CORONARY COLLATERAL VASODILATOR ACTION OF N-ETHOXYCARBONYL-3-MORPHOLINOSYDNONIMINE (SIN-10) IN HEART WITH CHRONIC CORONARY INSUFFICIENCY IN DOGS

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Effects of the sydnonimine derivatives on cardiovascular system have been studied (1–5), and the results in our laboratory are summarized as follows: 1) the substitution by the group containing nitrogen with -N-N- linkage in the 3-position (SIN-series) showed vasodilator and consequently hypotensive effects resembling to those of nitrites, and 2) N-acylation of imino group of sydnonimine made the compounds stable chemically (6), which probably resulted in gradual development and prolongation of the hypotensive effects as well as in decreased toxicity (4). These findings prompted us to study the effects of the derivatives on coronary collateral circulation, because nitroglycerin, the most important antianginal agent, was reported to dilate the collateral vessels which developed as a result of chronic myocardial ischemia (7, 8), and because the effects of agents on the collateral circulation have received considerable attention as one of the approaches to evaluate antianginal activity in laboratory experiment (9).

The present study was carried out to investigate the effects of N-ethoxycarbonyl-3-morpholinosydnonimine (SIN-10) and related compounds on the coronary collateral circulation.

METHODS

Male mongrel dogs weighing 8 to 13 kg were used.

The effects of agents on the coronary collateral circulation were evaluated in the heart with chronic coronary insufficiency. The preparation of the coronary insufficiency was accomplished according to the technique of Harris (10) with a few modifications. The animals were treated with intramuscular 400,000 units of penicilline prior to the operation. Under anesthesia with intravenous 30 mg/kg of sodium pentobarbital and artificial respiration, the chest was opened semiaseptically through the fourth intercostal space. The anterior descending branch of the left coronary artery was dissected free from the surrounding tissues as near the origin as possible, and constricted with a silk thread so that the blood flow is somewhat limited. For that purpose, the arterial branch was ligated together with a stainless wire of 1.0 to 1.5 mm in diameter, which was soon removed after the ligation. The chest was tightly closed and air was removed from the chest cavity. The
incisure was covered with bandage of synthetic resin (Novectan Spray, Yoshitomi, Co.). For one week after the operation animals were treated with 50 mg of tetracycline HCl given orally twice a day mixed with diet. Following five to eight weeks were adequate to use these animals for the experiment.

The chest was opened as usual and the anterior descending branch was dissected free and ligated completely at the distal part to the constriction previously done. The blood pressure was measured from the left carotid artery and change in the lead II ECG was observed for 30 minutes. Then, as shown in Fig. 1, a polyethylene cannula was orthogradely inserted into the arterial branch for the measurement of retrograde pressure (RP). Retrograde flow (RF) through the cannula for 10 or 15 seconds was intermittently measured with graduated cylinder according to the method of Anrepp and Häusler (11). RP and RF were taken as indices to evaluate the magnitude and the hemodynamic behaviour of the collateral circulation. In addition, cRF, which was expressed as ml/min/100 mmHg mean blood pressure (MBP), was considered to normalize the changes in systemic blood pressure. Systemic blood pressure and RP were recorded by use of pressure transducers (MP-24T, Nihon Kohden). Heart rate was registered with a tachometer (RT-2, Nihon Kohden) triggered by R waves of ECGs. All these parameters were recorded on a polygraph recorder (RM-150, Nihon Kohden).

The agents tested were SIN-10, 3-morpholino sydnonimine HCl (SIN-1) and N-nitroso-N-morpholinoamino-acetonitrile (SIN-1A) (Fig. 2).

![Fig. 1. Schematic representation of heart. See the text for details of experimental setup.](image)

![Fig. 2. Structures of sydnonimine derivatives tested. See the text for nomenclature of the compounds signified with symbolic names.](image)
RESULTS

I) Development of collateral circulation after coronary artery constriction

Of 25 animals, 5 died from ventricular fibrillation within 30 minutes after the operation limiting the blood flow through the left anterior descending artery, and 3 from unknown cause within several days. In most of the animals a severe attack of ventricular arrhythmia was observed immediately or within several hours after the operation. Fourteen animals showed the mean blood pressure of 104.5±14 mmHg and the mean RP and RF of 62.4±21 mmHg and 14.3±1.0 ml/min, respectively. Therefore, the coronary insufficiency seemed to be well compensated by the development of the collateral circulation. ECG showed no change at all even after the ligation of the anterior descending branch constricted previously. No sign of myocardial necrosis caused by coronary insufficiency was observed in 11 hearts, but in 3 thin fibrous scar was detected on the anterior wall of the left ventricle as a sign of necrosis.

