Effects of Radical Scavengers, TA248 and TA276, on Stunned Myocardium in Dogs: Involvement of K\textsubscript{ATP} Channels

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ABSTRACT—TA248 (7-((\beta-D-glucopyranosyloxy)-4-hydroxy-3-octyloxy-2H-1-benzopyran-2-one) and TA276 (sodium 7-hydroxy-3-octyloxy-2H-1-benzopyran-2-one-4-oxide) were newly developed as radical scavengers. In vitro, TA276 scavenged both superoxide anions (\cdot O\textsubscript{2}\textsuperscript{-}) and hydroxyl radicals (\cdot OH). TA248 also trapped \cdot O\textsubscript{2}\textsuperscript{-}, but had less activity on \cdot OH. In vivo, left ventricular contractile functions were determined in pentobarbital-anesthetized open-chest dogs. A regional portion of the left ventricular wall was made ischemic for 20 min by ligating the left anterior descending coronary artery and then reperfused for 60 min. TA248 (3 mg/kg) and TA276 (3 mg/kg) injected i.v. 10 min before occlusion significantly improved myocardial stunning that is contractile dysfunction observed after reperfusion following brief ischemia. Glibenclamide (1 mg/kg) injected i.v. 20 min before occlusion significantly worsened the myocardial stunning. Pretreatment with glibenclamide completely abolished the beneficial effect of TA276 on myocardial stunning, whereas it only partially attenuated that of TA248, showing some improvement even in the presence of glibenclamide. Because of the incomplete scavenging activity of TA248, residual \cdot OH may play some roles in improvement of myocardial stunning with TA248 in the presence of glibenclamide. We speculate that the \cdot OH may eject glibenclamide from its binding site on K\textsubscript{ATP} channels, leading to opening of the channels.

Keywords: K\textsubscript{ATP} channel, Myocardial stunning, Radical scavenger

Oxygen radicals, such as superoxide anion (\cdot O\textsubscript{2}\textsuperscript{-}), hydroxyl radical (\cdot OH), and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) are involved in the pathogenesis of various tissue injuries, including ischemic heart diseases (1). Radical scavengers eliminate reactive oxygen species and protect the tissue from their harmful effects. However, several reports have shown the beneficial effects of low concentrations of hydrogen peroxide or a small amount of free radicals on the ischemic myocardium (2 – 5). Tokube et al. (2) and Yao et al. (5) have demonstrated that opening of cardiac ATP sensitive potassium (K\textsubscript{ATP}) channels is involved in the protection of ischemic myocardium by reactive oxygen species. K\textsubscript{ATP} channels have been identified in various organs and tissues, including cardiac muscle (6), pancreatic \beta cells (7), and vascular smooth muscle (8). The intracellular level of ATP primarily modulates opening of K\textsubscript{ATP} channels (6). In the heart, ischemia causes activation of the K\textsubscript{ATP} channel due to reduction of the tissue ATP level in the myocardium and coronary smooth muscle (9). Because the activation or opening of K\textsubscript{ATP} channels causes hyperpolarization of the cell and then reduces Ca\textsuperscript{2+} influx through the voltage-dependent Ca\textsuperscript{2+} channels, it decreases myocardial contractility and dilates the coronary artery (10, 11). The former decreases myocardial energy demand and the latter increases energy supply. Therefore, K\textsubscript{ATP} channels may be involved in the intrinsic mechanisms of myocardial protection during ischemia and reperfusion.

We (12) have synthesized novel antioxidants, TA248 (7-((\beta-D-glucopyranosyloxy)-4-hydroxy-3-octyloxy-2H-1-benzopyran-2-one) and TA276 (sodium 7-hydroxy-3-octyloxy-2H-1-benzopyran-2-one-4-oxide), and reported in dogs that these compounds improve myocardial stunning that is the contractile dysfunction during reperfusion following brief ischemia. In the present study, therefore, we examined the mechanisms by which TA248 and TA276 improved the myocardial stunning by the use of glibenclamide, an inhibitor of K\textsubscript{ATP} channels.
MATERIALS AND METHODS

This investigation conforms to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido College of Pharmacy (published in 2001).

