Rabbit Hearts for the Critical Evaluation of Drugs to Reduce the Size of Experimentally Produced Acute Myocardial Infarction

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SUMMARY

Using more than 500 rabbits, we found that the rabbit heart is a good model for the evaluation of drugs which affect acute myocardial infarction (AMI) size. When the ratio of the epicardial coloration area to the long axis length of the left ventricle was controlled immediately after the ligation of the left anterior descending coronary artery and small branches of the left circumflex artery, it was possible to estimate the size of the ischemic region because AMI region in rabbit heart was always transmural. The necrotic region in the left ventricle was determined by phosphorylase histochemistry 24 hours after the operation. The incidence of arrhythmia and death following the operation was negligible. Then, we evaluated several drugs to examine their effects on AMI size. Propranolol (1, 2, and 4 mg/Kg) and verapamil (0.25, 0.5, and 1.0 mg/Kg) reduced AMI size, although the mortality and AMI size increased at higher doses of verapamil. Another Ca\textsuperscript{2+} antagonist, diltiazem (2 mg/Kg) and an adenosine potentiator, dilazep (2 mg/Kg) also decreased AMI size, while nicardipine, a water soluble, photoresistant nifedipine analogue (0.01, 0.05, and 0.1 mg/Kg) did not show a significant effect. These data suggest that this rabbit model is useful for assessing drug effects on AMI size and that the mechanism(s) of action of nicardipine may differ from other Ca\textsuperscript{2+} antagonists.
Additional Indexing Words:
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THE prevention of heart failure in patients with acute myocardial infarction (AMI) has become even a more important consideration than the management of arrhythmias. Experimental trials to prolong tissue viability until an occluded coronary artery is recanalized or additional collateral channels develop have been performed in a dog AMI model system. The administration of a beta blocker, a Ca\(^{2+}\) antagonist, and a coronary dilator have been reported to reduce ischemic injury.

For the screening of drugs which might be beneficial for the salvage of ischemic myocardium, both the production of a constant mass of ischemic tissue and the exact quantification of AMI size are essential. Dog heart is not suitable for this, because the distribution of coronary arteries is so variable among dogs. In this study we have established simple and reliable methods for these purposes using the rabbit. This paper describes several aspects of the rabbit model that are superior to other experimental AMI preparations and confirms the beneficial effects of propranolol and verapamil in reducing AMI size. We also present data regarding the effects of dilazep, an adenosine potentiator and possible Ca\(^{2+}\) channel blocker, and nicardipine, a photo-resistant nifedipine analogue, on the extent of AMI.

Materials and Methods

Production of a constant mass of ischemic myocardium:
Male albino rabbits weighing 1.6 to 2.1 Kg were anesthetized with sodium pentobarbital (35 mg/Kg, i.v.). A left thoracotomy was performed under positive pressure respiration using a Harvard respirator. According to the degree of the development of the left anterior descending coronary artery (LAD) three ligation procedures were employed: I. In animals with a well developed LAD (about 20% of the animals), the LAD was ligated 4 to 5 mm distal to the origin of the left coronary orifice, immediately distal to the first diagonal artery, with #6-0 nylon thread. Immediately after the ligation, cyanotic coloration was clearly visible and the border between the normal and ischemic zones was traced on a plastic film. The area was measured by planimetry, and divided by the distance from the atroventricular groove to the left ventricle apex (L in Fig. 1-A). To normalize the cyanotic area in relation to the whole left ventricle area, a coefficient was obtained by dividing the cyanotic area by L. Usually this coefficient was between 0.75 and 0.8. II.
Fig. 1. Production of a constant mass of ischemic myocardium by coronary artery ligation in the rabbit. The epicardial coloration area shown by the broken line in the epicardium was measured by planimetry after tracing on a plastic film and divided by the distance from atrioventricular groove to the left ventricle apex in diastole (L). The resultant coefficient was adjusted to between 0.75 and 0.8. According to the degree of left anterior descending coronary artery development, the rabbit hearts were classified into 3 subgroups: well developed (A), poorly developed (B), and undeveloped (C).

![Diagram showing A, B, and C subgroups of heart with ischemic area marked by broken line and measurement of epicardial coloration area](image)

Protocols for drug administration:

- **Propranolol (Inderal®, Sumitomo Chemicals)**: 1/2 of total dose
- **Dilazep (Komelian®, Kowa)**: 1/8 of total dose
- **Diltiazem (Herbesser®, Tanabe)**: 1/5 of total dose
- **Verapamil (Vasoran®, Eisai)**: 2/5 of total dose
- **Nicardipine (Perdipine®, Yamanouchi)**: Sacrifice

Fig. 2. Protocol for drug administration.

