Effects of treatment with oral isosorbide dinitrate on platelet function *in vivo*; a double-blind placebo-controlled study in patients with stable angina pectoris

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- 1 A randomised double-blind placebo-controlled cross-over study was performed to investigate the effects of oral isosorbide dinitrate (ISDN; 20 mg twice daily for 2 weeks) on various aspects of platelet function *in vivo* in 20 patients with stable angina pectoris. Measurements were performed at rest and after platelet activation by physical exercise (bicycle ergometry).
- 2 Compared with placebo, treatment with ISDN significantly decreased systolic blood pressure at rest by 7 (–14 to –1) mm Hg (mean and 95% CI) and tended to increase exercise capacity by 7 (–1 to 14) W and attenuate perceived chest pain during maximal work. The dosage was high, as judged by side-effects reported (mainly headache). Compliance was good, as assessed by electronic counter equipped tablet bottles (Medication Event Monitoring System); only one patient had a compliance rate below 60%.
- 3 Exercise significantly increased platelet aggregability as measured by filtragemetry *ex vivo*; the time taken for platelet aggregates in whole blood drawn directly from an antecubital vein to occlude a microfilter was significantly decreased from 155 to 95 s (antilog of mean log values). Platelet secretion *in vivo* also increased, as indicated by significant elevations of β -thromboglobulin in plasma; from 22 to 35 ng ml^{–1} ($P = 0.006$).
- 4 ISDN treatment did not inhibit platelet function. Relative to placebo, filtragemetry readings (ISDN/placebo ratios; mean and 95% CI) were not altered either at rest (1.05 (0.83 to 1.32)) or immediately after exercise (0.98 (0.80 to 1.20)). Similarly, β TG in plasma was unaltered by ISDN treatment; 1.09 (0.98 to 1.21) at rest, and 1.04 (0.82 to 1.30) immediately after exercise. The urinary excretions of β TG and the thromboxane metabolite 11-dehydro thromboxane B₂ were not reduced by ISDN treatment.
- 5 It is concluded that treatment with ISDN, despite expected clinical effects, does not affect platelet function *in vivo* in patients with stable angina pectoris.

Keywords isosorbide dinitrate platelet aggregation physical exercise β -thromboglobulin filtragemetry ischaemic heart disease

Introduction

The organic nitrate nitroglycerin, and its followers isosorbide dinitrate (ISDN) and isosorbide-5-mononitrate, have been reported to exert platelet inhibitory effects when administered *in vivo* [1–7]. Such effects

would be highly desirable in the treatment of ischaemic heart disease (IHD), and the concept that organic nitrates inhibit platelet function is gaining increased support [8]. It should, however, be realized

that there are few controlled studies on the matter, and that several investigators have been unable to document significant platelet inhibition after nitrate administration [9–12]. On the other hand, platelet function is complex and platelet attenuating effects of nitrates may not be revealed with insensitive *in vitro* techniques or when measurements are performed only at rest. In healthy volunteers ISDN tended to attenuate platelet responses *in vivo* to adrenaline infusion, without any effect in the basal state [12].

Atherosclerotic diseases, such as IHD, are associated with defective endothelial function [13] resulting in decreased release of antiplatelet (and antithrombotic) factors like prostacyclin [14] and nitric oxide (NO) [15, 16]. Although not fully proven, IHD is considered to be associated with platelet hyperactivity, possibly due to endothelial dysfunction. Therapy with organic nitrates (and other nitrovasodilators which act through the release of NO) may substitute for failing endothelial function by NO donation [14], and it is possible that the antiplatelet effects of organic nitrates are more readily revealed in the presence of significant atherosclerosis than in healthy volunteers.

Physical exercise may precipitate coronary ischaemia in patients with IHD, and heavy exertion has been reported to be associated with a five-fold increase in the risk of myocardial infarction during the subsequent 2 h [17]. In this context it is of interest that exercise has been reported to activate platelets *in vivo* [18–20]. It may be hypothesized that drugs which attenuate platelet activation in response to exercise are beneficial in the treatment of IHD.

