Isosorbide Dinitrate Blocks Thromboxane Synthesis Caused by CO₂ in Dog Heart-Lung Preparation

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Abstract—Effects of isosorbide dinitrate (ISDN) on coronary flow and arterial prostaglandin (PG) concentrations were investigated, using dog heart-lung preparations. Two kinds of gases (low and high CO₂ gases) were used for the artificial respiration. Low CO₂ gas contained 55% O₂ and 0.2% CO₂, whereas high CO₂ gas contained 55% O₂ and 8% CO₂. Administration of ISDN into a blood reservoir at high CO₂ caused an increase in coronary sinus blood flow, which was blocked by indomethacin, but not at low CO₂. In the absence of ISDN, the arterial concentration of thromboxane (TX) B₂ was larger at high CO₂ than at low CO₂. ISDN attenuated such an increase in TXB₂ concentration caused by CO₂. In the absence of ISDN, the arterial concentration of 6-keto PGF₁α was altered by neither CO₂ nor ISDN, but slightly increased with time. Indomethacin lowered the concentrations of 6-keto PGF₁α and TXB₂. These results suggested that the arterial CO₂ tension enhanced the TXA₂ synthesis and that ISDN inhibited such a relation between CO₂ and TXA₂ synthesis. Additionally, the vasodilatory effects of PG₁₂ was enhanced by elevating the arterial CO₂ tension. Thus, the increase in canine coronary flow at high CO₂ in the presence of ISDN may be related to the inhibitory effects of ISDN on the TXA₂ synthesis enhanced by the high arterial CO₂ tension and the facilitatory effects of CO₂ on the PG₁₂-induced vasodilation.

As recently reported, nitrates affect the metabolic pathway of prostaglandins (PGs) (1, 2). Several studies were made to determine whether the antianginal action of nitrates was mediated by PGs (3–5). On the other hand, cyclooxygenase metabolites such as thromboxane (TX) A₂ are synthesized in ischemic heart diseases (6–8); and a thromboxane (TX) synthetase inhibitor, such as OKY-046, has been demonstrated to be effective in the treatment of angina pectoris or myocardial infarction (9, 10).

Controversial results have been reported with respect to the involvement of PGs in the increase in coronary flow. Morcillio et al. (11) suggested that infusion of nitroglycerin resulted in an increase in coronary flow, which was blocked by indomethacin. However, Panzenbeck et al. (5) showed that intracoronary infusions of nitroglycerin and nitroprusside resulted in an increase in coronary flow by a mechanism which was independent of PG release.

Previously (12), we showed that the increase in coronary sinus blood flow caused by isosorbide dinitrate (ISDN) was affected by the inspiratory CO₂ tension and was abolished by cyclooxygenase inhibitor. In the present study, we further studied, using dog heart-lung preparation, how the concentrations of PGs were modified by ISDN and CO₂.

Materials and Methods

Experimental preparation: Eighteen mongrel dogs, weighing 7–12 kg, were anesthetized with 25 mg/kg of pentobarbital sodium, i.v., after preanesthesia with 10 mg/kg of ketamine, i.m. Heart-lung preparations were made according to the Krayer-Mendez
modifications of the original Starling method (13). Venous and arterial cannulae were inserted into the superior vena cava and the brachiocephalic artery, respectively. For priming the extracorporeal circuit, larger dogs which were anesthetized with 10 mg/kg of ketamine, i.m. and 25 mg/kg of pentobarbital sodium, i.v., were exsanguinated. Collected blood was heparinized and diluted with a 10% dextran solution so that hemoglobin concentration was in the range of 8–10 g/dl. The blood temperature was kept at 37°C.