$\cdot O_2^-$ and $\cdot OH$ scavenging activities of TA248 and TA276

The radical scavenging effects of TA248 and TA276 on $\cdot O_2^-$ and $\cdot OH$ generated in vitro were examined. For $\cdot O_2^-$ determination, TA compounds dissolved in 2% ethanol were added into a cuvette containing 5 μM 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA), 0.1 mM xanthine, and 0.08 μM catalase in 50 mM Tris-HCl buffer (pH 7.0). The intensity of CLA-dependent chemiluminescence was recorded for 4 min at 380 nm by a spectrofluorometer after addition of 0.01 unit/mL xanthine oxidase. For $\cdot OH$ determination, $\cdot OH$ generated by the Fenton reaction (13) was trapped by 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) that is a spin trap reagent. The Fenton reaction was initiated by addition of 9 μM FeSO$_4$ and 90 μM H$_2$O$_2$ into the mixture of 94.5 mM DMPO, TA compounds dissolved in 3% acetonitrile, and 100 mM potassium-phosphate buffer. After shaking, the mixture was transferred to an ESR cuvette and measured by an ESR spectrometer (JES-RE1X ESR spectrometer; JEOL, Ltd., Tokyo). We calculated the scavenging rate (% inhibition) of TA248 and TA276 on $\cdot O_2^-$ and $\cdot OH$.

Animal preparations

Forty-one healthy mongrel dogs of either sex weighing 14.3 ± 1.2 kg were used. Animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated into the trachea and ventilated with room air by using a respirator. After a left thoracotomy, the main trunk of the left anterior descending coronary artery was dissected free from the adjacent tissues and encircled with a silk thread ligature. The coronary flow was determined by a magnetic flow probe positioned proximal to the ligature. Myocardial contraction was assessed as segment shortening by sonomicrometry at the region that would be ischemic. The value of segment shortening was calculated as (DSL – SSL)/DSL, where DSL is the diastolic segment length, and SSL is systolic segment length (14, 15). The values obtained after TA compound injection were normalized to values obtained just before the injection (% segment shortening). Left ventricular pressure and its first derivative (LVPd/dt) were measured through a cannula inserted into the left ventricular cavity from the apex; data are not shown but used for determination of DSL and SSL. Arterial blood pressure and heart rate were also monitored.

Experimental protocols

After control observations were completed, either glibenclamide at 1 mg/kg or 0.1 ml/kg 100% dimethylsulfoxide (DMSO) as vehicle was injected i.v. from the left femoral vein over 30 s (the first injection). Ten minutes after the first injection, 3 mg/kg TA248, 3 mg/kg TA276 or 100% DMSO as vehicle for TA compounds was injected i.v. (the second injection). The dose used here was thought to be a submaximal dose to exert the beneficial effects of TA248 and TA276 on myocardial stunning in dogs (12). Then, 10 min after the second injection, the left anterior descending coronary artery was ligated with the ligature for 20 min and released for 60 min. A transmural full-thickness sample of the myocardium was removed from the region that had been ischemic 60 min after reperfusion. The myocardial samples were immediately frozen with clamps previously chilled in liquid N$_2$. The frozen sample was used for determination of the levels of ATP and lactate, according to standard enzymatic procedures (16), and determination of the level of thiobarbituric acid reactive substance (TBARS) (17).

The venous blood sample was taken from animals treated with glibenclamide before and 20 min after glibenclamide injection. The level of blood glucose was not affected by glibenclamide at the dosage used in the present study. DMSO, TA248 and TA276 did not alter the blood glucose level in the presence of glibenclamide.

