

Induction and Circumvention of Nitrate Tolerance Applying Different Dosage Intervals

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There is increasing evidence that constant nitrate plasma levels, as induced by at least three-times-daily ingestions of isosorbide dinitrate in sustained-release form, lead to an attenuation or even complete loss of the anti-ischemic effects (nitrate tolerance). Therefore, the dependence of tolerance development on dosage intervals according to once-daily and twice-daily ingestions was assessed. Tablets of isosorbide dinitrate (80 mg) in sustained-release form were administered once-daily at 8 A.M. (dosage interval 24 hours) or twice-daily at 8 A.M. and 8 P.M. (dosage interval 12 hours), as well as at 8 A.M. and 2 P.M., respectively (maximal dosage interval 18 hours). A total of 34 patients with angiographically proven coronary artery disease, a history of stable, exercise-dependent angina pectoris, and a reproducible, exercise-induced ST-segment depression of at least 0.15 mV (1.5 mm), who initially showed a response to 80 mg of isosorbide dinitrate, were enrolled. The anti-ischemic effects of isosorbide dinitrate on exercise-induced ischemia were objectively determined by the measurement of exercise-induced ST-segment depression before as well as two, six, and 12 hours after the ingestion at the first and the 15th day of the studies. Since the dosage interval of 12 hours resulted in constant plasma levels, the initially beneficial anti-ischemic effects of isosorbide dinitrate were considerably attenuated after two weeks of treatment. In contrast, the once-daily regimen with its intermittent peaks and valleys of nitrate plasma levels showed identical anti-ischemic effects at the 15th day as compared with the first day. Ingestions at 8 A.M. and 2 P.M. also circumvented the development of nitrate tolerance, however, combined with an even more pronounced anti-ischemic effect after 12 hours as compared with the once-daily regimen. Thus, the circumvention of nitrate tolerance requires a daily "nitrate-poor" interval. The best compromise between a maximal possible anti-ischemic effect and the circumvention of tolerance development was found for the "eccentric" dosage regimen in which the tablets were ingested in the morning and early afternoon.

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Despite the widespread use of coronary artery bypass surgery and balloon dilatation, drug therapy still plays a major role in the treatment of myocardial ischemia. Nitrates have been used sublingually for more than 100 years to curtail anginal episodes and orally for more than 25 years to prevent symptomatic episodes [1,2]. Nitrates are highly effective in all forms of angina and act, in contrast to beta blockers and calcium antagonists, by reducing the preload via venodilatation [3,4]. Nitrates may improve hemodynamics independently of the myocardial contractility; thus, in contrast to beta blockers and calcium antagonists, nitrates are

relatively safe [5]. Adverse effects, such as headache, nausea, and hypotension, are usually restricted to the initial phase of therapy only. Thus, nitrates seem to be the "ideal" anti-ischemic medication.

Towards the end of the last decade, however, there was increasing evidence of a relevant attenuation or even loss of the anti-ischemic effects following oral long-term treatment (nitrate tolerance). Nitrates were ingested in all these studies at least three times a day, suggesting a relationship between constant plasma levels and the development of tolerance [6–12].

Because at that time there were no studies assessing the nitrates according to a less than three-times-a-day prescription, we first investigated the hemodynamic and anti-ischemic effects of isosorbide dinitrate administered in different dosage intervals, according to once- and twice-daily regimens [13]. The goal of our studies was to determine whether a new regimen may prevent the development of tolerance. In order to achieve the longest possible duration of action of a single tablet, we administered the highest single dosage of isosorbide dinitrate in sustained-release form available at that time (80 mg) [13]. This article summarizes our studies addressed to nitrate tolerance in conjunction with the determined plasma levels of isosorbide dinitrate as well as its metabolites isosorbide-2-mononitrate and isosorbide-5-mononitrate.

PATIENTS AND METHODS

Inclusion Criteria. The criteria for enrollment were based on a history of stable, exercise-dependent angina pectoris, angiographically proven coronary artery disease (75 percent or greater stenosis of at least one of the three major coronary arteries), and a reproducible, exercise-induced ST-segment depression of at least 0.15 mV (1.5 mm or greater). Since angina pectoris is highly subjective and an unreliable parameter for the assessment of myocardial ischemia [14], we choose exercise-induced ST-segment depression as the decisive parameter for assessing the anti-ischemic effects. All patients had to show a response to nitrate, with a "good" nitrate response defined according to the following criteria: reduction of the exercise-induced ST-segment depression by greater 1 mm, combined with an absolute ST-segment depression of no more than 1 mm, two hours after the ingestion of an 80-mg tablet of isosorbide dinitrate in sustained-release form.

Patients with inconclusive exercise electrocardiograms (e.g., resting ST-segment abnormalities, bundle-branch blocks, pacemakers, digitalis, and preexcitation syndromes, among others) and those with atrial fibrillation or complex and/or frequent arrhythmias were excluded as were patients with a documented myocardial infarction within three months prior to enrollment. Informed consent was obtained from all patients. Further prerequisites were a history of no or minimal nitrate headache and a high likelihood of good cooperation. Patients with known arterial hypertension controlled by beta blockers were not enrolled in the study.

During a single-blind washout period of four days, placebo tablets visually identical with the active drug were ingested and all anti-ischemic medication was discontinued with only sublingual nitroglycerin allowed. Patients complaining of resting angina during this period were excluded from further investigation.

Simultaneous recordings of exercise electrocardiography and exercise radionuclide ventriculography were obtained two, six, and 12 hours after ingestion of the drug on the first and the 15th days. Two different protocols were performed with isosorbide dinitrate (Isoket retard 80, Pharma Schwarz, West Germany): The dosage intervals of 24 hours (8 A.M., once daily) and 12 hours (8 A.M. and 8 P.M., twice daily) were studied according to a randomized and, with respect to the dosage interval, double-blind design. After the 12-hour exercise test of the first day, patients were randomly assigned to one of two groups, receiving isosorbide dinitrate either twice daily (dosage interval of 12 hours) or once daily (dosage interval of 24 hours with placebo in the evening). At the end of the treatment phase of two weeks, the anti-ischemic response was again evaluated at two, six, and 12 hours after the ingestion of the single tablet of 80 mg of isosorbide dinitrate. The study administering the tablets at 8 A.M. and 2 P.M. for two weeks was performed separately in an analogue manner. On Day 1 and Day 15, the second isosorbide dinitrate tablet was ingested at 2 P.M. In both studies, the results of the 15th day were compared with those of the first day to assess the development of tolerance. All objective parameters were evaluated by different observers blinded with respect to the day and time of data acquisition.