The blood level in the reservoir was set throughout the experiments at 100 mm above the level of the opening of the superior vena cava to the right atrium. Venous resistance between the blood reservoir and venous cannula was adjusted so that an equilibrium point, i.e., a crosspoint of cardiac output and venous return curves (14), was kept on a venous return curve, which intersected the abscissa at 100 mmH2O of right atrial pressure (RAP) and the ordinate at 500 ml/min of cardiac output (CO) in the RAP-CO relation diagram (15). Cardiac output was measured using an electromagnetic flowmeter (Nihon Kohden, MF-25), the probe of which was placed on the venous side of the extracorporeal circuit. Lungs were ventilated artificially by a respirator (Natsume Seisakusho), with a tidal volume of 20 ml/kg and an end-expiratory pressure of 20 mmH2O. Frequency of respiration was 16 breaths/min. Pulmonary arterial pressure (PAP), left atrial pressure (LAP) and RAP were measured with pressure transducers (Nihon Kohden, LPU-0.1), and heart rate was monitored by a tachometer (Nihon Kohden, RT-5) triggered by an electrical signal of the aortic pressure. PAP was measured from the pulmonary artery in the right upper lobe.

Measurement of coronary sinus blood flow: To measure the coronary sinus blood flow (CSBF), a Morawitz cannula was inserted in the coronary sinus via the right atrial appendage. CSBF was measured by a square wave electromagnetic flowmeter (Nihon Kohden, MF-25), and the blood was allowed to return to the right atrium, through the venous cannula inserted into the superior vena cava. Cardiopulmonary nerves (16), vagi and phrenic nerves were severed. These maneuvers were necessary to prevent a significant increase in arterial 6-keto PGF1α concentration and to forestall a spontaneous gradual increase in CSBF.

Protocol: Twelve dogs were divided into 2 groups: i.e., with and without the administration of ISDN. Five dogs were treated with ISDN and the other seven dogs were not. To change the CO2 tension, room air, pure oxygen and pure CO2 gases were adequately blended to use as the inspiratory gas. CO2 gas in the inspiratory tract of the respirator was adjusted to be 0.2% for low CO2 gas and 8% for high CO2 gas. In both low and high CO2 gases, oxygen gas was mixed in at 55%. In every preparation, the basal condition throughout the experiments was maintained with low CO2 gas, and when needed, the inspiratory gas was changed to high CO2 gas. As shown in Fig. 1, such an exposure to high CO2 gas was repeated three times in every preparation. The first and second exposures to the high CO2 gas were performed 20 and 60 min after the heart-lung preparation was completed. In the ISDN-untreated group, the inspiratory gas was changed twice to the high CO2 gas, and then, a thromboxane
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The synthesis of thromboxane was blocked by ISDN 161 synthetase inhibitor, OKY-046 (30 mg), administered into the reservoir. Thereafter, the third exposure to the high CO₂ gas was performed. In the ISDN treated group, after control data were obtained by changing the inspiratory gas (the first exposure to high CO₂ gas), ISDN (100 μg) was injected into the blood reservoir, and the second exposure was performed. After one set of data with ISDN was obtained, indomethacin (1 mg) was administered into the reservoir at high CO₂. Arterial blood was withdrawn from the aortic cannula carefully under each steady state in hemodynamics, for later analyses of blood gases and PG's. Drug solutions administered into the blood reservoir were mixed with blood in the reservoir (approx. 500 ml). By this means, drugs at the final concentrations will have reached the lungs within 15 sec, as estimated from the blood flow (400 ml/min).

Four dogs were exposed to the high CO₂ gas during an infusion of PGI₂ (10 μg/ml) into the left atrium. An effect of high CO₂ gas on the coronary vasodilation caused by PGI₂ was examined during its infusion.

In addition, two dogs, ISDN was administered into the left atrium under the respiration with high or low CO₂ gas, before and after the treatment with indomethacin (1 mg).