Despite being a very important factor for the reliability of a clinical trial, patient compliance to the drug regimen is often neglected [21]. In the case of organic nitrates, false negative results may be due to either poor compliance or 'over-compliance', as overdosing may decrease the therapeutic effect of the drug, due to the development of nitrate tolerance [3]. At present, one of the best methods for measuring patient compliance is the use of microprocessors incorporated into the tablet containers [22].

In the present study we investigated, in a placebo-controlled double-blind randomized cross-over fashion, the effects of 2 weeks treatment with an oral long acting nitrate (isosorbide dinitrate, ISDN) on various aspects of platelet function *in vivo* in patients with angina pectoris. Platelet function was assessed at rest and after platelet activation by physical exercise. Patient compliance was assessed by a computerized device (MEMS) [see e.g. 23] which records the openings of a special bottle in which the medication is dispensed.

Methods

Subjects and treatment

Twenty patients were included in the study. Inclusion criteria for the study were: male sex, stable exercise induced angina (verified by a pretrial diagnostic exer-

cise test), work capacity ≥ 100 W, and antecubital veins allowing technically good sampling for platelet function parameters. Patients with myocardial infarction within the last year, liver or kidney disease, respiratory disease, significant valvular stenosis, systolic blood pressure below 100 mm Hg, abnormal blood counts, or need for alterations of concomitant drug therapy during the study were excluded, as were patients on oral anticoagulants.

The patients were randomized in a block size of four to receive treatment with 20 mg isosorbide dinitrate given twice daily at 07.00 h and 13.00 h (in order to allow a nitrate free interval; Sorbangil®, Kabi-Pharmacia, Uppsala, Sweden) or placebo, in a double-blind cross-over fashion. Ten patients were randomized to each order (of the 16 patients that completed the study (see below) eight patients received placebo and eight patients received active treatment during the first treatment phase). The experiments were performed after 2 weeks treatment with either drug. The patients were instructed to ingest half a tablet twice daily during the first 2 days of each treatment phase, in order to minimize problems with ISDN induced headaches. Concomitant antiischaemic therapy during the trial included treatment with β -adrenoceptor blockers (four patients), calcium antagonists (six patients) or both (four patients). Two individuals also received ACE inhibitors, two subjects were on diuretics and one patient was treated with an α_1 -adrenoceptor antagonist (doxazosin). No medication other than the study drug was changed during the trial.

Sublingual short-acting nitroglycerin consumption and symptoms were reported in a patient diary. The patients were instructed not to take any platelet active drugs during the study (starting 2 weeks before the first treatment phase). The subjects were also instructed to abstain from tobacco and caffeine containing beverages on the days of the experiments. The study was approved by the Ethics Committee of the Karolinska Hospital and written informed consent was obtained from all patients.

Experimental protocol

The patients arrived in the laboratory at noon and were served a standardized light lunch. The afternoon dose was ingested under the observation of a nurse, 1 h after finishing the meal. After another 60 min of rest in the supine position heart rate and blood pressure (sphygmomanometry) were measured, and filtagometry measurements and blood sampling were performed. The ensuing exercise test was carried out on a computer assisted bicycle ergometer (Siemens EM 840 connected to a Siemens Megacart ECG apparatus, Siemens AB, Solna, Sweden) starting at a workload of 30 W with increments of 10 W min^{-1} , and terminated upon the occurrence of limiting symptoms (moderate chest pain, shortness of breath, or fatigue) or other indications of severe coronary ischaemia, such as a fall in blood pressure or substantial ST-segment depression on the ECG. Perceived chest-pain during the exercise test was estimated according to a 10-graded category-ratio scale [24]. Filtagometry

and blood sampling were repeated immediately upon termination of the exercise test.

For measurements of urinary excretion products night urine from the night preceding the experiment, and day urine covering the exercise test, were collected separately in flasks.