Animal groups

Animals were divided into 6 groups; DMSO-treated (DMSO, n = 6), 3 mg/kg TA248-treated (TA248, n = 9), 3 mg/kg TA276-treated (TA276, n = 8), 1 mg/kg glibenclamide+DMSO-treated (glibenclamide+DMSO, n = 6), 1 mg/kg glibenclamide+3 mg/kg TA248-treated (glibenclamide+TA248, n = 6), and 1 mg/kg glibenclamide+3 mg/kg TA276-treated (glibenclamide+TA276, n = 6) groups. In the present study, 56 dogs were used. However, 15 out of 56 dogs died shortly after the onset of reperfusion because of ventricular fibrillation; 5 out of 11 dogs in the DMSO group, 2 out of 11 dogs in the TA248 group, 1 out of 9 dogs in the TA276 group, 3 out of 9 dogs in the glibenclamide+DMSO group, 3 out of 9 dogs in the glibenclamide+TA248 group, and 1 out of 7 dogs in the glibenclamide+TA276 group. There was no significant difference in incidence of ventricular fibrillation among groups.

Drugs

TA248 and TA276 were synthesized at the General Laboratories, Dainippon Ink and Chemicals, Inc. (Sakura). DMSO, glibenclamide, xanthine and xanthine oxidase were purchased from Sigma Chemicals (St. Louis, MO, USA). CLA and DMPO were from Tokyo Kasei Kogyou (Tokyo) and Labotec Co., Ltd. (Tokyo), respectively. Other chemicals used were of analytical or high-performance liquid
chromatography grade.

Statistical analyses

All values are means ± S.E.M. The significance of differences between groups was evaluated by one-way analysis of variance followed by the unpaired *t* test with Dunnett’s procedure. Difference within groups in myocardial segment function was compared by the paired Student’s *t* test. *P* values less than 0.05 were considered statistically significant.

RESULTS

Radical scavenging activities of TA248 and TA276

The scavenging rates (% inhibition) of TA248 and TA276 on ·O₂⁻ and ·OH generated in vitro are summarized in Table 1. Both TA248 and TA276 scavenged ·O₂⁻ in a dose-dependent manner. TA276 also eliminated ·OH in a dose-dependent manner. However, the scavenging effect of TA248 on ·OH was neither complete nor in a dose-dependent manner.

Myocardial stunning

Effect of TA248 and TA276 on myocardial stunning in the absence and presence of glibenclamide is shown in Figs. 1 and Fig. 2, respectively. In the DMSO group, % segment shortening decreased significantly and fell below 0% during ischemia, indicating bulging. Reperfusion returned the % segment shortening that had been decreased by ischemia toward the pre-ischemic level, but the recovery was incomplete (stunning phenomenon). TA248 and TA276 significantly increased the recovery of myocardial contractile dysfunction during reperfusion after ischemia. In the glibenclamide+DMSO groups, glibenclamide did not modify the % segment shortening before ischemia. The % segment shortening during ischemia appeared to be low as compared to that in the DMSO group, although the difference was not significant. During reperfusion, the myocardial contractile dysfunction in the presence of glibenclamide significantly worsened as compared to that in the absence of glibenclamide. The protective effect of TA276 on the stunned myocardium was completely abolished by glibenclamide (Fig. 2). Recovery of % segment shortening during reperfusion in the glibenclamide+TA276 group was equivalent to that in the glibenclamide+DMSO group. Glibenclamide also attenuated the recovery of % segment shortening in the glibenclamide+TA248 group as compared to that in the TA248 group (Fig. 1). However, recovery of myocardial % segment shortening during reperfusion in the glibenclamide+TA248 group was greater than that in the glibenclamide+DMSO group.

Table 1. Inhibitory effects of TA248 and TA276 on appearance of chemiluminescence due to ·O₂⁻ and ESR spectra due to ·OH

| Concentration | TA248 | TA276 | TA248 | TA276 |
|---------------|-------|-------|-------|-------|
| Solvent       | 0     | 0     | 0     | 0     |
| 0.03 mM       | 9.7 ± 3.4 | 14.6 ± 3.1 | –     | –     |
| 0.1 mM        | 35.7 ± 2.8 | 49.6 ± 2.0 | 9.8 ± 2.9 | 1.9 ± 2.1 |
| 0.3 mM        | 60.9 ± 2.1 | 76.8 ± 1.4 | 34.4 ± 1.9 | 11.1 ± 2.9 |
| 1.0 mM        | 85.3 ± 0.8 | 94.0 ± 0.5 | 51.4 ± 1.5 | 21.2 ± 2.9 |
| 3.0 mM        | –     | –     | 44.9 ± 1.9 | 50.8 ± 1.7 |
| 10.0 mM       | –     | –     | 41.8 ± 2.3 | 80.6 ± 1.3 |

Data are expressed as % inhibition (means ± S.E.M. of 8 – 10 observations).

