

# Landiolol, esmolol and propranolol protect from ischemia/reperfusion injury in isolated guinea pig hearts

*[Le landiolol, l'esmolol et le propranolol protègent des lésions résultant de l'ischémie/reperfusion dans des cœurs de cobaye isolés]*

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**Purpose:** Beta blockers are thought to exert beneficial effects on the ischemic heart. The authors examined the effects of landiolol (ONO 1101), a highly selective  $\beta_1$  antagonist, propranolol, a non-specific  $\beta$  blocker, and esmolol, a selective  $\beta_1$  antagonist, on postischemic contractile recovery. Drugs were given prophylactically.

**Methods:** Ischemia-reperfusion in isolated guinea pig hearts was induced by stopping the perfusion for 45 min and reperfusing for 60 min. Hearts ( $n = 7$  in each group) were treated with or without propranolol (1 or 10  $\mu\text{M}$ ), esmolol (5 or 50  $\mu\text{M}$ ), or landiolol (20, 100 or 500  $\mu\text{M}$ ) ten minutes before inducing ischemia.

**Results:** At the end of reperfusion, left ventricular pressure (LVP) recovered to  $64 \pm 3\%$  of the baseline value in the control group. With 1 and 10  $\mu\text{M}$  propranolol, LVP recovered to  $90 \pm 5\%$  and  $100 \pm 6\%$  of the baseline value at 60 min after reperfusion, respectively. Fifty  $\mu\text{M}$  but not 5  $\mu\text{M}$  of esmolol resulted in restoration of LVP to  $97 \pm 17\%$  of the pre-ischemic value at 60 min after reperfusion. In hearts pretreated with 100 and 500  $\mu\text{M}$  landiolol, LVP was restored to  $109 \pm 5\%$  and  $104 \pm 5\%$  of the baseline value, respectively. Landiolol 100  $\mu\text{M}$  did not depress LVP in the pre-ischemic period.

**Conclusions:** The present study shows that landiolol, an ultra-short-acting cardioselective  $\beta_1$  blocker, has cardioprotective effects on ischemia-reperfusion injury in isolated guinea pig hearts. All three  $\beta$  blockers were equally protective but the intermediate dosage of landiolol preserved LVP during the pre-ischemic period.

**Objectif :** On croit que les bêtabloquants ont des effets bénéfiques sur le cœur ischémié. Les auteurs ont vérifié les effets du landiolol (ONO 1101), un antagoniste  $\beta_1$  hautement sélectif, du propranolol, un  $\beta$  bloquant non spécifique, et de l'esmolol, un antagoniste  $\beta_1$  sélectif, sur la récupération contractile postischémique. Les médicaments ont été administrés préventivement.

**Méthode :** L'ischémie-reperfusion a été induite dans des cœurs de cobaye isolés en stoppant la perfusion pendant 45 min et en reperfusant pendant 60 min. Les cœurs ( $n = 7$  dans chaque groupe) ont été traités avec ou sans propranolol (1 ou 10  $\mu\text{M}$ ), esmolol (5 ou 50  $\mu\text{M}$ ) ou landiolol (20, 100 ou 500  $\mu\text{M}$ ) dix minutes avant l'induction de l'ischémie.

**Résultats :** À la fin de la reperfusion, la pression ventriculaire gauche (PVG) avait retrouvé  $64 \pm 3\%$  de sa valeur de base dans le groupe témoin. Avec 1 et 10  $\mu\text{M}$  de propranolol, la PVG a été récupérée respectivement à  $90 \pm 5\%$  et  $100 \pm 6\%$ , 60 min après la reperfusion. La dose de 50  $\mu\text{M}$ , non celle de 5  $\mu\text{M}$ , d'esmolol a été suivie de la restauration de la PVG à  $97 \pm 17\%$  de la valeur préischémique, 60 min après la reperfusion. Dans les cœurs prétraités avec 100 et 500  $\mu\text{M}$  de landiolol, la PVG a été restaurée à  $109 \pm 5\%$  et à  $104 \pm 5\%$  de sa valeur de base, respectivement. La dose de 100  $\mu\text{M}$  de landiolol n'a pas causé de baisse de la PVG en période préischémique.