Measurements of prostaglandins: For the quantitative analysis of prostaglandins, arterial blood samples (10 ml) treated with indomethacin (35.6 μg) and EDTA-2K (12 mg), kept at 4°C, were centrifuged to separate the serum, which was stored at −30°C for later analysis. The plasma was extracted according to Jaffe's method (17) with 3 ml of petroleum ether, followed by centrifugation (3000 rpm, 10 min). To remove proteins and lipids, the aqueous layer was exposed to 3 ml of 3:3:1 ethyl acetate: isopropanol: 0.1 M HCl and 2 ml of ethyl acetate. The purification of each PG was performed with high-performance thin-layer chromatography (Silica G 13749, 10×20 cm, Merck) for 6-keto PGF₁α and TXB₂. After extraction of each fraction, PG content was measured by radioimmunoassay. The recovered radioactivities in both 6-keto PGF₁α and TXB₂ fractions were in the range of 65–72%. The analyses were carried out at Mitsubishiyuka Laboratory of Medical Science, Tokyo.

Drugs used: ISDN (E-0291) was kindly provided by Eisai Co., Ltd. It was dissolved in a concentration of 0.5 mg/ml in saline. Indomethacin was purchased from Sigma Chemical Company and dissolved in 90% ethanol in a concentration of 10 mg/ml. Thromboxane A₂ synthetase inhibitor, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate (OKY-046), was a gift from Ono Pharmaceutical Company, and it was dissolved at 50 mg/ml in saline. PGI₂ sodium (Funakoshi Pharmaceutical Co.) was dissolved with Tris buffer (pH 9.1) at 4°C, just before use. CSBF and arterial blood pH were not influenced by the Tris buffer vehicle injected at the same flow rate of infusion.

Statistical analysis: Data were expressed as mean and standard error. For each parameter, we performed two-way analysis of variance. Then, to compare the two mean values, a difference of their means was examined with Tukey's wholly significant difference. When necessary, a t-test was also conducted between the two means. The level of significance was taken as 0.05.

Results

Changes in blood gases and pH: Table 1

| Inspiratory CO₂ | Pao₂ (mmHg) | Paco₂ (mmHg) | pH |
|-----------------|-------------|--------------|----|
| 0.2% (36)       | 361±19      | 7.8±0.6      | 7.67±0.08 |
| 8% (36)         | 341±13      | 65.4±1.3**   | 7.21±0.05** |

Data were expressed as values of the mean±S.E. Number in parentheses indicates the number of experiments. **: P<0.01, compared with values obtained in low CO₂ respiration.
Fig. 2. Typical recordings of hemodynamic parameters in canine heart-lung preparation. Percentage of CO₂ in the inspiratory gas was changed during the experiment as shown at the top. Arrows indicate the additions of isosorbide dinitrate (ISDN) and indomethacin into the blood reservoir. HR: heart rate, CSBF: coronary sinus blood flow, PAP: pulmonary arterial pressure, LAP: left atrial pressure, RAP: right atrial pressure.

shows PaO₂, PaCO₂ and pH values of the arterial blood. PaO₂ values were in the range of 300-400 mmHg and were not altered by changing the inspiratory gases. PaCO₂ values were less than 10 mmHg for low CO₂ gas and 65.4±1.3 mmHg (n=36) for high CO₂ gas.

Changes in hemodynamic parameters: Figure 2 shows the recordings of hemodynamic parameters during an experiment with the administration of ISDN. When CO₂ tension in the inspiratory gas was increased to 5% and then to 8%, PAP, LAP and RAP increased stepwise. In each step, it took approximately 4 min to reach a steady state. These changes were seen both before and after the administration of ISDN. No remarkable change in CSBF was observed before the administration of ISDN, whereas CSBF increased stepwise after that. Such an increase in CSBF caused by elevating the inspiratory CO₂ tension could be observed repeatedly for at least one hour after the administration of ISDN. Indomethacin completely abolished the increase in CSBF, without affecting the other hemodynamic parameters. When ISDN was injected into the left atrium at high CO₂ (Fig. 3), the response of CSBF was biphasic: a fast response appeared immediately after the injection, and it was followed by a slow-phase response. The latter was blocked by the pretreatment with indomethacin, whereas the former was unaffected. At low CO₂, the slow-phase response was not observed.
When CO₂ tension was elevated, PAP and LAP significantly increased both before and after the treatment with ISDN (P<0.05) as shown in Fig 4. Heart rate was hardly altered. Figure 5 shows the changes obtained in experiments with (dotted line) and without the administration of ISDN (solid line). CSBF was not changed by elevating the CO₂ tension before the treatment with ISDN, but was increased from 21.1±3.8 ml/min to 41.3±6.9 ml/min (n=5) after that (P<0.05). The increase in CSBF (20.2±8.1 ml/min, n=5) was significantly greater than that (-0.6±3.4 ml/min, n=7) obtained at a similar time in experiments without the administration of ISDN (P<0.05). Indomethacin inhibited such an increase in CSBF at high CO₂, lowering CSBF significantly (P<0.05) from 40.2±5.9 ml/min to 19.1±5.7 ml/min (n=5).