Patients' Characterization. Of the 34 patients included, 10 patients received isosorbide dinitrate once daily, 12 patients received the drug twice daily at 8 A.M. and 8 P.M., and 12 patients received the medication twice daily at 8 A.M. and 2 P.M. These groups did not differ with respect to age and number of stenosed vessels as well as baseline heart rate, systolic blood pressure, and the double-product at rest and during exercise (Table I). Maximal workload and ST-segment depression were identical during baseline exercise tests in these groups. Since the 8 A.M./2 P.M. study contained six patients with previous myocardial infarction, left ventricular ejection fraction was slightly lower at rest and during exercise as compared with those of the other patients (Table I).

Exercise Tests. Exercise tests were performed on an electronically braked bicycle ergometer, which is self-adjusting and provides constant workloads, while the patient was in a semi-supine position (30° inclination) with the legs below heart level. The attending staff and environment were kept as constant as possible. Depending on the individual exercise capacity (as derived from previous stress tests), exercise was started with 50 or 80 watts and, if possible, increased automatically by a programmable computer (ELP 500, Bosch) by 30 watts every three minutes. The endpoint of the baseline exercise test, i.e., following the washout period, was defined as angina pectoris associated with ST-segment depression of at least 1.5 mm. The subsequent exercise tests were then performed until this individual workload had been reached. The 12-lead electro-

TABLE I Baseline Characteristics of the Patients in the Three Groups*

	Dosage Regimen		
	8 A.M.	8 A.M. + 8 P.M.	8 A.M. + 2 P.M.
Number of patients enrolled	10	12	12
1-/2-/3-vessel disease	4/3/3	5/4/3	5/5/2
Number of patients with previous myocardial infarction	0	0	6 [†]
Age (years)	50 ± 10	53 ± 9	55 ± 9
Workload (watts)	80 ± 32	98 ± 38	97 ± 15
Exercise-induced ST-segment depression (mm)	2.1 ± 0.6	2.4 ± 0.7	2.1 ± 0.7
EF-rest (percent)	60 ± 7	60 ± 8	53 ± 7 [†]
EF-exercise (percent)	52 ± 8	52 ± 16	43 ± 11 [†]
HR-rest (beats/minute)	72 ± 13	67 ± 12	72 ± 13
HR-exercise (beats/minute)	121 ± 23	130 ± 21	125 ± 18
RR _{syst} -rest (mm Hg)	144 ± 7	125 ± 22	135 ± 5
RR _{syst} -exercise (mm Hg)	176 ± 23	187 ± 17	182 ± 15
Rate-pressure-product-rest (mm Hg/minute)	1,042 ± 2,178	8,561 ± 2,495	9,544 ± 1,475
Rate-pressure-product-exercise (mm Hg/minute)	21,576 ± 6,137	24,474 ± 4,901	22,868 ± 4,176

* Tablets of isosorbide dinitrate 80 mg in sustained-release form were ingested for two weeks either once daily at 8 A.M. or twice daily at 8 A.M. and 8 P.M. or at 8 A.M. and 2 P.M. The groups did not differ with respect to the number of patients studied, the number of patients with one-, two-, or three-vessel disease, the mean age, and the control values at the first day for maximal workload achieved, the exercise-induced ST-segment depression, the heart rate (HR) at rest or during exercise, the systolic blood pressure (RR_{syst}) at rest or during exercise as well as the rate-pressure-product at rest or during exercise. Because the 8 A.M./2 P.M. group contained six patients with previous myocardial infarction, left ventricular ejection fraction (EF) at rest and during exercise in these patients was slightly but significantly lower than those of the other groups.

[†] $p < 0.05$.

cardiogram (Schiller, Switzerland) was documented with a chart speed of 50 mm/second at 1.5 and 2.5 minutes of each workload stage. ST-segment depression was measured 80 milliseconds after the J-point in the precordial lead that showed maximal ST-depression during the control exercise test. Heart rates were determined from the strips recorded at 50 mm/second. Blood pressures were measured using standard cuff sphygmomanometry, always on the right arm and by the same person.

Radionuclide ventriculography was performed at rest and simultaneously with the electrocardiogram during exercise. After standard stannous in vivo labeling of red blood cells with approximately 25 mCi (925 MBq) of technetium 99m, the data were acquired with a low-energy, all-purpose collimator and a 10-inch mobile Anger gamma camera (Dyna Camera 4, Picker), automatically setting an asymmetric energy window of 15 percent at 140 keV and using a microprocessor for the control of homogeneity. The data were stored on 80-megabyte disks of the dedicated on-line computer (MDS/A²). Using the equilibrium multiple-gated acquisition technique (MUGA), 21 frames per cardiac cycle were generated, rejecting each premature beat as well as the two postextrasystolic contractions. Ventriculograms were acquired for six minutes at rest and for the last two minutes of each exercise step in a modified left anterior oblique position that best isolated the left ventricle. After temporal and spatial smoothing of the images, digitized in a 64 × 64 matrix, the left ventricular ejection fraction was calculated by a count-rate method applying a semi-automatic, clinically validated algorithm. Briefly, the left ventricular border was automatically determined by the computer within a rectangle placed by the operator arbitrarily around

the left ventricle. The algorithm used identifies the left ventricular border on a frame-by-frame basis, using constant thresholds and second-derivative criteria. Each frame was reviewed by the operator. For background correction, the region of interest was automatically set five pixels inferolateral to the left ventricular free wall of the end systolic frame. Care was taken to avoid surrounding structures unrelated to background activity.

