Inhalation of Carbon Dioxide Enhances the Coronary Vasodilating Action of Isosorbide Dinitrate in the Dog

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Accepted November 18, 1986

Abstract—Two kinds of inspiratory gases, 100% O₂ and 81% O₂ + 19% CO₂, were used for the artificial respiration. Elevating the inspiratory CO₂ tension enhanced the decrease in coronary vascular resistance caused by isosorbide dinitrate (100 µg/kg), without any change in heart rate and systemic arterial pressure. Such a change in coronary vascular resistance was prevented by indomethacin. The results suggested an interaction between isosorbide dinitrate and CO₂, which was mediated by prostaglandins.

Nitrates relax the vascular smooth muscles by their direct action. Besides the direct action on the vasculature, recent studies have revealed that nitrates also affect the metabolic pathway of prostaglandins (PG's) (1, 2), indirectly increasing the coronary flow (3). On the other hand, an increase in coronary flow, mediated by some PG's, might also be caused by elevating the arterial PCO₂ (4). Therefore, we assumed that there may be some interaction between isosorbide dinitrate (ISDN) and CO₂, which serves to increase the concentration of some vasodilative prostaglandin at a local area and increases the coronary flow.

We used 10 mongrel dogs, weighing 7–12 kg, and anesthetized them with 25 mg/kg pentobarbital sodium, i.v., after preanesthesia with ketamine 10 mg/kg, i.m. After opening the chest, the lungs were ventilated artificially by a respirator, with a tidal volume of 20 ml/kg and an end expiratory pressure of 20 mmHg. The frequency of respiration was 16 breaths/min. Heparin was used to prevent blood coagulation.

To measure the coronary sinus blood flow (CSBF), a Morawitz cannula was inserted into 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 returned to the right atrium. Right atrial pressure and systemic arterial pressure (RAP and SAP) were measured using pressure transducers (Nihon Kohden, LPU-0.1 and LPU-0.5), and the heart rate was monitored by a tachometer (Nihon Kohden, RT-5) triggered by an electrical SAP signal.

In every preparation, the effects of one-shot injection of 100 µg/kg ISDN, i.v., were examined first. The basal condition throughout the experiments, was maintained with an inspiratory gas of 100% O₂ (low CO₂ gas); and when needed, the inspiratory gas was changed to 81% O₂ + 19% CO₂ (high CO₂ gas). The procedure to change the inspiratory gases was performed by a gas blender (MERA, MF-2). After control data were obtained by changing the inspiratory gas, animals were treated with ISDN; and thereafter, the inspiratory gas was changed. The inspiratory gas was changed 2–5 times before and after treatment with ISDN. The mean value for each period was calculated. The dose of ISDN was 100 µg/kg, which was large enough to produce the maximum response of CSBF to CO₂. In five of 10 dogs, indomethacin of 1 mg/kg was administered i.v. after
completing the first experiments. Again, ISDN was injected in the same way, and inspiratory gas was changed to high CO₂ gas. In the other 5 dogs, after the first experiments, the secondary injection of ISDN was followed by the administration of a thromboxane synthetase inhibitor, OKY046 5 mg/kg, i.v., during a respiration with high CO₂ gas.

ISDN (E-0291) was kindly provided by Eisai Co., Ltd. Indomethacin (Sigma) was dissolved with 90% ethanol at a concentration of 10 mg/ml. A thromboxane A₂ synthetase inhibitor, (E)-3-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate (Ono Pharmaceutical Co., OKY046), was dissolved at a concentration of 50 mg/ml in saline. The significance of differences between the means was determined by Student's t-test.

Figure 1 shows the recordings of hemodynamic parameters during the first experiments. When CO₂ content in the inspiratory gas was increased from 0% to 19%, RAP increased. Such a change was seen both before and after the administration of ISDN. However, no remarkable change in CSBF was observed before the administration of ISDN, whereas CSBF increased after that. There was a latency of 5 min for the appearance of the CSBF response. CSBF increased gradually and leveled off in 4–5 min. More than 30–40 min after ISDN was administered, the incremental response of CSBF to CO₂ disappeared.Possibly, the concentration of ISDN in the blood might be lowered below the effective concentration by metabolism and excretion.

Figure 2A shows the results obtained before and after the administration of ISDN. The data in the presence of ISDN were obtained within 30 min after the administration. When CO₂ ratio was elevated in the inspiratory gas, RAP significantly increased after the treatment with ISDN (P<0.05). CSBF was not increased by elevating the inspiratory CO₂ tension before the administration of ISDN, but it was significantly increased thereafter from 46±2 to 84±6 ml/min (P<0.01). The coronary vascular resistance, calculated as (SAP-RAP)/CSBF, was significantly decreased (P<0.05). These results indicated an interaction between ISDN and CO₂ in terms of their vasodilatory effects. No remarkable changes in heart rate.