In rabbits with a poorly developed LAD (about 75% of the animals), a few branches of the left circumflex artery (LCX) supplying the ischemic area were ligated as well as the LAD. The number of ligations was selected to adjust the index described above to the same coefficient value, i.e. 0.75 to 0.8 (Fig. 1-B). III. When the LAD was not present (about 5% of cases), several branches of the LCX supplying the anterior surface of the left ventricle were ligated, keeping the index at a constant level (Fig. 1-C). After practicing on about 20 animals, estimation of the ischemic area became very consistent and accurate, although the exact area was still determined as described earlier.

Protocol of drug administration:

Propranolol (Inderal®, Sumitomo Chemicals), verapamil (Vasoran®, Eisai), dilazep (Komelian®, Kowa), diltiazem (Herbesser®, Tanabe), and nicardipine (Perdipine®, Yamanouchi) were dissolved in physiological saline (10 ml). As summarized in Fig. 2, one half of the total dose of all drugs ex-
cept verapamil was administered intravenously 5 min before the ligation, 1/4 was infused for 1 hour after the ligation and 1/8 was given at 2 and 3 hours after the ligation. In the case of verapamil, the protocol was modified, because of its powerful negative inotropic action. In the control group, physiological saline was administered.

Quantification of infarction size:
Rabbits were anesthetized again with sodium pentobarbital (35 mg/Kg) 24 hours after coronary ligation and sacrificed. The heart was removed, washed with saline and frozen at $-20^\circ$C for 30 min to make it easy to slice. Two mm thick slices were obtained parallel to the atrioventricular groove. One sample for histochemistry using the phosphorylase reaction was prepared from one slice, and another for the usual histological study using haematoxylin-eosin staining. After staining, slices were photographed on color film (Fig. 3). The infarction size was determined by measuring the infarcted area and whole ventricular area, including the interventricular septum, by planimetry (Konton Messgerate®). After adding the area of all slices, infarction size was expressed as the percentage of the left ventricular size.

Miscellaneous:
Statistical analysis was performed by Student's unpaired t test and p values less than 0.05 were considered to be the statistically significant difference
of AMI size, compared with the control study.

**Results**

*Production of constant mass of myocardial ischemia:*

Compared with dog coronaries, the distribution and the length of the left anterior descending coronary artery (LAD) of rabbits are less variable. However, they are not constant (Fig. 1). Immediately after LAD ligation the epicardial surface of unperfused region became cyanotic and was clearly demarcated from the control region. By measuring the cyanotic area with planimetry, coronary arteries were ligated to equalize the ratio of cyanotic area to the length of the left ventricle. Arrhythmias did not occur, and all rabbits survived the operation.

Twenty-four hours after the operation, rabbits were sacrificed and the heart was removed, sliced and the infarct area in each slice was clearly distinguished from the intact myocardium using the phosphorylase reaction (Fig. 3). Intact myocardium stained purple with iodine, while the necrotic area was unstained. This histological method was simpler and more sensitive than
triphenyl tetrazolium chloride staining, and the unstained area was in good agreement with the haematoxylin eosin stain (data not shown).

AMI size was determined from 47 rabbits in 4 groups of trials. Each experiment was performed on a different day. AMI size after saline infusion ranged from 14 to 15% of the left ventricular volume, and showed good reproducibility (Fig. 4). To examine this method of evaluating drug effectiveness in reducing AMI size, drugs which have been reported to be effective for AMI size reduction in other animals were administered, as well as newly developed drugs.

**Effect of beta antagonist and Ca^{2+} channel blockers on AMI size:**

After drug administration, heart coloration and arrhythmias were assessed. Propranolol (4 mg/Kg), diltiazem (2 mg/Kg), and verapamil (0.5 and 1.0 mg/Kg) induced an enlargement of the left ventricle volume. The negative inotropic action caused by verapamil was especially prominent: one animal in 8 and 2 in 6 died within 20 min after the drug administration by 0.5 and 1.0 mg/Kg, respectively (Fig. 5). Dilazep is reported to be a powerful adenosine potentiator and may have a Ca^{2+} channel blocking action. This drug (2 mg/Kg), diltiazem (2 mg/Kg), nicardipine, a water soluble and photoresistant nifedipine analogue at 0.01, 0.05, and 0.1 mg/Kg and verapamil (0.01 mg/Kg) changed the epicardial color to bright red, although the exact measurement of the coronary dilating action was not re-
Fig. 6. Effect of nicardipine, a photoresistant, water soluble nifedipine analogue that is a powerful coronary dilator. Note that nicardipine administration (0.01, 0.05, and 0.1 mg/Kg) did not significantly reduce AMI size, even when prominent coronary dilatation was demonstrated.