Filtragometry measurements

The *ex vivo* filtragometry technique, described in detail previously [25, 26] has been shown to measure platelet aggregates in blood continuously drawn from an antecubital vein and thus to reflect platelet aggregability *in vivo*. Each reading requires a new venepuncture by a 19 G Butterfly needle. To prevent coagulation heparin (final concentration 5 i.u. ml⁻¹) is infused into the tubing system leading the blood to the apparatus. This concentration of heparin does not influence filtragometry assessments of platelet function [25]. The time (tA; aggregation time) taken to occlude 25% of the pore area of a nickel filter (pore size 20 µm; diameter of filter surface 2.0 mm) is measured. Aggregation time is inversely related to platelet aggregability. Thus, a shortened tA reflects increased aggregability. Previous validation of the method has shown that blood cells other than platelets are not responsible for the filter occlusion (scanning electron microscopy of the filter), and that acetylsalicylic acid prolongs filtragometry readings [25, 27]. Information regarding the patient's subjective health state during the treatment phases (e.g. if the patient experienced headache or increased angina) was kept secret to the filtragometrist during the investigation.

Patient compliance

An electronic counter, placed in the cap of the bottle containing the pills, was used to monitor patient compliance. This computerized counter (Medication Event Monitoring Systems, MEMS, Apex Ltd, Zug, Switzerland) records the exact day, hour and minute each time the bottle is opened. Compliance rate was calculated as follows: [number of days during which doses were taken as prescribed/number of days observed] × 100%.

β-thromboglobulin and 11-OH thromboxane B₂ measurements

Sampling for measurements of βTG in plasma was performed according to a validated procedure [28]. In brief, an antecubital vein was punctured with a Wasserman 18 G stainless-steel needle. After discarding the first 2 ml, 8 ml of blood was allowed to drip into ice-cooled sampling tubes containing 0.8 ml of an anticoagulant and platelet stabilizing solution (final concentrations: 9 mmol l⁻¹ EDTA, 1 mmol l⁻¹ theophylline and 1.4 µmol l⁻¹ prostaglandin E₁). All samples were immediately centrifuged at 15 000 g (4° C, 30 min). Plasma was carefully removed from the midportion of the supernatant (to avoid lipid contamination) and immediately stored at -80° C.

High molecular weight fractions (HMW) contain-

ing β-thromboglobulin in urine (UβTG) were obtained by gel filtration on Sephadex G-25 columns (PD-10; Pharmacia Fine Chemicals, Uppsala, Sweden) as recently described and validated [29]. HMW UβTG and plasma βTG immunoreactivities were analysed by a commercially available r.i.a.-kit (IM-88, Amersham, UK) with modifications described previously [29].

Urinary excretion of 11-dehydro thromboxane B₂ was analysed using a commercially available EIA kit (Cayman Chemicals Company, Ann Arbor, MI, USA). Prior to the analysis, the urines were extracted over Bond Elut Certify II® columns (Varian Sample Preparation Products, Harbour City, CA, USA). The eluates were vacuum centrifuged to dryness, and resuspended and incubated in EIA-buffer at room-temperature overnight (in order to convert the thromboxane metabolite into its open-ring form) before analysis.

Other biochemical assays and blood cell counts

Urinary creatinine was measured by the Jaffé reaction, using a Monarch 2000 automated analyzer (Instrumentation Laboratories, IL Test™ 181615-60, Lexington, MA, USA).

Platelet counts and median platelet volume in whole blood (MPV; anticoagulated with EDTA, final concentration 10 mmol l⁻¹) were counted 2 h after the experiments by use of a semi-automatic cell counter (Cellanalyzer 460, Medonic AB, Solna, Sweden).

Statistical methods

Based on power calculations (using pooled data from repeated filtragometry measurements in 28 male healthy volunteers at rest) it was found that a sample size of 15 individuals was required to detect a 20% difference in resting filtragometry measurements at the 5% significance level ($\alpha = 0.05$, $\beta = 0.20$). Considering that some patients may drop out, or have to be excluded (e.g. due to non-compliance, side-effects or technical difficulties), twenty patients were included in the study.

Data are presented as means ± s.e. mean, or as means (95% confidence intervals; CIs). Chest pain data are presented as medians (25th–75th percentiles). Due to asymmetrical distribution, filtragometry and plasma βTG data were logarithmically transformed prior to statistical evaluation. These data are shown as mean log values and the antilogarithm of the corresponding mean log values. When expressing treatment effects, these variables are shown as the means and CIs of ISDN/placebo ratios. Two-way analyses of variance (ANOVA), repeated measures design including the effects of treatment (ISDN and placebo) and all time points (i.e. rest and exercise), were used to compare treatment conditions. The significance level of the interaction term of the two-way of ANOVA is given. Student's paired *t*-test or Wilcoxon's signed rank test were used to test changes over time during each treatment condition. When Wilcoxon's test was used (*n.b.* chest pain and anginal

episodes), data are presented as median and 25th–75th percentiles. $n = 16$ unless otherwise stated.