Treatment with OKY-046 in the control group caused an increase in CSBF: CSBF changed from 20.5±3.3 ml/min to 30.8±4.3 ml/min (n=7) at low CO₂, and from 21.6±3.4 ml/min to 74.5±10.3 ml/min (n=7) at high CO₂ (P<0.01). In the presence of OKY-046, CSBF was significantly increased by changing the inspiratory gas from the low CO₂ gas to the high CO₂ gas (P<0.01).

Prostaglandins in arterial blood: In Fig. 5, changes in the arterial concentrations of TXB₂ and 6-keto PGF₁α are also shown. In the experiments without the administration of ISDN, TXB₂ concentration was increased significantly by elevating the CO₂ tension (P<0.05): from 426±53 pg/ml to 761±96 pg/ml (n=12). Pretreatment with ISDN inhibited such an increase. The TXB₂ concentrations obtained at high CO₂ (512±61 pg/ml, n=5) were significantly lower than those (943±149 pg/ml, n=7) obtained at a similar time in experiments without the administration of ISDN (P<0.05).

On the other hand, 6-keto PGF₁α concentrations tended to increase with time, in both experiments with and without the administration of ISDN. No significant change was caused by elevating the CO₂ tension. Additional treatment with indomethacin, in experiments with the administration of ISDN, lowered the concentration of TXB₂ and 6-keto PGF₁α to 257±101 pg/ml (P<0.05) and to 889±257 pg/ml (P<0.05), respectively, at high CO₂. Additional administration of OKY-046 in experiments without the administration of ISDN caused a significant decrease in TXB₂ concentration at low and high CO₂ (P<0.05) to 256±46 pg/ml and 206±44 pg/ml, respectively. On the other hand, the 6-keto PGF₁α concentration was further increased up to 1387±177 pg/ml and 1552±429 pg/ml (n=7) at low and high CO₂, respectively, but could not be distinguished from the spontaneous large increase, which often appeared after 120 min.

Effects of carbon dioxide on PGI₂-
induced coronary vasodilation: We performed four other experiments with an infusion of PGI₂ into the left atrium, in four dogs. As shown in Fig. 6, when the inspiratory CO₂ tension was increased during the infusion of PGI₂ at a rate of 10 μg/ml, the increase in CSBF was greatly enhanced. Similar recordings were obtained in the other dogs.

Discussion

The hypothesis that PG mediates the coronary vasodilation caused by nitrates has recently been examined in in vivo experiments. Panzenbeck et al. (5) infused nitroglycerin at a rate of 60 μg/min into the coronary artery to eliminate the effects of drug-induced systemic hypotension. They found that cyclooxygenase blockade did not prevent the increase in coronary arterial flow, suggesting that PG does not act as a local vasodilation intermediate. Our present study, however, showed that the coronary vasodilation which could be inhibited by indomethacin depended on the arterial CO₂ tension, confirming our previous findings obtained in in vivo experiments (12). Actually, the increase in CSBF was not caused at low CO₂, but at high CO₂. Failure to demonstrate a participation of PG in the nitroglycerin-induced vasodilation may be attributed to a low CO₂ gas, such as room air, used for the inspiration. It is still possible that in the ischemic heart diseases which are accompanied by a local elevation in CO₂ tension (19), nitrates affect the blood flow at a high CO₂ area via a change in PG metabolism. In the present study, we measured the PG concentrations in arterial blood, because the CO₂ tension in arterial blood is more con-
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Elevation in CO₂ tension caused an increase in the TXB₂ concentration. The site of action of CO₂ is still obscure from this study. According to Förster (3), nitroglycerin does not affect the TXA₂ biosynthesis in homogenates of guinea pig lungs, but it does in platelet-rich plasma. Similar findings were also reported by Levin et al. (1). The platelet is a possible site for the action of nitrates, and Kerry and Paton (20) showed evidence that CO₂-treated platelets were rendered up to eight times more sensitive to sodium arachidonate in the aggregation response. Whether TXA₂ mediates such a phenomenon is still to be investigated.

