO in very close proximity and might increase vascular oxidative stress without increasing total vascular superoxide formation (6). Although aortic generation of NO from tissue ISMN as measured by GC/MS did not change superoxide detection by lucigenin (suggesting that peroxynitrite formation from tissue ISMN is unlikely), increased vascular radicals and/or oxidants other than superoxide are likely involved in intimal lesion formation and endothelial dysfunction in CHOL-ISMN.

Increased vascular superoxide in hypercholesterolemia had apparently little impact on the vasodilator activity of ISMN, but we observed a moderate nitrate tolerance in CHOL-ISMN where superoxide was not increased as compared with controls. This confirms our previous findings on nitrate tolerance induced by eccentric ISMN (26) and provides further evidence for multifactorial mechanism of in vivo nitrate tolerance that includes superoxide-dependent (30) and -independent pathways (26). An eccentric dosing regimen for ISMN as used in our previous study had no effect on vascular superoxide but initated nitrate tolerance when its minimal plasma concentrations reached a threshold 14.5 $\pm$ 4 ng/ml.

Recently, it has been demonstrated that the extent of endothelial dysfunction predicts acute coronary events in coronary artery disease (31). This finding suggests that an improvement of endothelial function (e.g., by reducing vascular superoxide) may delay the atherosclerotic disease

process. In this context, one may speculate on potential clinical implications of our results. We have shown previously that pentaerythrithyl tetranitrate inhibits the induction (12) and the progression (18) of atherosclerosis in hypercholesterolemic rabbits. It has been reported that the cholesterol-fed rabbit produces atherosclerotic lesions, which are quite similar to human atherosclerosis (32). This may suggest some clinical relevance of our results, although animal experiments have significant limitations. A recently published large clinical trial has shown that nicorandil, which predominantly acts as a nitrovasodilator but also opens potassium channels (33–35), can improve the outcome of patients with stable angina pectoris and additional risk factors by reducing major coronary events (36). This suggests that the nitrate-like activity of nicorandil contributed to its prognostic effect.

Although we used a high dose of ISMN, which initated nitrate tolerance even at eccentric dosing, we believe that the results of our study add another piece of evidence on potentially inhibitory effects of nitrates on the development of atherosclerosis. To achieve this effect, it is obviously important to avoid severe nitrate tolerance, as inducible with continuous nitroglycerine (30), by strictly adhering oral nitrate therapy to the recommended intermittent dosing regimen (10). Finally, a randomized placebo-controlled prospective clinical investigation is needed to elucidate the effects of organic nitrates on prognosis in the setting of human atherosclerosis.

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