Other hemodynamics

Changes in systolic and diastolic blood pressures, heart rate, and coronary flow throughout the experiment are summarized in Table 2. DMSO, TA248 and TA276 in the presence and absence of glibenclamide did not affect blood pressures and heart rate before ischemia, during ischemia and after reperfusion. Coronary flow was decreased by ischemia to 0 mL/min and then returned beyond the pre-ischemic level 5 min after the onset of reperfusion, indicating reactive hyperemia. The reactive hyperemia in the glibenclamide+DMSO and glibenclamide+TA276 groups was significantly attenuated as compared to that in the DMSO group. However, there was no significant difference in the coronary flow 60 min after reperfusion between the groups.

Metabolic parameters

The tissue levels of ATP, lactate and TBARS in the 60-min reperfused myocardium are shown in Table 3. The levels of ATP in the TA248 and TA276 groups appeared to be higher than that in the DMSO group, although there was no significant difference between them. The level of TBARS in the TA276 group was significantly lower than that in the DMSO group. There was no difference in the lactate level between groups. Glibenclamide alone did not affect the levels of ATP and lactate. In the presence of glibenclamide, TA248 appeared to increase the ATP level, whereas it significantly decreased the lactate level as compared to the values in the glibenclamide+DMSO group. TA276 did not change the ATP and lactate levels appreciably. The levels of TBARS in the glibenclamide+TA248 and glibenclamide+TA276 groups were significantly higher than the respective values in the TA248 and TA276 groups.
Fig. 1. Effect of TA248 on myocardial contraction before and during ischemia and after reperfusion in the presence and absence of glibenclamide. First, either DMSO (left panel) or glibenclamide (1 mg/kg) (right panel) was injected i.v. After 10 min of the first injection, DMSO (circle) or TA248 (3 mg/kg, triangle) was injected i.v. Ischemia was induced by ligating the left anterior descending coronary artery 10 min after the second injection. After 20 min of ischemia, the ligated coronary artery was released, so that ischemic myocardium was reperfused for 60 min. *P<0.05, compared with the DMSO group in each panel. †P<0.05, compared with the DMSO group in the left panel.

Fig. 2. Effect of TA276 on myocardial contraction before and during ischemia and after reperfusion in the presence and absence of glibenclamide. Experimental protocol is the same as Fig. 1. Ten minutes after DMSO (left panel) or glibenclamide (right panel) injection, DMSO (circle) or TA276 (3 mg/kg, square) was injected i.v. *P<0.05, compared with the DMSO group. †P<0.05, compared with the DMSO group in the left panel.
Table 2. Hemodynamic data obtained before (Base) and 10 min after glibenclamide injection, 10 min after TA compound injection, 20 min after ischemia, and 5 and 60 min after reperfusion