II) Effects of SIN-10 and related compounds on coronary collateral circulation

The hemodynamic changes in systemic and coronary collateral circulation caused by intravenous administration of SIN-10 in a dose of 100 μg/kg were shown in Fig. 3. The level of RP was a little less than that of systemic blood pressure (BP), indicating a marked development of the collateral circulation. RF was about 14 ml/min. The systemic blood pressure decreased gradually following the intravenous SIN-10 and reached the maximum hypotension within 20 to 30 minutes. At the time, systolic and diastolic pressure decreased by 20 and 10 mmHg, respectively, and the hypotension was characterized by a decrease in pulse pressure. On the other hand, RP did not change significantly. CRF increased from 16.1 to 19.2 ml/min/100 mmHg MBP 15 minutes after SIN-10. Fig. 4 summarizes the effects of SIN-10 in doses of 100 and 250 μg/kg. The systemic blood pressure and

![Fig. 3. Effects of intravenous 100 μg/kg of SIN-10 on systemic and coronary collateral hemodynamics. Note the unchangeableness of RP in spite of hypotension and the increased cRF after SIN-10. ECG (II) : the lead II electrocardiogram ; BP : systemic blood pressure ; HR : heart rate ; cRF : the RF normalized the changes in systemic blood pressure ; RP : retrograde pressure.](image-url)
FIG. 4. Summarized results of intravenous 100 and 250 pg/kg of SIN-10. The same description can be applied as in Fig. 3, although BP and RP are shown in mean values, and cRF in percent change. Abbreviations are the same as in Fig. 3.

FIG. 5. Effects of intravenous 50 pg/kg of SIN-1A on systemic and coronary collateral hemodynamics. Note the marked increase in RF even during hypotension, and less decrease in RP than in BP. RF: retrograde flow; Other abbreviations are the same as in Fig. 3.
RP are shown as mean values, and the changes in cRF by percentage of the control level. It is clear that falls of RP were less than those of systemic blood pressure. While falls of systemic blood pressure were by 20 (10-35) and 26 (18-40) mmHg, those of RP were by 5.1 (2-10) and 8.3 (6-15) mmHg on intravenous 100 and 250 µg/kg of SIN-10, respectively. On the other hand, cRF increased in all animals except for one, in which cRF was not affected. The increases in cRF appeared several minutes after the injection and the maximum effect was attained in 10 to 20 minutes. In 3 of 4 animals the increases in cRF lasted for more than one hour. The heart rate tended to decrease slightly, but sometimes increased slightly only at the early stage of hypotension.

The effects of intravenous injection of 50 µg/kg of SIN-1A are illustrated in Fig. 5. The systemic blood pressure decreased abruptly by more than 40 mmHg in systole, while RP did by only around 20 mmHg. The increase in RF was observed even while the systemic blood pressure was significantly lowered, and thus the increase of cRF was definite. Intravenous 100 µg/kg of SIN-1 showed the similar changes in these parameters, but the hypotension occurred more rapidly than with SIN-10 and lasted much longer than with SIN-1A. Increase in RF was found even during the hypotension.

DISCUSSION

Nitrites are the oldest and still the most important antianginal agents since Lauder Brunton introduced their availability in the clinical medicine (12). Extensive efforts have been made to develope new, ideal antianginal agents. The estimation of antianginal activity in the laboratory experiment has been carried out in terms of coronary vasodilator activity in vitro and in vivo in normal hearts. However, question still remains to be settled whether the coronary vasodilator activity is an essential prerequisite for the manifestation of the antianginal action (9, 13-15), and measurement of total coronary flow may be somewhat misleading until more is known regarding the types of vessels primarily involved in dilatation by agents. It was proposed to reevaluate experimentally the antianginal agent from several standpoints besides the effect on total coronary flow in anesthetized normal animals (9). In the present study, the authors brought to a focus on the modification of hemodynamic behaviour of coronary collateral circulation by sydnonimine derivatives. The collateral vessels in the heart with acute coronary insufficiency were described to be relatively refractory to the pharmacological agents (16), and effects of agents were studied in chronically ischemic hearts in the present study.