Statistical analyses were performed using the commercially available computer programmes Statview II™ and Super ANOVA (versions 1.03 and 1.1 respectively; Abacus Concepts Inc., Berkley, CA, USA).

Results

Twenty male patients with stable exercise induced angina pectoris, mean age 60.5 years (range 49–70 years), were included in the study. Three individuals discontinued the trial during the active treatment phase due to severe headache. One subject was excluded due to a symptomatic vasovagal reaction during blood sampling. Among the patients completing the study six subjects reported headache during the active treatment phase, one patient reported heartburn, and one patient experienced palpitations. One individual reported headache during the placebo phase.

Patient compliance data (Figure 1)

In two patients the MEMS devices were defective and data were therefore lost. As can be seen from Figure 1, overall drug compliance was good. On the day of the experiment all patients ingested their study drug as prescribed (i.e. one tablet in the morning and one tablet 1 h prior to the investigation). The day before the experiment, four patients missed taking the study drug on one occasion during the active phase. No patient ingested short-acting nitroglycerin on either of the two experimental days.

Exercise capacity, anginal symptoms and nitroglycerin consumption

After placebo treatment the reasons for termination of the exercise test were: chest pain (five patients),

Figure 1 Distribution of compliance rates for drug regimens during active treatment (■) and placebo (□) in 14 patients. See Methods and Results for further information.

tiredness in the legs (five patients), dyspnoea (three patients), ECG changes (two patients) and a fall in blood pressure (one patient). After ISDN treatment the corresponding reasons were: dyspnoea in six patients, chest pain and tiredness in legs in three patients each, ECG changes, fall in blood pressure and pain in a knee in one patient each. Data regarding the specific reason for termination of the test were missing in one patient.

There was a tendency towards a higher exercise capacity during ISDN (7, –1 to 14 W). During ISDN treatment the perceived chest pain at maximal exercise was 1.5 (0–3; median value, and 25th and 75th percentile); this tended to be less than during placebo treatment (2 (0.5–3.5)). Coronary ischaemia on exercise, as measured by the maximal ST-segment depression during exercise, did not differ between treatments (the difference between the active and placebo treatments was 0 (–1 to 0) mm; mean and 95% CI). According to patient diaries the total use of short-acting nitroglycerin tablets was low (0–2 tablets) during both treatment phases. The number of anginal episodes were 1 (0–3 episodes) during ISDN treatment, and 1 (0–4.5 episodes) in the placebo phase.

There were no significant relationships between coronary ischaemia (assessed as ST-segment depression on ECG during exercise), and increases in platelet aggregability (filtragometry) or platelet secretion (β TG levels in plasma) during exercise, either when platelet responses were calculated as relative or absolute changes.

Cardiovascular variables are summarized in Table 1.

Haematological variables

As expected, platelet counts increased significantly in response to exercise (from $210 \pm 10 \times 10^9 \text{ l}^{-1}$ to $244 \pm 12 \times 10^9 \text{ l}^{-1}$, and from $224 \pm 10 \times 10^9 \text{ l}^{-1}$ to $255 \pm 12 \times 10^9 \text{ l}^{-1}$ during placebo and ISDN treatment, respectively). The mean increase of platelets in response to exercise was $5 (-19 \text{ to } 9) \times 10^9 \text{ l}^{-1}$ lower

Table 1 Cardiovascular variables at rest (reclining and in the upright position on the ergometer bicycle, respectively), and during maximal exercise. Data are presented as mean $\pm$ s.e. mean

	Rest (reclining)	Rest (on bike)	Exercise (maximal)
<i>Heart rate (beat min⁻¹)</i>			
Placebo	61 $\pm$ 3	63 $\pm$ 3	130 $\pm$ 5
ISDN	64 $\pm$ 4	69 $\pm$ 4	130 $\pm$ 4
<i>Systolic blood pressure (mm Hg)</i>			
Placebo	134 $\pm$ 5	131 $\pm$ 5	205 $\pm$ 7
ISDN	129 $\pm$ 5*	123 $\pm$ 5	211 $\pm$ 7†
<i>Diastolic blood pressure (mm Hg)</i>			
Placebo	83 $\pm$ 2		
ISDN	81 $\pm$ 2		

* $P < 0.05$ refers to placebo vs active treatment at rest as calculated by a paired t -test.