| Treatment | Parameter | Base | Glibenclamide injection | TA compound injection | Ischemia | 5 min after reperfusion | 60 min after reperfusion |
|-----------|-----------|------|--------------------------|-----------------------|----------|------------------------|------------------------|
| DMSO (n = 6) | SP (mmHg) | 166 ± 19 | – | 171 ± 16 | 165 ± 16 | 168 ± 16 | 161 ± 15 |
| | DP (mmHg) | 112 ± 16 | – | 120 ± 15 | 117 ± 15 | 118 ± 15 | 113 ± 13 |
| | HR (beats/min) | 166 ± 11 | – | 155 ± 8 | 160 ± 11 | 158 ± 10 | 160 ± 12 |
| | CF (mL/min) | 13 ± 4 | – | 13 ± 3 | 0 | 39 ± 9 | 13 ± 3 |
| TA248 (n = 9) | SP (mmHg) | 139 ± 7 | – | 144 ± 7 | 139 ± 8 | 139 ± 8 | 145 ± 7 |
| | DP (mmHg) | 103 ± 7 | – | 110 ± 8 | 107 ± 8 | 107 ± 9 | 111 ± 7 |
| | HR (beats/min) | 167 ± 13 | – | 163 ± 12 | 165 ± 12 | 164 ± 11 | 166 ± 10 |
| | CF (mL/min) | 13 ± 3 | – | 13 ± 3 | 0 | 31 ± 6 | 12 ± 3 |
| Glibenclamide+DMSO (n = 6) | SP (mmHg) | 162 ± 5 | – | 171 ± 6 | 165 ± 5 | 164 ± 6 | 164 ± 8 |
| | DP (mmHg) | 122 ± 6 | – | 131 ± 6 | 126 ± 6 | 127 ± 6 | 127 ± 7 |
| | HR (beats/min) | 173 ± 12 | – | 167 ± 11 | 166 ± 11 | 165 ± 11 | 168 ± 11 |
| | CF (mL/min) | 13 ± 2 | – | 13 ± 2 | 0 | 27 ± 4 | 13 ± 2 |
| Glibenclamide+TA248 (n = 6) | SP (mmHg) | 159 ± 4 | 164 ± 5 | 161 ± 6 | 165 ± 7 | 169 ± 7 | 162 ± 4 |
| | DP (mmHg) | 116 ± 8 | 125 ± 6 | 125 ± 6 | 124 ± 5 | 121 ± 7 | 122 ± 4 |
| | HR (beats/min) | 185 ± 16 | 180 ± 16 | 178 ± 16 | 181 ± 16 | 178 ± 15 | 182 ± 17 |
| | CF (mL/min) | 8 ± 1 | 7 ± 1 | 8 ± 1 | 0 | 18 ± 4* | 7 ± 1 |
| Glibenclamide+TA276 (n = 6) | SP (mmHg) | 151 ± 14 | 152 ± 10 | 154 ± 10 | 148 ± 11 | 149 ± 10 | 148 ± 10 |
| | DP (mmHg) | 109 ± 10 | 118 ± 8 | 119 ± 9 | 113 ± 9 | 108 ± 9 | 113 ± 8 |
| | HR (beats/min) | 158 ± 10 | 147 ± 10 | 148 ± 10 | 153 ± 11 | 162 ± 13 | 157 ± 10 |
| | CF (mL/min) | 11 ± 3 | 9 ± 2 | 10 ± 2 | 0 | 24 ± 5 | 10 ± 2 |

All values are means ± S.E.M. n, the number of animals; DMSO, dimethylsulfoxide; SP, systolic blood pressure; DP, diastolic blood pressure; HR, heart rate; CF, coronary flow. *P<0.05, compared to the value in the DMSO group.

Table 3. Metabolic data in the 60-min reperfused myocardium following 20-min ischemia

| Treatment | n | ATP μmol/g | Lactate μmol/g | Thiobarbituric acid reactive substance nmol malondialdehyde/mg protein |
|-----------|---|------------|---------------|---------------------------------------------------------------|
| DMSO      | 6 | 3.10 ± 0.20 | 1.03 ± 0.18 | 0.20 ± 0.02 |
| TA248     | 9 | 3.72 ± 0.27 | 1.34 ± 0.28 | 0.18 ± 0.04 |
| TA276     | 8 | 3.48 ± 0.32 | 0.84 ± 0.07 | 0.13 ± 0.01* |
| Glibenclamide+DMSO | 6 | 3.40 ± 0.34 | 1.51 ± 0.36 | 0.27 ± 0.05 |
| Glibenclamide+TA248 | 6 | 3.84 ± 0.25 | 0.60 ± 0.10* | 0.29 ± 0.04* |
| Glibenclamide+TA276 | 6 | 3.16 ± 0.39 | 1.71 ± 0.98 | 0.24 ± 0.03* |