Nitrate Plasma Levels. In the 8 A.M. versus 8 A.M./8 P.M. study, blood samples were withdrawn according to standard requirements into precooled, heparinized glass tubes (−20°C). After immediate centrifugation (4,000 revolutions/minute for 10 minutes at −4°C), they were kept frozen (−40°C) until assay. Plasma was analyzed for isosorbide dinitrate and its metabolites isosorbide-5-mononitrate and isosorbide-2-mononitrate by a gas chromatographic electron-capture detection method (Hewlett Packard 5880 A) [15]. Plasma levels were measured six and 12 hours after ingestion at the first and 15th day in the once-daily and 8 A.M./8 P.M. groups.

Compliance. To enhance compliance, each patient received the tablets in a package individually inscribed with the date, day, and time for each single tablet to be ingested. In addition, a diary of documentation of the ingestion of each single tablet was given to every patient. Compliance was further enhanced during this period by telephone calls to the patients as well as to their physicians. For assessment of compliance from the second to the 14th day (the tablets of the first and 15th day were ingested in the presence of the investigators), the riboflavin fluorescence method was used: To each 80-mg isosorbide dinitrate tablet (and to each placebo tablet) 10 mg of riboflavin was added.

The patients were asked to avoid all food with a relatively high riboflavin content and to fill all of the provided vials with urine about two to four hours after ingestion of each tablet.

Statistical Analysis. All exercise parameters were compared at maximal comparable, intra-individually identical workloads. Statistical analysis of the effects on Day 1 and Day 15 was performed by comparing the values after ingestion with the baseline values according to the two-tailed Wilcoxon test for matched pairs. The possibility of tolerance development was calculated by the Mann-Whitney test, comparing the areas under the curves (resulting from the baseline values and the measurements at two, six, and 12 hours) from Day 1 to those of Day 15. Probabilities were considered significant at the $p < 0.05$ level. The p values were corrected using Bonferroni's adjustment. All data are expressed as mean \pm 1 SD.

RESULTS

During the treatment phase of two weeks, compliance as determined from the urine specimens was 95 percent with respect to the ingestion of each single tablet.

Once Daily at 8 A.M. No development of tolerance was observed with regard to the hemodynamics at rest and during exercise as well as to the anti-ischemic effects.

Heart rate: At rest, the heart rate increased significantly two hours after the first ingestion of isosorbide dinitrate (+24 percent) and remained elevated after six hours (+20 percent) as well as after 12 hours (+7 percent, Table II). After two weeks of treatment, there was a nearly identical accelerated heart rate after ingestion of isosorbide dinitrate. During exercise, there was an increase in heart rate on the first day of 6 percent at two hours, 9 percent at six hours, and 5 percent at 12 hours (Table III). On the 15th day, similar changes were recorded after isosorbide dinitrate therapy (Table III).

Blood pressure: The systolic blood pressure decreased significantly after two hours (−9 percent), after six hours (−13 percent), and after 12 hours (−8 percent, Table II). The corresponding values at the 15th day are also listed in Table II. During exercise, the systolic blood pressure did not change significantly on the first day or on the 15th day (Table III). The diastolic blood pressures are depicted in Tables II and III.

Rate-pressure product: The rate-pressure product on the first day two hours after ingestion was elevated to +13 percent. After six hours, there was no difference as compared with the control values (Table II). Also during exercise, there was a tendency for the rate-pressure product to increase two hours after ingestion (+11 percent), after six hours (+9 percent), and after 12 hours (+13 percent). After the two weeks of treatment, the changes following isosorbide dinitrate ingestion at rest and during exercise were quite similar (Table II).

Left ventricular ejection fraction: During resting conditions, the left ventricular ejection fraction slightly in-

TABLE II Results at Rest*

	8 A.M.			8 A.M. + 8 P.M.			8 A.M. + 2 P.M.					
	Baseline	2 Hours	6 Hours	12 Hours	Baseline	2 Hours	6 Hours	12 Hours	Baseline	2 Hours	6 Hours	12 Hours
Heart rate (beats/minute)												
Day 1	72 ± 13	89 ± 15 [†]	87 ± 14 [†]	78 ± 18	67 ± 12	84 ± 14 [†]	83 ± 14 [†]	77 ± 14 [†]	72 ± 13	86 ± 15 [†]	86 ± 14 [†]	84 ± 12 [†]
Day 15	67 ± 10	83 ± 12 [†]	80 ± 16 [†]	80 ± 11 [†]	65 ± 7	77 ± 13 [†]	73 ± 10 [†]	74 ± 8 [†]	72 ± 13	78 ± 13	81 ± 12	80 ± 11
Systolic RR (mm Hg)												
Day 1	144 ± 7	131 ± 14 [†]	125 ± 16 [†]	132 ± 13 [†]	125 ± 22	122 ± 19	116 ± 15 [†]	123 ± 14	135 ± 5	124 ± 7 [†]	124 ± 9 [†]	128 ± 11 [†]
Day 15	143 ± 10	140 ± 9	127 ± 12 [†]	148 ± 12	137 ± 17	133 ± 14	127 ± 11	136 ± 14	140 ± 13	128 ± 11	129 ± 11	127 ± 13
Diastolic RR (mm Hg)												
Day 1	98 ± 4	95 ± 8	88 ± 10 [†]	91 ± 10	87 ± 11	87 ± 10	85 ± 10	89 ± 9	87 ± 6	79 ± 7 [†]	77 ± 5 [†]	80 ± 8
Day 15	99 ± 7	95 ± 7	90 ± 10 [†]	99 ± 10	92 ± 8	90 ± 9	90 ± 7	94 ± 10	85 ± 7	82 ± 8	81 ± 5	82 ± 9
Rate-pressure product (mm Hg/minute × 1,000)												
Day 1	10.4 ± 2.2	11.7 ± 2.5	10.7 ± 1.9	10.3 ± 2.7	8.6 ± 2.5	10.2 ± 2.3 [†]	9.6 ± 2.4	9.5 ± 2.6	9.5 ± 1.6	10.9 ± 2.2	10.6 ± 2.1	10.7 ± 2.4
Day 15	9.5 ± 1.4	11.6 ± 2.2	10.2 ± 2.5	11.9 ± 1.9	8.9 ± 1.9	10.3 ± 2.2	9.2 ± 1.4	10.1 ± 1.9	9.8 ± 2.4	10.1 ± 2.0	10.5 ± 2.0	10.1 ± 2.3
LV-EF (percent)												
Day 1	60 ± 7	62 ± 8	64 ± 10 [†]	65 ± 7 [†]	60 ± 8	63 ± 10 [†]	62 ± 6	64 ± 7 [†]	53 ± 7	61 ± 7 [†]	60 ± 9 [†]	61 ± 10 [†]
Day 15	60 ± 6	63 ± 6	64 ± 8 [†]	63 ± 8	59 ± 6	64 ± 9 [†]	64 ± 6 [†]	63 ± 8 [†]	54 ± 8	60 ± 6 [†]	60 ± 7 [†]	62 ± 9 [†]