† $P < 0.05$ refers to placebo vs ISDN including effects over time (i.e. rest on bike and exercise) as calculated by a 2-way ANOVA.

Figure 2 Effects of dynamic exercise (symptom limited exercise test) on platelet aggregability as measured by filtragometry during ISDN treatment (●) and placebo (○) in 15 patients. The y-axis shows filtragometry readings expressed as mean log values $\pm$ s.e. mean (log tA); a logarithmic scale (tA) shows the corresponding antilogarithmic mean values. The x-axis shows the exercise capacity (mean $\pm$ s.e. mean). Filtragometry measurements were performed at rest (0 W) and immediately after exercise.

during ISDN treatment. MPV remained unchanged during the placebo experiments (9.4 ± 0.2 fl) but tended to decrease in response to exercise during ISDN treatment by 0.2 (-0.3 to 0) fl. MPV differed significantly between treatments when calculated by 2-way ANOVA ($P = 0.02$).

Filtragometry measurements (Figure 2)

Filtragometry measurements could not be obtained in one patient, due to technical problems with the apparatus.

Exercise significantly shortened filtragometry measurements (antilog of mean log values) from 155 to 95 s (log tA: 2.19 ± 0.08 to 1.98 ± 0.06 ; $P = 0.005$, $n = 15$). During ISDN treatment the corresponding values were 162 and 89 s (log tA: 2.21 ± 0.07 to 1.95 ± 0.06 ; $P = 0.005$, $n = 15$). The ISDN/placebo ratios were 1.05 (0.83 to 1.32) at rest, and 0.98 (0.80 to 1.20) during exercise.

In contrast to our findings in healthy volunteers [12], there were no relationships between ISDN induced vasodilation (i.e. the decrease in systolic blood pressure at rest, calculated as placebo values minus ISDN values) and the filtragometry readings—either at rest or during platelet activation by exercise (the platelet activating effect was expressed either as the percent decrease in tA (exercise divided by rest), or the corresponding algebraic difference (i.e. rest minus exercise readings)). Individuals who responded with headache to the ISDN treatment were also subject to subgroup analyses; platelet responses in these patients were not significantly different during ISDN treatment compared with placebo. Exclusion of the patient showing poor drug compliance (i.e. the individual with a compliance rate of less than 30%; see Figure 1) did not affect the results.

There were no apparent order effects with respect to filtragometry or plasma β TG data (see below)

Figure 3 The effect of placebo compared with ISDN treatment, on the excretions of β TG (upper panel) and 11-dehydro thromboxane B_2 (lower panel) in urine in 16 patients during the night preceding the experiment and the day of the experiment (covering the exercise test). Data are presented as mean $\pm$ s.e. mean. P values are derived from Student's t -test for paired data (within treatments) or 2-way ANOVA (between treatments).

when data from the two treatment sequences were compared.

β TG- and thromboxane-measurements (Figure 3)

Sampling for plasma β TG was not possible due to practical problems in one individual. After placebo treatment β TG in plasma (antilog of mean log values) increased from 22 to 35 ng ml^{-1} (log values: 1.35 ± 0.04 to 1.54 ± 0.07 ; $P = 0.006$) after exercise. During ISDN treatment β TG in plasma increased from 27 to 38 ng ml^{-1} (log values: 1.43 ± 0.05 to 1.58 ± 0.09). The ISDN/placebo ratios were 1.09 (0.98 to 1.21) at rest, and 1.04 (0.82 to 1.30) during exercise.