All values are means ± S.E.M. n, the number of animals; ATP, adenosine triphosphate; DMSO, dimethylsulfoxide. *P<0.05, compared with the corresponding DMSO group. **P<0.05, compared with the corresponding TA compound group.
DISCUSSION

It is believed that the more oxygen radicals are trapped, the better effects of the scavenger are expected (18–21). However, some investigators have reported the protective effects of ·OH and $\text{H}_2\text{O}_2$ at the $\mu\text{M}$ concentration on the ischemic myocardium (2, 3). That this controversy exists is puzzling to us. Tokube et al. (22) have demonstrated that ·OH competes with glibenclamide for binding to the K$_{\text{ATP}}$ channel. The K$_{\text{ATP}}$ channel is known as one of the intrinsic mechanisms to protect the myocardium against ischemic insults (23, 24). In fact, when K$_{\text{ATP}}$ channels were blocked by glibenclamide, % segment shortening worsened during ischemia and reperfusion (Figs. 1 and 2), and reactive hyperemia observed 5 min after reperfusion reduced (Table 2).

We confirmed that TA248 and TA276 improved the myocardial stunning that is contractile dysfunction observed during reperfusion after brief ischemia in dogs. Because pretreatment with glibenclamide attenuated the effects of TA248 and TA276 on myocardial stunning (Figs. 1 and 2), opening of the K$_{\text{ATP}}$ channel may be involved in their effects. TA248 and TA276 may open more K$_{\text{ATP}}$ channels during ischemia/reperfusion. However, the extent to which glibenclamide attenuated the effect of TA276 on stunned myocardium was different from that of TA248. The recovery of % segment shortening during reperfusion in the glibenclamide+TA276 group was the same level as that in the glibenclamide+DMSO group (Fig. 2), whereas the recovery in the glibenclamide+TA248 group was higher than that in the glibenclamide+DMSO group (Fig. 2). Even in the presence of glibenclamide, TA248 still showed an ability to improve the myocardial stunning when compared to that in the glibenclamide+DMSO group.

As shown in Table 1, TA248 scavenges ·OH partially, whereas TA276 completely scavenges ·OH in vitro. Both TA compounds scavenge ·O$_2^-$ to the same extent. Scavenging activity of TA248 for ·OH was less than that of TA276. As a result, the level of TBARS was significantly lowered by TA276, but not by TA248 in the myocardium in vivo (Table 3). Taking the radical scavenging activity of TA248 into account, ·OH at a certain low concentration that remains in the myocardium may prevent the binding of glibenclamide to K$_{\text{ATP}}$ channels. Therefore, a part of the K$_{\text{ATP}}$ channels of the myocardium may be able to open even in the presence of glibenclamide. On the other hand, K$_{\text{ATP}}$ channels are completely blocked by glibenclamide when ·OH is exhausted by treatment with TA276. The net effect of TA248 and TA276 on myocardial stunning may be based on the balance between protection due to opening of K$_{\text{ATP}}$ channels and deterioration due to ·OH. In the case of the glibenclamide+DMSO group, because a large amount of oxygen radicals is extremely harmful for the heart, pre-vention of the glibenclamide-K$_{\text{ATP}}$ channel binding with ·OH may be masked. A schematic illustration is shown in Fig. 3. However, the blood concentration of TA276 in vivo may not be high enough to exhaust ·OH from the myocardial tissue. According to a simple calculation, the dose of 3 mg/kg could be equivalent to 0.08 mM for TA248 and 0.12 mM for TA276 in blood.