* Heart rate, systolic and diastolic blood pressure (RR), rate-pressure product, and left ventricular ejection fraction (LV-EF) at the end of the washout period (Day 1) and at the end of the treatment phase (15th day). The resting values were obtained before (baseline) as well as two, six, and 12 hours after the ingestion of a single 80-mg tablet of isosorbide dinitrate. During the different treatment phases, the tablets were ingested once daily at 8 A.M. or twice daily at 8 A.M. and 8 P.M. or at 8 A.M. and 2 P.M.

[†] $p < 0.01$, compared with baseline.

[‡] $p < 0.05$, compared with baseline.

TABLE III Results during Exercise*

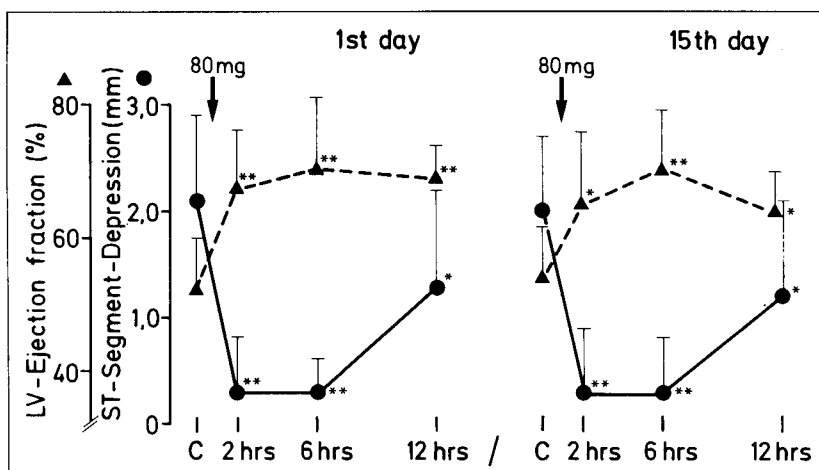
	8 A.M.				8 A.M. + 8 P.M.				8 A.M. + 2 P.M.			
	Baseline	2 Hours	6 Hours	12 Hours	Baseline	2 Hours	6 Hours	12 Hours	Baseline	2 Hours	6 Hours	12 Hours
Heart rate (beats/minute)												
Day 1	121 ± 23	128 ± 19	132 ± 16	127 ± 17	130 ± 21	142 ± 18 [†]	140 ± 18 [†]	132 ± 18	125 ± 18	128 ± 28	128 ± 27	130 ± 29
Day 15	117 ± 14	125 ± 21	124 ± 17	123 ± 14	133 ± 18	135 ± 17	132 ± 15	134 ± 14	127 ± 16	123 ± 26	134 ± 15	129 ± 19
Systolic RR (mm Hg)												
Day 1	176 ± 23	187 ± 18	177 ± 21	191 ± 17	187 ± 17	194 ± 19	190 ± 21	184 ± 18	182 ± 15	180 ± 17	182 ± 30	180 ± 26
Day 15	179 ± 25	190 ± 16	185 ± 22	191 ± 21	191 ± 18	196 ± 16	197 ± 16	193 ± 23	184 ± 17	182 ± 16	182 ± 19	181 ± 23
Diastolic RR (mm Hg)												
Day 1	108 ± 13	102 ± 12	96 ± 11 [†]	98 ± 9	109 ± 10	102 ± 12	98 ± 14	101 ± 15	108 ± 14	96 ± 16	92 ± 12 [†]	98 ± 16
Day 15	109 ± 7	107 ± 8	98 ± 8 [†]	108 ± 6	110 ± 13	108 ± 11	105 ± 14	110 ± 13	109 ± 13	100 ± 15	104 ± 12	103 ± 13
Rate-pressure product (mm Hg/minute × 1,000)												
Day 1	21.6 ± 6.1	24.1 ± 4.8	23.5 ± 4.7	24.3 ± 4.7	24.5 ± 4.9	27.5 ± 4.3 [†]	26.7 ± 4.7	24.4 ± 4.8	22.8 ± 4.2	24.2 ± 3.9	23.7 ± 7.1	23.4 ± 6.8
Day 15	20.9 ± 4.1	23.9 ± 5.6	23.1 ± 5.3	23.7 ± 4.5	25.4 ± 4.3	26.6 ± 4.5	26.1 ± 3.7	25.9 ± 4.3	23.6 ± 4.3	23.4 ± 3.5	24.3 ± 4.4	23.1 ± 4.2
LV-EF (percent)												
Day 1	52 ± 8	67 ± 9 [‡]	70 ± 11 [‡]	68 ± 6 [‡]	52 ± 16	62 ± 13 [‡]	62 ± 12 [‡]	61 ± 12 [‡]	43 ± 11	62 ± 12 [‡]	59 ± 8 [‡]	60 ± 12 [‡]
Day 15	54 ± 8	65 ± 11 [†]	70 ± 9 [‡]	64 ± 6 [†]	54 ± 11	56 ± 12	58 ± 12	56 ± 14	45 ± 12	61 ± 11 [‡]	58 ± 10 [‡]	59 ± 11 [‡]
ST-segment depression (mm)												
Day 1	2.1 ± 0.6	0.3 ± 0.5 [‡]	0.3 ± 0.4 [‡]	1.3 ± 0.9 [†]	2.4 ± 0.7	0.7 ± 0.7 [‡]	0.9 ± 0.6 [‡]	2.0 ± 0.6	2.1 ± 0.7	0.3 ± 0.4 [‡]	0.8 ± 0.7 [‡]	0.8 ± 0.6 [‡]
Day 15	2.0 ± 0.7	0.3 ± 0.6 [‡]	0.3 ± 0.5 [‡]	1.2 ± 0.9 [†]	2.2 ± 0.8	1.3 ± 0.8 [†]	1.5 ± 0.8 [†]	2.2 ± 0.8	2.1 ± 0.8	0.4 ± 0.6 [‡]	0.8 ± 0.7 [‡]	0.8 ± 0.7 [‡]