AMI size in this series was 14.8±0.5% (S.E.M.) of the left ventricle muscle volume in the control group. Propranolol at 1.0, 2.0, and 4.0 mg/Kg reduced the AMI size by 7.4, 26.4 (p<0.05), and 27.7% (p<0.05) from the control group, respectively (Fig. 5), demonstrating clear dose dependency. Both dilazep and diltiazem (2 mg/Kg) also showed a significant decrease by 17.6 and 19.2% (p<0.05), respectively. In contrast, verapamil induced a 33.1% reduction (p<0.01) at 0.25 mg/Kg, but the AMI size increased at higher doses, showing 18.9 and 5.4% reduction at 0.5 and 1.0 mg/Kg, respectively. This may result from a cardiac inhibitory action of the drug.

Among Ca²⁺ antagonists, nifedipine has been reported to be the most potent coronary dilator. However, it is inconvenient to administer because of its water insolubility and lability to light. Thus, we utilized its analogue, nicardipine. The drug induced a marked coronary dilating action at the...
doses employed (0.01, 0.05, and 0.1 mg/Kg) but did not alter the AMI size (Fig. 6).

DISCUSSION

Prevention of heart failure is one of the most important objectives in the therapy of AMI, and the prognosis of AMI patients has been shown to be dependent on AMI size. Many investigations have been directed to limit AMI size, including drug therapy using a beta blocker, a Ca$^{2+}$ antagonist or coronary dilators. However, some of these drugs are not directly applicable to humans, both because they might cause a negative inotropic action on the heart, and because the doses used in the animal model are harmful to man.

In this study, we tried to establish an animal model of AMI for drug screening. Rabbits were useful for the following reasons. They are easy to handle and the heart size is moderate. Arrhythmias and death after coronary occlusion are minimal in rabbits, compared with other species. The ischemic region in rabbit is transmural (Fig. 2), so that cyanotic coloration is more marked than in dogs. As mentioned earlier, the distribution, size and length of the coronary arteries are less variable in rabbits than in dogs.

When the LAD artery alone was ligated, the infarcted mass in the left ventricular muscle ranged from 4 to 25%. However, when small branches of the LCX artery were ligated in addition to the LAD artery, a constant AMI size was obtained. A histochemical method using the phosphorylase reaction has been demonstrated to be sensitive and was reported to show clear demarcation of AMI area as early as 3 hours after the coronary ligation. The time required for staining and measurement of AMI size could be shortened compared with other histological methods.

Previously, we have presented the hypothesis that Ca$^{2+}$ entry exceeding a physiological level may activate calcium-activated neutral protease. This protease is included in the cytosol of muscle cells and degrades phosphorylase kinase, troponin, tropomyosin, and eu-actinin. These proteins are very important in glycogen metabolism, the regulation of muscle contraction and relaxation, and the preservation of highly organized sarcomere structure. Thus, the degradation of these proteins by the protease may cause an irreversible loss of contractility. Using tissue cultured cardiac myocytes, Ca$^{2+}$ entry preceded myocardial cell destruction under hypoxic and glucose-depleted conditions (T. Toyo-oka et al., submitted). Verapamil blocked the Ca$^{2+}$ entry as well as myocardial cell degradation under these conditions (T. Toyo-oka et al., submitted). The tissue culture did not con-
tain a vascular system and the effects of coronary flow could be neglected. These data and the present beneficial effect of some, and not all, Ca\textsuperscript{2+} channel blockers to reduce AMI size are consistent with the scheme described above. Furthermore, a synthetic protease inhibitor of calcium-activated neutral protease also reduced AMI size.\textsuperscript{19)}

The present study suggests the new methods using rabbit heart and obtaining a constant surface area of cyanotic epicardial coloration are useful for the evaluation drug effects on the extent of AMI and that the effects of nикаrpidine are different from other Ca\textsuperscript{2+} antagonists.

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