![Fig. 1](image-url) A typical recording of hemodynamic parameters in a canine in vivo preparation which represents the effects of isosorbide dinitrate (ISDN) in the presence and absence of CO₂ in the inspiratory gas. SAP, RAP, CSBF, and HR indicate systemic arterial pressure, right atrial pressure, coronary sinus outflow, and heart rate, respectively. Inspiratory gas was changed from 100% O₂ to 81% O₂+19% CO₂ before and after the administration of ISDN at 100 μg/kg, i.v. Carbon dioxide increased CSBF soon after the ISDN treatment, but the increase was no longer observed 50 min thereafter.
Fig. 2. Effects of isosorbide dinitrate (ISDN) on the hemodynamic parameters in the presence and absence of CO₂ in the inspiratory gas, before (A) and after (B) the treatment with indomethacin, and the modification caused by additional administration of thromboxane synthetase inhibitor (OKY046) (C). Experiments in (A) were followed by a treatment with 1 mg/kg indomethacin (B) or by an additional administration of 5 mg/kg OKY046 after the second injection of 100 µg/kg ISDN (C). Numbers of experiments in (A), (B) and (C) are 10, 5 and 5, respectively. In (A) and (B), the open circles with the solid line indicate the results before the administration of ISDN, and the closed circles (broken line) indicate the results after that. Experiments in (C) were carried out with an inspiratory gas of 81% O₂+19% CO₂. Values are expressed as means±SE. *P<0.05 vs. with 100% O₂ inspiration, **P<0.01 vs. with 100% O₂ inspiration. †P<0.05 vs. without OKY046.

Indomethacin inhibited the increase in CSBF and the decrease in coronary vascular resistance which were induced by ISDN (Fig. 2B). The increase in RAP caused by CO₂ was also inhibited. The negative inotropic effect of CO₂ is mediated by intracellular acidosis of the myocardium (5) on one side and by activation of chemoreflexes (6) on the other side. It is still to be investigated which was affected by indomethacin. From the former result, an indirect action of nitrate on the coronary vasculature mediated by PG's has been suspected.

Figure 2C shows the effect of OKY046 on the coronary vasculature in the presence of ISDN under the respiratory condition with high CO₂ gas. Both the increase in CSBF and the decrease in coronary vascular resistance were further enhanced by OKY046. Since the dose of ISDN was high enough to produce the maximum response to CO₂, the result indicated that there was a difference in the vasodilative mechanism between ISDN and OKY046.

An increase in PaCO₂ or intracellular acidosis is known to elevate the coronary flow (4, 5, 7), although the change was very small, as shown by Rooke and Sparks (8). Pickard and Mackenzie (9) reported that cerebral vasculature was dilated by the elevation of CO₂ ratio in the inspiratory gas and that such a vasodilatation was inhibited by indomethacin, suggesting a mediation of...
PG’s. ISDN is also known to modify the blood concentrations of PG’s (10); i.e., the increase in PG12 (1) and the decrease in thromboxane A2 (2). As shown in the present study, there seemed to be an interaction between ISDN and CO2 in the response of CSBF, which was inhibited by indomethacin. Possibly, the interaction between them might have modified the blood concentration of PG’s more than the single treatment with CO2 or ISDN.

Panzenbeck et al. (11) reported that nitroglycerin and nitroprusside when infused into the coronary artery of dogs caused increases in coronary blood flow by a mechanism which was independent of PG release. However, it is suspected that they might have used room air for the artificial respiration, because the CO2 tension in air is relatively low.

Morcillio et al. (3) showed that an i.v. infusion of nitroglycerin (10 µg/min·kg−1) produced significant decreases in systemic arterial pressure and coronary vascular resistance, the latter of which was diminished by indomethacin. During the infusion, the PGE concentration in coronary sinus blood was significantly increased, whereas that in left atrial blood was not changed, suggesting that the origin of PGE was the coronary vasculature. Although the above investigators used room air for the respiration, the infusion of nitroglycerin produced a decrease in mean circumflex arterial resistance. Panzenbeck et al. (11), however, suspected that such a decrease in the vascular resistance was induced by the autoregulatory compensation in the decrease in systemic driving pressure in the face of a substantial reflex tachycardia.

In the present study, SAP and heart rate were hardly affected by ISDN or CO2. Hence, it is unlikely that the autoregulatory vasodilatation had occurred in our experiments.

It has been demonstrated that thromboxane synthetase inhibitor decreases the blood concentration of thromboxane A2; and in contrast to that, it elevates the concentration of PGI2, a vasodilator. If one assumes that ISDN fully lowered the concentration of thromboxane A2 and increased that of PGI2, the additional injection of the thromboxane synthetase inhibitor might not increase CSBF any further. However, the result was that OKY046 further raised CSBF in the presence of ISDN under the respiration with high CO2 gas. It is likely that the modification of ISDN on the PG metabolism is absolutely different from that of OKY046. OKY046 interacts on thromboxane synthetase, whereas ISDN may act at some site in the PG metabolic pathway, in a facilitatory manner with CO2.

From these results, we also speculate that the effects of nitrates on PG metabolism, resulting in the indirectly-induced coronary vasodilatation, may be efficient in special cases of respiratory insufficiency.

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