During the placebo phase the urinary excretion of β TG was significantly higher during the day compared with night (2.9 ± 0.4 and 2.1 ± 0.2 ng mmol^{-1} creatinine, respectively; $P = 0.008$), as observed previously [29]. During ISDN treatment this diurnal pattern was abolished (the mean difference between day and night was -0.1 (-0.8 to 0.5) ng mmol^{-1} creatinine). When day and night values were combined (as mean values) the urinary excretion of β TG tended

to be higher (+0.4 (0 to 0.8) ng mmol⁻¹ creatinine) during ISDN compared with placebo treatment.

The excretion of 11-dehydro thromboxane B₂ in urine was similar during the day and at night during placebo treatment (mean difference -2 (-27 to 23) ng mmol⁻¹ creatinine). During ISDN treatment the excretion tended to be attenuated during the day compared with at night (-37 (-80 to 5.2) ng mmol⁻¹ creatinine). When day and night values were combined there was no significant difference in the urinary excretion of 11-dehydro thromboxane B₂ between the treatments; the mean difference was -11 (-63 to 42) ng mmol⁻¹ creatinine.

Exclusion of the individual showing poor compliance (i.e. a compliance rate below 30%, see Figure 1) did not influence the results.

Urinary creatinine levels did not differ significantly between treatments (the mean values were 11 mmol l⁻¹ at night, and 10 mmol l⁻¹ during the day, for both placebo and ISDN treatment).

Discussion

The present study does not support the concept of an antiplatelet effect of ISDN, at least not when administered orally and over a period of time. Thus, ISDN treatment did not significantly inhibit any aspect of platelet function investigated, either at rest or after platelet activation by physical exercise. Our power calculations based on healthy volunteers indicated a possibility of detecting a 20% difference between treatments for filtragemetry measurements at rest. The confidence intervals of the present study suggest a slightly lower statistical power for resting measurements, but adequate power for the exercise measurements. Our data indicate that ISDN does not importantly influence platelet reactivity to exercise. This is of interest since physical exertion has been implicated in the precipitation of myocardial infarction [17]. Our investigation was performed in a placebo-controlled, double-blind fashion, and we used several methods in order to assess different aspects of platelet function *in vivo*. Furthermore, drug compliance was checked with the MEMS system. Despite these precautions we could not find any platelet attenuating effects of oral treatment with ISDN.

It should, however, be emphasized that several previous studies have shown significant platelet inhibiting effects of organic nitrates when administered intravenously, often at rather high doses. Diodati *et al.* [1] reported significant platelet inhibitory effects of intravenous nitroglycerin (titrated to reduce mean arterial blood pressure by 10 mm Hg) in patients with unstable coronary artery disease; ADP- and thrombin-induced aggregation were attenuated when assessed by whole blood impedance aggregometry. Using filtragemetry *ex vivo*, Karlberg *et al.* [2] found antiplatelet effects of intravenous nitroglycerin (titrated to decrease systolic blood pressure by 20%) in healthy volunteers, and Ferrario *et al.* [3] reported reduced collagen-induced aggregation in whole blood

ex vivo in patients with cardiomyopathy when nitroglycerin was administered intravenously in a similar fashion. Intravenously administered ISDN has also been found to inhibit platelet aggregation significantly. When given to patients with stable angina pectoris the number of circulating platelet aggregates was reduced, and ADP- and adrenaline-induced aggregation *in vitro* were attenuated [5]. Individual dose titration and high doses may have increased the possibility of detecting antiplatelet effects of nitrates in the above-mentioned studies. However, all of these positive studies were performed in an uncontrolled fashion.