In normal dog hearts, the magnitude of the collateral circulation was different from animal to animal, and was generally sparse. And following occlusion of the anterior descending branch of the left coronary artery, systemic blood pressure decreased, and various degrees of ventricular arrhythmia inversely related to the magnitude of the collateral circulation were observed (17). In the hearts subjected to the experiment in the present study, the values of RP and RF were not only higher than those in normal hearts, but also there was no change in systemic blood pressure and ECG patterns after occlusion of the constricted artery. The macroscopic examination of plastic cast of coronary trees
of the hearts revealed presence of the collateral vessels of more than 500 \mu in diameter between anterior descending and circumflex branches of the left coronary artery. Therefore, the coronary insufficiency seemed to be compensated by abundant development of the collateral circulation.

Hemodynamic behaviour of the coronary collateral circulation in response to agents were evaluated with changes in RP, RF and cRF. These parameters are primarily determined by magnitude of the collateral circulation, and dependent on aortic blood pressure (16). Furthermore, RP is the result of the interaction of aortic perfusion pressure, which was substituted by the systemic blood pressure in the present study, the collateral blood flow to be delivered to the ischemic area and total resistance which consisted of the vascular resistances anatomically arranged in series of the conductive vessels leading to the collateral vessels, of the total collateral vessels and of the peripheral vessels in the ischemic area. Therefore, only RP does not necessarily give the informations about the hemodynamic behaviour of the collateral circulation. The blood flow to be delivered through the collateral vessels to the ischemic area can not be directly determined. In the present study, RF was taken as the estimate of the collateral blood flow.

SIN-1 is relatively unstable in weak alkaline solution and decomposition ensues to yield SIN-1A (18). The increased RF by SIN-1A and SIN-1 even at the stage of hypotension suggests that the agents dilate primarily the coronary collateral vessels. SIN-10, N-acylated and chemically stable derivative of SIN-1, showed gradually developed and prolonged hypotension which was characterized by decrease in pulse pressure resulting from more depression during systole than during diastole. In constant flow perfusion experiments, SIN-10 decreased slightly the perfusion pressure of the coronary artery, but decreased the total coronary inflow in association with the fall of systemic blood pressure in autoperfusion experiments (5). In the present study, RP was not affected in spite of the hypotension by intravenous 100 and 250 \mu g/kg of SIN-10, although RF did not consistently increase. The relative unchangeableness of RP in spite of hypotension and the increased cRF reflect the coronary collateral vasodilator action of the agent. The results, therefore, suggest that SIN-10 has an ability to promote the blood redistribution toward the ischemic area with coronary insufficiency. Although coronary collateral vasodilator activity has not yet been conclusively disclosed of all the antianginal agents, it is interesting to know whether or not the hemodynamic effects of SIN-10, especially the collateral vasodilator activity, are favorable for the manifestation of therapeutic activity in angina pectoris.

SUMMARY

The effects of SIN-10 and related compounds on the coronary collateral circulation were studied in the heart with chronic coronary insufficiency in dogs. Chronic coronary insufficiency was produced experimentally by constriction of the left anterior descending coronary artery. The magnitude and the changes in collateral circulation were estimated by measurement of retrograde pressure (RP) and retrograde flow (RF) from the insufficient coronary artery.
On intravenous administration of SIN-10 (100–250 \( \mu g/kg \)), which had no increasing action of total coronary inflow by both intra-coronary and intravenous routes in normal hearts, RP was not affected in spite of hypotension and cRF, which was the RF value divided by systemic blood pressure and multiplied by 100, increased in the hearts with chronic coronary insufficiency. The results suggest that the fall of perfusion pressure due to hypotension can be compensated enough to promote the blood redistribution toward the area with coronary insufficiency. SIN-1A (50 \( \mu g/kg \) i.v.) and SIN-1 (100 \( \mu g/kg \) i.v.) had qualitatively similar, but more rapid developing cardiovascular actions than SIN-10 in insufficient hearts.

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