* Heart rate, systolic and diastolic blood pressure (RR), rate-pressure product, left ventricular ejection fraction (LV-EF), and exercise-induced ST-segment depression at the end of the washout period (1st day) and at the end of the treatment phase (15th day). The exercise values were obtained before (baseline) as well as two, six, and 12 hours after the ingestion of a single 80-mg tablet of isosorbide dinitrate. During the different treatment phases, the tablets were ingested once daily at 8 A.M. or twice daily at 8 A.M. and 8 P.M. or at 8 A.M. and 2 P.M.

[†] p < 0.05, compared with baseline.

[‡] p < 0.01, compared with baseline.

Figure 1. Exercise-induced ST-segment depression and simultaneously recorded left ventricular ejection fraction at maximal comparable workload before (C) as well as two hours, six hours, and 12 hours after the ingestion of an 80-mg tablet of isosorbide dinitrate in sustained-release form. Measurements were obtained after the washout phase (first day) as well as after the treatment period (15th day), during which the tablets were ingested once daily at 8 A.M. This regimen showed no development of tolerance regarding the initially beneficial effects on exercise-induced ST-segment depression or left ventricular ejection fraction.



creased after the ingestion of isosorbide dinitrate at the first and the 15th day (Table II). There was no attenuation of the nitrate-induced left ventricular ejection fraction changes during exercise at the 15th day as compared with the first day (Table III and Figure 1).

ST-segment depression: On the first day, the exercise-induced ST-segment depression was significantly reduced (−83 percent) two hours after ingestion of the medication (Table III and Figure 1). After six hours, the value was nearly identical (−86 percent), and after 12 hours the ST-segment depression was still significantly different from the control value (−39 percent). On the 15th day, the control ST-segment depression was similar to that of the first day. Two hours after ingestion of the medication, the exercise-induced ST-segment depression showed almost the same improvement (83 percent) (Table III and Figure 1). After six hours, a similar result was obtained (−86 percent). The value for 12 hours was still significantly different from the control conditions (Table III and Figure 1).

Plasma levels: On the first day six hours after ingestion, the isosorbide dinitrate levels were 26 ± 13 ng/ml, those of isosorbide-2-mononitrate were 97 ± 44 ng/ml, and those of isosorbide-5-mononitrate were 449 ± 217 ng/ml. Twelve hours after ingestion, the corresponding values were 6 ± 4 ng/ml, 33 ± 19 ng/ml, and 313 ± 94 ng/ml. Following the treatment phase of two weeks (Figure 2), after six hours the isosorbide dinitrate (25 ± 7 ng/ml) and isosorbide-2-mononitrate (96 ± 36 ng/ml) plasma levels were within the range of the first day. This was also true for the plasma levels after 12 hours for isosorbide dinitrate (9 ± 7 ng/ml) and isosorbide-2-mononitrate (35 ± 9 ng/ml). The isosorbide-5-mononitrate plasma levels at the 15th day were slightly higher than those of the first day at six hours (485 ± 216 ng/ml, not significant and at 12 hours (385 ± 76 ng/ml, $p < 0.05$).

Twice Daily at 8 A.M. and 8 P.M. There was a signifi-

cant ($p < 0.05$) development of tolerance with regard to the nitrate-induced increase in exercise heart rate as well as concerning the left ventricular ejection fraction during exercise. The initially positive anti-ischemic effects on exercise-induced ST-segment depression were also considerably attenuated.

Heart rate: At rest, the heart rate increased significantly two hours after the first ingestion of isosorbide dinitrate (+24 percent) and remained elevated after six

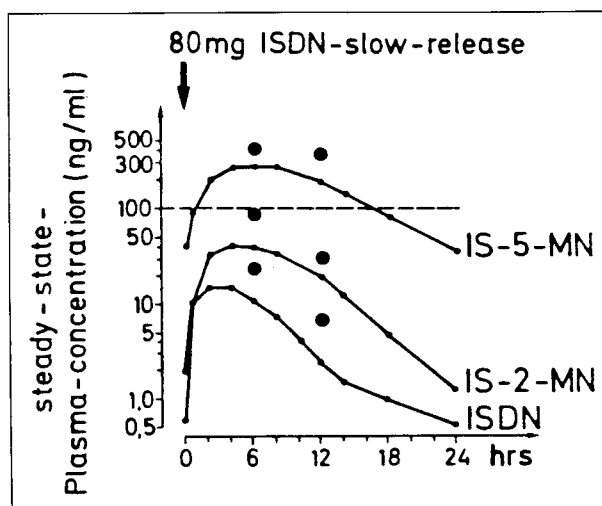


Figure 2. Plasma levels of isosorbide dinitrate (ISDN) and its metabolites isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) during steady-state conditions after the treatment phase of two weeks of ingesting 80-mg tablets of isosorbide dinitrate in sustained-release form once daily at 8 A.M. Plasma levels were determined in the patients with coronary artery disease of the presented study (large circles) and compared with the results obtained in 12 healthy volunteers (small circles) following an identical protocol [29]. (For further details, please see text).