**Conclusion :** La présente étude montre que le landiolol, un  $\beta_1$  bloquant cardioselectif à action très brève, a des effets cardioprotecteurs sur les lésions résultant de l'ischémie-reperfusion dans des cœurs de cobaye isolés. Les trois  $\beta$  bloquants ont été également protecteurs, mais la dose intermédiaire de landiolol a diminué la PVG pendant la période préischémique.

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**L**ANDIOLOL hydrochloride (ONO-1101) is a newly developed highly cardioselective  $\beta$  blocker with a potency ratio ( $\beta_1/\beta_2$ ) of 255, compared with 33 for esmolol and 0.68 for propranolol.<sup>1</sup> Although this agent is more potent than esmolol as a  $\beta$ -antagonist,<sup>1,2</sup> the cardiodepressant effect of landiolol is less than that of esmolol.<sup>3</sup> In addition, it has a short duration (half-life of four minutes) of activity, enabling rapid recovery after cessation of administration by rapid hydrolysis of its ester link.<sup>1</sup> It has neither intrinsic sympathomimetic activity nor significant membrane-stabilizing activity.<sup>1</sup>

Beta adrenoceptor blocking agents have been shown to have cardioprotective effects on ischemic and hypoxic hearts, including attenuation of arrhythmias,<sup>4</sup> improvement in ventricular function, and an overall decrease in mortality in patients with ischemic heart disease.<sup>5,6</sup> Beta adrenoceptor blockers have also been used as an alternative to cardioplegic arrest during coronary artery bypass surgery to reduce ischemic damage.<sup>7,8</sup> Although the exact mechanisms by which cardioprotective agents act are not yet fully understood,  $\beta$  blockers are thought to exert beneficial effects on the ischemic heart by lowering myocardial oxygen consumption as a consequence of reduced contractility and heart rate (HR).<sup>9</sup> Their beneficial effects are also thought to be due to their antioxidant<sup>10-12</sup> and, in the case of propranolol, membrane-stabilizing<sup>13</sup> properties. Recently,  $\beta$  blockers have also been reported to preserve the sarcoplasmic reticular (SR) function and gene expression in ischemia-reperfusion (I/R) rat hearts.<sup>14</sup> These findings suggest that  $\beta$  blockers would be beneficial for the treatment of I/R-induced myocardial injury.

Although the cardioprotective effects of propranolol and esmolol are well known, the effects of landiolol on contractility in hearts subjected to I/R are not well known. The purpose of this study was 1) to determine the cardioprotective effects of landiolol on functional recovery in isolated guinea pig hearts subjected to I/R, and 2) to compare the effects of landiolol (a  $\beta_1$ -specific blocker), propranolol (a nonspecific  $\beta$  blocker) and esmolol (a  $\beta_1$ -specific blocker) on functional recovery in isolated guinea pig hearts subjected to I/R.

## Materials and methods

### *Preparation and measurements*

This investigation conformed to the guidelines for care and use of laboratory animals published by the National Institutes of Health and was approved by our institutional Animal Care Committee. As described previously,<sup>15</sup> male English short-haired guinea pigs (250–300 g) received *ip* ketamine 20 mg and 1,000 U of heparin and

were decapitated after they had become unresponsive to noxious stimulation. After thoracotomy, the inferior and superior vena cava were sectioned and the aorta was cannulated distal to the aortic valve. Each heart was perfused in a retrograde fashion via the aorta with a cold oxygenated, modified Krebs-Ringer's solution equilibrated with 95% oxygen and 5% carbon dioxide and was then rapidly excised. Perfusate was disk-filtered (5  $\mu$  bore size) in-line and had the following composition (in mM): 137 Na<sup>+</sup>, 5 K<sup>+</sup>, 1.2 Mg<sup>+</sup>, 2.5 Ca<sup>2+</sup>, 134 Cl<sup>-</sup>, 15.5 HCO<sub>3</sub><sup>-</sup>, 1.2 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 11.5 glucose, 2 pyruvate, 16 mannitol, and 0.05 ethylene-diamine-tetraacetic acid. Perfusate and bath temperatures were maintained at 37°C by a thermostatically controlled water circulator (Thermo pump™, Taitec CO., Koshiya, Japan). Isovolemic development (systolic-diastolic) of left ventricular pressure (LVP), spontaneous atrial HR, and aortic inflow, which indicates coronary inflow (CF), were measured continuously. LVP was measured by using a pressure transducer (DTX Plus Press™, Ohmeda, WI, USA) connected to a thin, saline-filled latex balloon (LB-3™, MARS Inc., Sapporo, Japan) inserted into the left ventricle (LV) through the left atrium. Balloon volume was adjusted to maintain a diastolic LVP of 0 mmHg during the initial control period so that any increase in diastolic LVP reflected an increase in left ventricular wall stiffness or diastolic contracture. The volume of the balloon was unchanged during the experiment. CF (aortic) was measured continuously at a constant temperature and at a normal aortic perfusion (gravity) pressure of 55 mmHg by a transit-time, self-calibrating, in-line ultrasonic flowmeter (Research Flowmeter Transonic T106X; Transonic System, Ithaca, NY, USA) placed directly into the aortic inflow line.