With respect to antiplatelet effects of oral treatment with organic nitrates, the literature is divided. Sublingual nitroglycerin (300 µg) has been reported to inhibit platelet function when evaluated by ADP-induced aggregation in PRP [4], whereas a double-blind randomized crossover trial showed no effects of transdermally administered nitroglycerin (20 mg) on ADP induced aggregation in whole blood [11]. Gerzer *et al.* [7] reported that platelet activating factor (PAF) induced platelet aggregation *in vitro* in platelet rich plasma (PRP) was more strongly attenuated after a single oral dose of isosorbide-5-mononitrate (20 mg; ISMN) than after placebo in healthy volunteers. However, Daví *et al.* [30] could not find any antiaggregating effects of ISMN (20 mg twice daily) in a randomized double-blind and placebo-controlled study in patients with stable angina, when tested with various platelet agonists (including PAF) in PRP. With respect to ISDN treatment, one uncontrolled study reported platelet inhibiting effects in patients with stable angina pectoris [6]. The authors reported decreased ADP-induced aggregation in PRP, a reduction of circulating platelet aggregates, and a slight reduction of plasma βTG, after 4 weeks' treatment with 100 mg ISDN daily. However, the present study does not support the idea of significant antiplatelet effects of oral ISDN treatment. It seems as if the antiplatelet effects of organic nitrates reported in uncontrolled studies are difficult to reproduce in controlled studies.

There was a modest effect of ISDN treatment on exercise capacity and angina in the present study. It may be argued that this was due to a low dosage of ISDN. However, 20 mg ISDN twice daily caused headache in almost half of the patients (nine out of 20), and in three subjects (i.e. in 15% of the patients) the headache was so severe that it led to withdrawal from the study. Thus, a higher dosage of ISDN would not have been possible. The modest effect on exercise induced anginal symptoms was, rather, related to few symptomatic events in the placebo test. It should be kept in mind that angina pectoris is a condition which is very sensitive to the placebo effect [31].

Results concerning levels of βTG in plasma and the urinary excretion of βTG and 11-dehydro thromboxane B₂ support the contention that ISDN did not significantly inhibit platelet function *in vivo*. During the placebo phase the urinary excretion of βTG was lower during the night, a phenomenon which has been observed by us previously [29], and is in accordance with the concept of diurnal variation in platelet func-

tion [32]. The observed increase in urinary β TG excretion at night, and the tendency towards increased excretion when day and night data were combined suggest that ISDN, if anything, activates platelets slightly.

Physical exercise increased platelet aggregability and platelet secretion *in vivo* significantly and to a similar extent during treatment with placebo and ISDN. Conflicting data have previously been reported with regard to responses of platelet function to dynamic exercise [for a review see 33]. Discrepancies may, at least in part, be due to methodological difficulties and differences [33], and the present findings of enhanced platelet aggregability and secretion *in vivo* are in line with previous findings by us in patients with stable angina pectoris [20]. The clinical importance of the platelet activating effect of exercise in the context of IHD is not known, but platelet activation may, especially in combination with decreased fibrinolytic capacity [34], increase the susceptibility to thrombotic complications during heavy exercise.

Increased platelet size has previously been linked to poor outcome of ischaemic heart disease [35]. In the present study the platelet size response to exercise, measured as median platelet volume (MPV), was significantly altered by ISDN treatment. Whereas MPV was unaffected by exercise during the placebo phase, it decreased following exercise during ISDN treatment. The small but significant increase in mean platelet size in response to exercise previously reported by others [36–39] could thus not be reproduced in our experimental set up; we have no explanation for this discrepancy. The decrease in MPV following exercise after ISDN treatment is also difficult to explain. As platelet count and size are factors regulated by splenic blood flow [40], it may be hypothesized that the vasodilating properties of ISDN may have had an influence. However, viewed against the lack of effects of ISDN treatment on the func-

tional platelet parameters studied (i.e. secretion and aggregability) it seems unlikely that the observed effect on MPV has any biological importance.

In a previous study in healthy volunteers, we found tendencies towards an antiplatelet effect of ISDN, as the blood pressure reducing effect of ISDN was related to its effect on platelet aggregating responses to adrenaline infusion [12]. Such trends were not found in the present study, but blood pressure measurements were performed 2 weeks apart and the comparison is thus less reliable. There was no relationship between proneness to headache and treatment effects on platelet function.

In conclusion, oral administration of the long-acting organic nitrate ISDN did not importantly affect platelet function *in vivo* in patients with IHD. Exercise activates platelet aggregation and secretion in such patients. If orally ingested ISDN has antiplatelet properties they are likely to be weak. In our opinion, the idea that organic nitrates have important antiplatelet effects *in vivo* should rest on positive randomized controlled studies showing clear-cut differences between active treatment and placebo. At present, convincing investigations of such a nature are lacking.

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