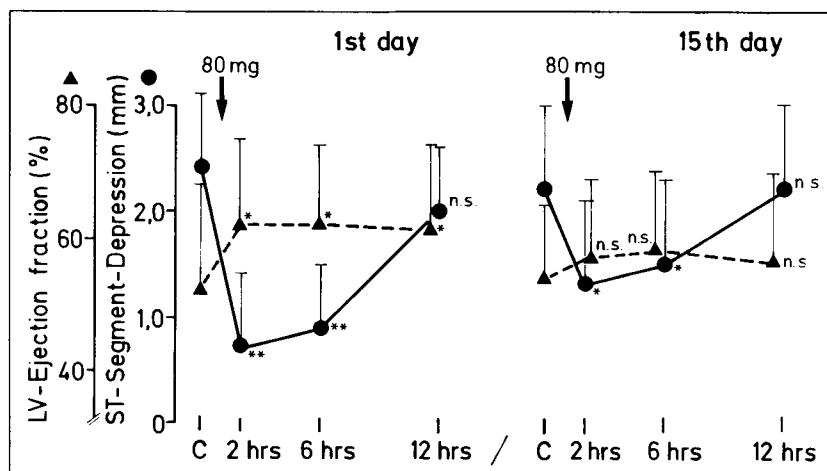


Figure 3. Exercise-induced ST-segment depression and simultaneously recorded left ventricular ejection fraction at maximal comparable workloads before (C) as well as two hours, six hours, and 12 hours after the ingestion of an 80-mg tablet of isosorbide dinitrate in sustained-release form. Measurements were obtained after the washout phase (first day) and after the treatment period (15th day) during which the tablets were ingested twice daily at 8 A.M. and 8 P.M. After two weeks, there was a considerable attenuation of the initial positive nitrate effects of ST-segment depression and exercise left ventricular ejection fraction.

(+23 percent) and 12 hours (+14 percent, Table II). After two weeks of treatment, the heart rate increase was still demonstrable with +19 percent (two hours), +12 percent (six hours), and +14 percent (12 hours, Table II). During exercise, there was also a significant increase in heart rate on the first day of +9 percent at two hours and +7 percent at six hours (Table III). On the 15th day, however, there was no significant change of the heart rate during exercise following the isosorbide dinitrate ingestion (Table III).

Blood pressure: The systolic blood pressure at rest did not change significantly after two hours but the change reached statistical significance at six hours (8 percent, Table III). The corresponding values at the 15th day are also listed in Table II. During exercise, the systolic blood pressure did not decrease significantly on the first day or on the 15th day (Table III). The diastolic blood pressures are shown in Tables II and III.

Rate-pressure product: The rate-pressure product during resting control conditions on the first day two hours after ingestion was significantly elevated (+19 percent, Table II). After six and 12 hours, there was no statistical difference from the control values (Table II). Also during exercise, statistical significance was reached only two hours after ingestion (+12 percent, Table III). There were no significant changes of the rate-pressure product at rest or during exercise on the 15th day (Table III).

Left ventricular ejection fraction: During resting conditions, the left ventricular ejection fraction slightly increased after the ingestion of isosorbide dinitrate on the first and on the 15th day (Table II). In contrast, there was no attenuation of the nitrate-induced left ventricular ejection fraction increase during exercise at the 15th day as compared with the first day (Table III and Figure 3).

ST-segment depression: At the first day, the exercise-induced ST-segment depression was significantly reduced to -72 percent at two hours after ingestion of the

medication (Table III and Figure 3). After six hours, the value was nearly identical (-63 percent). After 12 hours, the ST-segment depression did not reach statistical significance as compared with the control value (-16 percent, Table III and Figure 3). On the 15th day, the control ST-segment depression was within the range of that of the first day. Two hours after ingestion of the medication, the exercise-induced ST-segment depression was much more pronounced -42 percent (Table III and Figure 3). After six hours, a similar result was obtained showing a considerable attenuation of the initial positive effects of isosorbide dinitrate (Table III and Figure 3).

Plasma levels: On the first day six hours after ingestion, the isosorbide dinitrate levels were 12 ± 6 ng/ml, those of isosorbide-2-mononitrate were 62 ± 30 ng/ml, and those of isosorbide-5-mononitrate were 330 ± 127 ng/ml. Twelve hours after ingestion, the corresponding values were 5 ± 4 ng/ml, 43 ± 26 ng/ml, and 284 ± 54 ng/ml. Following the treatment phase of two weeks (Figure 4), six hours after the ingestion, the isosorbide dinitrate (20 ± 13 ng/ml) and isosorbide-2-mononitrate (89 ± 42 ng/ml) plasma levels were shown to have a tendency to be higher than those of the first day. After 12 hours, the isosorbide dinitrate and isosorbide-2-mononitrate levels (7 ± 3 ng/ml and 37 ± 10 ng/ml, respectively) were within the range of those on the first day. The isosorbide-5-mononitrate plasma levels on the 15th day were consistently higher than those of the first day at six hours (559 ± 188 ng/ml, $p < 0.05$) and at 12 hours (386 ± 108 ng/ml, $p < 0.05$).

Twice Daily at 8 A.M. and 2 P.M. Development of tolerance was only observed for the resting heart rate and blood pressure. The initially positive anti-ischemic effects remained unchanged.

Heart rate: At rest, the heart rate increased significantly two hours after the first ingestion of isosorbide dinitrate (+19 percent) and remained elevated after six

and 12 hours (Table II). After two weeks of treatment, this induction of an accelerated heart rate was attenuated (Table II). During exercise, there was no significant increase in heart rate on the first and the 15th day following ingestion of isosorbide dinitrate (Table III).

Blood pressure: The systolic blood pressure at rest decreased significantly after two hours (−9 percent) as well as at six and 12 hours (−8 percent, Table II). In contrast, on the 15th day, there was no significant change in the resting systolic blood pressure (Table II). During exercise, the systolic blood pressure did not decrease significantly on the first day or on the 15th day (Table III). The diastolic blood pressures are listed in Tables II and III.

Rate-pressure product: The rate-pressure product two hours after ingestion was elevated to +14 percent and after six and 12 hours to +11 percent (Table II). On the 15th day, there were similar changes following ingestion of isosorbide dinitrate (Table II). The exercise values are depicted in Table III.

Left ventricular ejection fraction: During resting conditions, the left ventricular ejection fraction increased after the ingestion of isosorbide dinitrate on the first day with nearly identical values at the 15th day (Table II). During exercise, the left ventricular ejection fraction significantly increased on the first day after ingestion of isosorbide dinitrate (Table III). There was no attenuation of the nitrate-induced left ventricular ejection fraction changes at the 15th day as compared with the first day (Table III).