Hearts were perfused with Krebs-Ringer's solution for a 15-min stabilization period. After pre-ischemic measurement of cardiac function, hearts ( $n = 7$  in each group) were treated with propranolol (1 and 10  $\mu$ M), esmolol (5 and 50  $\mu$ M), landiolol hydrochloride (ONO-1101; 20, 100, and 500  $\mu$ M) or no  $\beta$  blocker (control group) for ten minutes. Each  $\beta$  blocker was infused just above the perfusion cannula. The heart was then subjected to 45 min of global ischemic arrest at 37°C by clamping the aortic cannula. Finally, the hearts were reperfused for 60 min (37°C) for post-ischemic functional measurement. Cardiac mechanical function was estimated as LVP, HR, CF and rate-pressure product (RPP; LVP  $\times$  HR). In the preliminary experiments, 45 min of ischemia followed by 60 min of reperfusion were sufficient to produce a decrease in LVP to  $72 \pm 5\%$  of the pre-ischemic value.

Landiolol and esmolol were donated by Ono Pharmaceutical Co., Ltd. (Osaka, Japan) and Maruishi

Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Propranolol was purchased from Sigma Chemical Co. (St. Louis, USA).

#### Statistical analysis

Results are expressed as means  $\pm$  SEM. To determine differences in recovery of function between the groups, data were subjected to analysis of variance with repeated measures. Post hoc analyses were performed with Fisher's protected least significant difference test. The relationship between myocardial depressant effects of  $\beta$ -blockers and post-ischemic recovery in LVP was evaluated with analysis of covariance. A *P* value of less than 0.05 was considered statistically significant. The main results are summarized in the text and in the Figure.

#### Results

A Table showing the effects of propranolol, esmolol and landiolol on LVP, HR, RPP and CF is available as additional material on the Journal's web site at [www.cja-jca.org](http://www.cja-jca.org). Although pretreatment with the lowest doses of  $\beta$ -blockers showed no myocardial depression, the highest doses of  $\beta$ -blockers showed profound myocardial depression.

After 60 min of reperfusion, LVP was restored to  $64 \pm 1\%$ ,  $64 \pm 3\%$ , and  $64 \pm 3\%$  of the baseline value in the propranolol, esmolol, and landiolol groups without  $\beta$ -blockers, respectively (Figure). In hearts pretreated with 1 and 10  $\mu$ M propranolol, LVP was restored to  $90 \pm 5\%$  and  $100 \pm 6\%$  of baseline value, respectively. After 60 min of reperfusion, HR was restored to  $125 \pm 12\%$ ,  $94 \pm 6\%$  and  $94 \pm 5\%$  of the baseline value in the control, 1  $\mu$ M and 10  $\mu$ M propranolol group, respectively. The magnitudes of HR recovery from the corresponding pre-ischemic values were not significantly different from that in the control group. After 60 min of reperfusion, CF was restored to  $86 \pm 5\%$ ,  $92 \pm 4\%$  and  $65 \pm 7\%$  of the baseline value in the control, 1  $\mu$ M and 10  $\mu$ M propranolol group, respectively.