The arterial concentration of 6-keto PGF₁α was altered neither by CO₂ nor by ISDN. It is known, however, that ISDN or nitroglycerin enhances the PG₁₂ synthesis in the human thoracic aorta and coronary artery (21), microsomal fraction of pig aorta (3) or cultured endothelial layer (1, 22). Therefore, locally synthesized PG₁₂ may act on the vascular smooth muscles or platelets, causing vasodilation or inhibition of aggregates, respectively. In canine isolated lungs (23), the increase in perfusion flow caused a rapid increase in the pulmonary arterial pressure (15 Torr), followed by a small decrease (2.4 Torr) which corresponded to an increase in pulmonary venous 6-keto PGF₁α concentration (260 pg/ml). The present study showed that the high CO₂ tension in the inspiratory gas elevated the pulmonary arterial pressure, possibly augmenting the PG₁₂ synthesis. The time-dependent gradual increase in 6-keto PGF₁α concentration, observed in both ISDN-treated and untreated groups, indicated a gradual accumulation of PG₁₂ in blood, though the rate of rise was remarkably small as reported previously (16). The absolute value of the change may have been too small to be detected in the method used in this study.

Furthermore, PG₁₂-induced coronary vasodilation was enhanced by elevating the CO₂ tension. So far, no such studies have been performed elsewhere. The result suggested that the increase in CSBF caused by ISDN and OKY-046 was not only due to the increase in ratio of PG₁₂ to TXA₂ concentrations, but also to such a synergistic action of high CO₂ tension upon the vasodilatory effect of PG₁₂. It has been reported that PG₁₂ infusion into the left atrium at a rate of 25–35 ng/kg/min had no effect on either the transmural or regional blood flow in acutely ischemic canine myocardium (24). The infusion rate was extremely small, compared to that used in this study (10 μg/min). It is still to be investigated whether the PG₁₂-induced vasodilation is enhanced by high arterial CO₂ tension in the ischemic heart disease.

ISDN inhibited the increase in TXB₂ concentration at high CO₂, and coronary flow was increased at high CO₂ by ISDN or OKY-046. These results seemed to correspond to the fact that TXA₂ produced a contraction of isolated porcine coronary artery (25). However, when the TXB₂ concentration was increased by elevating the inspiratory CO₂ content in the absence of ISDN, no decrease in the coronary flow was observed. An explanation for such a controversial result is as follows: if TXA₂ constricts the coronary vasculature, a resultant decrease in coronary flow may be counteracted by the CO₂-induced augmentation of the increase in coronary flow caused by PG₁₂. If this is the case, ISDN may act as an indirectly-acting vasodilator at high CO₂, by inhibiting the increase in TXA₂ concentration at high CO₂; and a further increase in coronary flow may be made by the effect of high arterial CO₂ tension on the PG₁₂-induced vasodilation.

Whether the changes in PG metabolism caused by ISDN is related to the antianginal action is still obscure. Although this study provided evidence indicating that ISDN modified the arterial TXB₂ concentration at high CO₂, the sites of action of ISDN and CO₂ were not determined. Nevertheless, the decrease in the arterial TXA₂ concentration may possibly be a beneficial effect of ISDN on the blood flow and platelet aggregation in the coronary circulation.

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