ST-segment depression: On the first day, the exercise-induced ST-segment depression was significantly reduced to −87 percent at two hours and to −64 percent at six and 12 hours after ingestion of the medication (Table III). On the 15th day, the control ST-segment depression was within the range of that of the first day. Two hours after ingestion of the medication, the reduction of the exercise-induced ST-segment depression was nearly the same (−80 percent) (Table III). After six and 12 hours, a similar result was obtained (−60 percent).

COMMENTS

There is unanimous agreement on the development of tolerance with regard to nitrate-induced headache [16]. The reports on the development of tolerance to the hypotensive effects are almost as old as the application of nitrates themselves [17]. In fact, the possibility of the development of tolerance to reduction in blood pressure and increase in heart rate is generally accepted during oral long-term treatment, providing at least three-times-daily ingestions [18–20]. During the twice-daily ingestions of 80-mg tablets of isosorbide dinitrate, we observed in part an attenuated response of the heart rate and systolic blood pressure (Tables II and III).

In contrast, the development of tolerance with reference to the anti-ischemic (anti-anginal) effects is a matter of substantial controversy. A whole series of studies has

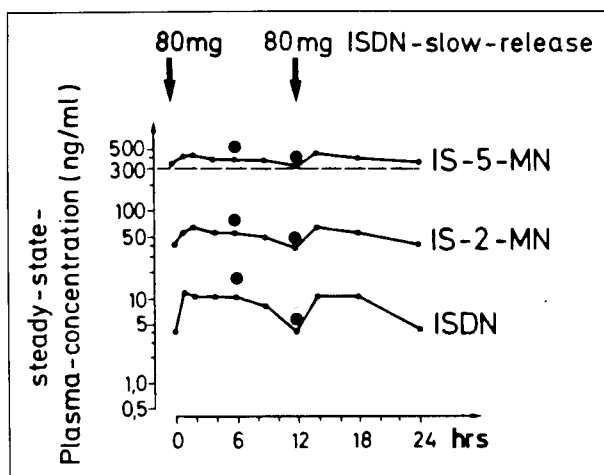


Figure 4. Plasma levels of isosorbide-dinitrate (ISDN) and its metabolites isosorbide-2-mononitrate (IS-2-MN) and isosorbide-5-mononitrate (IS-5-MN) during steady-state conditions after the treatment phase of two weeks of ingesting 80-mg tablets of isosorbide dinitrate in sustained-release form twice daily at 8 A.M. and 8 P.M. Plasma levels were determined in the patients with coronary artery disease of the presented study (large circles) and compared with the results obtained in 12 healthy volunteers (small circles) following an identical protocol [29]. (For further details, please see text).

failed to show any evidence of weakening or loss of efficacy in patients during long-term oral nitrate therapy with at least three-times-daily regimens. An unchanged nitrate-induced increase in exercise capacity was found in three studies with isosorbide dinitrate in non-sustained-release form (20 mg four times daily to 50 mg four times daily) for 12 to 40 weeks [21] and nitroglycerin (2.6 mg three times a day) in sustained-release form for 24 weeks [22] as well as 6.5 mg three to six times a day for 12 weeks [23]. Lee et al [24] found continuing efficacy of 40 mg of isosorbide dinitrate three times a day in non-sustained-release form after four weeks of treatment. Unattenuated anti-ischemic efficacy has also been described after four weeks of treatment with isosorbide dinitrate 80 mg six times a day [20]. In contrast, seven clinical studies demonstrated the development of tolerance during oral nitrate treatment. In 1969, Goldberg et al [9] reported that after four weeks of treatment with 10 mg of isosorbide dinitrate four times a day, there was no difference between placebo and the nitrate with respect to anginal frequency and exercise capacity. Similar studies in which isosorbide dinitrate was administered in non-sustained-release form four times daily and three times daily, respectively, were reported in 1970 and 1973 [7,11]. However, since the initial nitrate response was not evaluated in these studies, the possibility of having included patients who were unresponsive to nitrate [25] must be

taken into consideration. In 1980, the total disappearance of the anti-ischemic effect with respect to the exercise-induced ST-segment depression by the administration of 20 mg three times a day and 40 mg three times per day of isosorbide dinitrate in sustained-release form (nine patients) and 60 mg three times daily (10 patients) was reported [8]. Another study revealed the same result for 40 mg of isosorbide dinitrate four times per day in non-sustained-release form [26]. In 1982, Thadani et al [27] found that in 12 patients, subsequent to one week of therapy with 15 mg to 120 mg of isosorbide dinitrate four times a day in non-sustained-release form, there was a complete loss of the effects four hours after the ingestion.

As our results show, the development of tolerance with respect to the anti-ischemic effects can be induced by constant plasma levels and circumvented by a daily "nitrate-poor" interval. After ingestion of the tablets with dosage intervals of 12 hours for two weeks, the plasma levels of isosorbide-5-mononitrate were continuously higher than 386 ng/ml, those of isosorbide-2-mononitrate were above 37 ng/ml, and those of isosorbide dinitrate were above 7 ng/ml (Figure 4). Because of the short plasma half-life time of isosorbide dinitrate, the relatively high values for isosorbide dinitrate after 12 hours are surprising. These findings may be explained by an inhibited metabolism of isosorbide dinitrate due to its metabolites [28]. The administration of 80-mg tablets of isosorbide dinitrate once daily for two weeks produced maximal plasma levels of isosorbide-5-mononitrate of 485 ng/ml, of isosorbide-2-mononitrate of 96 mg/ml, and of isosorbide dinitrate of 25 ng/ml (Figure 2). Although the "trough" levels were not determined in this study, the minimum isosorbide-5-mononitrate plasma levels have been reported to be below 100 ng/ml in another study applying an identical dosage regimen [29] (Figure 2). Thus, intermittent changes in nitrate plasma levels prevent the development of tolerance with respect to the anti-ischemic effects.