A group in France has isolated and characterized endo-

![Fig. 3. Role of hydroxyl radical (·OH) in ischemic dog myocardium. Ischemic myocardial damage appears as a net balance between the harmful effect of radicals and the protective effect of hyperpolarization (upper panel). Glibenclamide (Gli) reduces K$^+$ conductance, leading to cell depolarization. Increase in the excitability may injure the myocardial cell, particularly under ischemic conditions. For example, Ca$^{2+}$ influx through the voltage-dependent Ca$^{2+}$ channel increases. Excess intracellular Ca$^{2+}$ may damage the ischemic myocardial cell. ·OH may inhibit glibenclamide-K$_{\text{ATP}}$ channel binding at least in part (middle panel). TA276 completely scavenges both ·OH and ·O$_2^-$. TA276 can abolish deleterious effects of radicals, while it also cancels the inhibition of glibenclamide-K$_{\text{ATP}}$ channel binding because of lack of ·OH (lower left panel). Although a small amount of ·OH that remains may be harmful to the TA248-treated heart, ·OH inhibits the glibenclamide-K$_{\text{ATP}}$ channel binding, opens K$_{\text{ATP}}$ channels, and causes myocardial protection due to hyperpolarization (lower right panel).](image-url)
sulfine, an endogenous ligand for the sulfonylurea receptor, in the pancreas and brain but not in the myocardium yet (25–28). This finding suggests that an endogenous glibenclamide-like substance exists under physiological conditions. Our above discussion should be extended to the conditions without glibenclamide. •OH at a certain concentration may interfere with the binding of endosulfine to K\textsubscript{ATP} channels. This speculation could provide the answer to a controversial question. Although many explanations have been proposed (29, 30), there is still a discrepancy in the concentration of ATP at which K\textsubscript{ATP} channels open between in vitro and in vivo experiments. The K\textsubscript{ATP} channel can open under a \textmu M concentration of ATP in vitro (6), whereas opening this channel in vivo might occur even at mM ATP (30). The myocardial level of ATP is decreased by ischemia, but still determined to be at a mM concentration (30). Even when the ATP level is high, •OH derived by ischemia pulls an endogenous ligand apart from K\textsubscript{ATP} channels, leading to opening of the channels. This hypothesis may also explain the fact that oxygen-derived free radicals augment the activation of K\textsubscript{ATP} channels induced by levercomakalim, a K\textsubscript{ATP} channel opener in vascular smooth muscle (31). The K\textsubscript{ATP} channels may open when the concentration of •OH is kept at an optimal level to prevent endosulfine-K\textsubscript{ATP} channel binding.

We failed to show a significant correlation between effects of TA compounds on mechanical function and metabolic function, although there were some tendencies; the level of ATP appeared to be high when TA compounds increased the recovery of myocardial contractile function; the level of TBARS was low in the myocardium when TA276 was used; and the level of TBARS did not change when glibenclamide attenuated the effects of TA compounds. Because TA276 did not show any improvement of contractile function during reperfusion in the presence of glibenclamide, the level of ATP was low, and the level of lactate was high as compared to those in the glibenclamide+TA248 group.

Glibenclamide at 1 mg/kg did not change the blood glucose level: 96 ± 19 to 108 ± 26 mg/dL in the DMSO group, 68 ± 9 to 67 ± 6 mg/dL in the glibenclamide+DMSO group, 81 ± 10 to 74 ± 11 mg/dL in the glibenclamide+TA248 group, and 116 ± 17 to 117 ± 21 mg/dL in the glibenclamide+TA276 group. We observed in similar experimental dogs that glibenclamide at 0.3 and 3 mg/kg significantly decreased the blood glucose level 45 min after the injection (30). Because the level of blood glucose in the present study was determined at 20 min after the injection, it would take a longer period to detect the glibenclamide-induced hypoglycemia.

In conclusion, K\textsubscript{ATP} channels could be involved, at least in part, in improvement of contractile function in the stunned myocardium with TA248 and TA276. •OH at a certain concentration may interfere with the binding of glibenclamide or glibenclamide-like substance to K\textsubscript{ATP} channels in the myocardium, resulting in opening of the channels. This may contribute to protect the myocardium against ischemic insults. When researchers invent an antioxidant like an anti-ischemic or anti-anginal drug, they need to consider the radical scavenging activity of the drug and the possible role of •OH remaining in the myocardium.

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