From a practical point of view, the clinical relevance of nitrate tolerance during oral therapy was often neglected. In our opinion, the "physiologic non-compliance" of patients may be principally responsible for this controversy. Since nitrate tolerance to the hemodynamic effects develops rapidly [13,16,30] and may be reversed within hours [13], the actual ingestion of each single tablet, i.e., the patients' compliance, is pivotal. An unreliable or irregular ingestion of tablets can blur the problem of nitrate tolerance in everyday practice. Unfortunately, most of the studies omitted to document patients' compliance [7,9,12,21,23]. Counting the tablets returned, or the documentation of tablet intake on the basis of patients' diaries, is not reliable. The assessment of plasma levels is not feasible in long-term studies on a day-by-day basis. A practical method for a daily compliance test is to deter-

mine the fluorescence in the patients' urine attributable to the riboflavin added to the tablets. In our studies, the compliance assessed by this method was 95 percent, probably higher than the average compliance expected for routine administrations. In addition, one should keep in mind that compliance not only refers to the fact of ingestion itself but also to the exact time of ingesting the prescribed dose. Obviously, it is not easy to ingest the tablets three-times-daily every eight hours for several weeks. Thus, it is conceivable that patients may have introduced their own nitrate-poor interval by modifying the study protocol. In fact, it was shown that a three-times-daily regimen with 30-mg dosages of isosorbide dinitrate and the last ingestion at 5 P.M. did not lead to the development of tolerance [31].

The practical relevance of nitrate tolerance was obvious, however, when constant plasma levels were maintained in even the unreliable patients. Thus, it is conceivable why many clinical studies testing transdermal nitrates reported the phenomenon of nitrate tolerance [32-34]. Furthermore, the experiences with nitroglycerin patches reveal that the changes in plasma levels seem to be much more important than the absolute height of the plasma levels. This hypothesis is underlined by the lack of relationship between the total daily dosage and the development of tolerance. On one hand, a relatively low dosage of 60 mg of isosorbide dinitrate per day led to a marked reduction in the anti-ischemic effects [8,27] and, on the other, a daily dosage of 160 mg of isosorbide dinitrate showed persistent anti-ischemic power (Table III). So the use of the term "high dosage" should not be automatically connected with "tolerance."

Causes of Development of Tolerance. To explain the phenomenon of nitrate tolerance one can exclude reduced absorption, an enhanced metabolism, or faster elimination of the administered nitrate or its active metabolites on the evidence of increased plasma levels during long-term treatment [12,35]. Counter-regulatory mechanisms during long-term therapy ("pseudotolerance" [36,37]) occurring as a reaction to nitrate-induced venous pooling may lead both to stimulation of the renin-angiotensin-aldosterone system with its concomitant fluid retention and vasoconstriction [38,39]. However, extreme withdrawal phenomena only occurred under extraordinary, nonclinical conditions [38,40]. On the basis of several findings, taken together with the failure of animal experiments to demonstrate a further rise in plasma renin levels during long-term application of isosorbide dinitrate [41], the renin-angiotensin-aldosterone system can hardly be considered responsible for nitrate tolerance, particularly since its development occurs without a considerable increase in body weight [42].

The anti-ischemic effect of nitrates on stable, reproducible, exercise-induced angina is primarily caused by

the preload reduction [3,4]. Therefore, a loss of the anti-ischemic effects can be seen as a reduction of its venous pooling capability. Thus, venous plethysmography has been used by Zelis and Mason [43] to demonstrate a loss of efficacy during long-term nitrate therapy.

The fundamental mechanism behind the development of tolerance following long-term therapy with nitrates must be regarded as a loss of their effect on the vessel's smooth muscle, which was observed in isolated vessel specimens [44,45]. The vasodilator properties of nitrates appear to be mediated by cyclic guanosine-monophosphate via the activation of guanylatecyclase [46–48]. Since *in vitro*, despite the induction of nitrate tolerance, the vessels remain responsive to cyclic guanosine-monophosphate, the loss of the effects during long-term nitrate application can be traced back to the reduced activation of guanylatecyclase [48]. Nitrates require sulfhydryl groups for the stimulation of guanylatecyclase [49]. Since cysteine represents the principal sulfhydryl donor [50,51], the development of nitrate tolerance may possibly be due to a rapidly occurring exhaustion of the "cysteine pool" with a subsequently reduced production of S-nitrosothiols [48,52]. This theory is supported by the clinical observation of enhanced nitroglycerin efficacy when used in conjunction with N-acetylcysteine in patients with coronary artery disease [53,54]. Packer et al [42] proved the reversibility of induced nitrate tolerance by adding N-acetylcysteine to the nitroglycerin infusion in patients with congestive heart failure. However, these findings may not be extrapolated to patients with coronary artery disease and exercise-induced ischemia; Parker et al [55] were not

able to reverse nitrate tolerance by the infusion of N-acetylcysteine.

Practical Implications. It is now accepted that the problem of nitrate tolerance with respect to the anti-ischemic effect is more common than previously thought [56]. Since a daily nitrate-poor interval is necessary to circumvent the attenuation of the anti-ischemic effect, we recommend since 1983 the intermittent administration of high single dosages of isosorbide dinitrate in sustained-release form [13]. In our studies, the best compromise between maximal possible anti-ischemic effects and the circumvention of development of tolerance was found for the "eccentric" dosage regimen in which the 80-mg tablets were ingested in the morning and early afternoon. According to this regimen, the maximal nitrate plasma level is achieved during the day. The problem of nocturnal silent myocardial ischemia should not be exaggerated, since silent episodes seem to be much more frequent during the day [57]. Whether the relatively low plasma levels during the night are still sufficient to prevent silent ischemic episodes is as yet unknown. In patients predominantly complaining of nocturnal angina, the therapeutic maximum can be shifted to the night.

Since it is obviously not possible to obtain maximal anti-ischemic protection for 24 hours with nitrates alone, combination therapy with a beta blocker or a heart rate-decreasing calcium antagonist should be recommended for each patient, if no contraindications exist. In order to optimize the anti-ischemic treatment, combination therapy should be preferred, even if patients receiving monotherapy with nitrates are free of angina [14,57,58].

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