In hearts pretreated with 50  $\mu$ M esmolol, LVP was restored to  $97 \pm 24\%$  of the baseline value. However, 5  $\mu$ M esmolol caused no significant change in LVP at 60 min after reperfusion compared with that in the control group. After pretreatment with 50  $\mu$ M esmolol, RPP decreased to  $75 \pm 7\%$  of the baseline value, and improved 60 min after reperfusion (Table). After 60 min of reperfusion, HR was restored to  $113 \pm 17\%$ ,  $98 \pm 21\%$  and  $135 \pm 20\%$  of the baseline value in the control, 5 and 50  $\mu$ M esmolol group, respectively. There was no significant difference in the magnitude of recovery in HR at 60 min after reperfusion

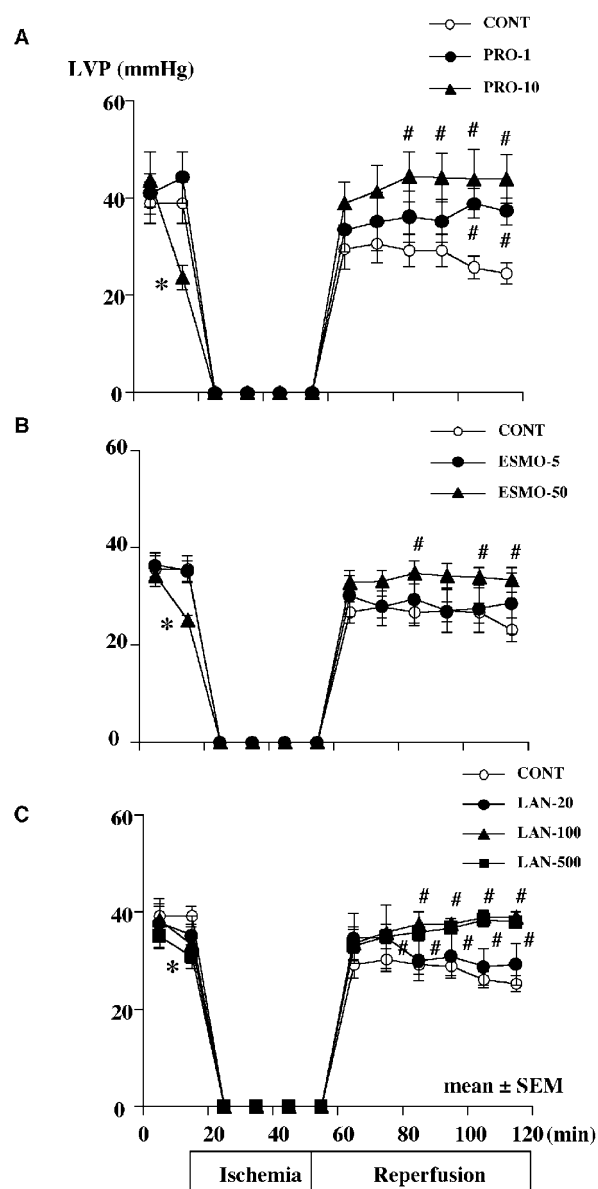


FIGURE 3 Time course of left ventricular pressure (LVP) before, during, and after global ischemia in propranolol (PRO; A), esmolol (ESMO; B), and landiolol (LAN; C) groups. CONT = control group. Values are expressed as mean  $\pm$  SEM. \**P* < 0.05 *vs* corresponding baseline value; #*P* < 0.05 *vs* control group. *n* = 7 in each group.

among the groups. After 60 min of reperfusion, CF was restored to  $85 \pm 7\%$ ,  $94 \pm 11\%$  and  $97 \pm 6\%$  of the pre-ischemic value in the control, 5 and 50  $\mu$ M esmolol group, respectively.

After 60 min of reperfusion, LVP was restored to  $64 \pm 3\%$ ,  $109 \pm 5\%$  and  $104 \pm 5\%$  of the baseline value in the control, 100 and 500  $\mu\text{M}$  landiolol group, respectively. However, 20  $\mu\text{M}$  landiolol did not significantly improve postischemic recovery in LVP. Only 500  $\mu\text{M}$  landiolol caused a significant decrease in RPP at ten minutes after drug administration, and showed a profound recovery 60 min after reperfusion. After 60 min of reperfusion, HR was restored to  $122 \pm 17\%$ ,  $118 \pm 4\%$ ,  $107 \pm 12\%$  and  $118 \pm 14\%$  of the baseline value in the control, 20, 100 and 500  $\mu\text{M}$  landiolol group, respectively. There was no significant difference in the magnitudes of recovery in HR at 60 min after reperfusion among the four groups. After 60 min of reperfusion, CF was restored to  $85 \pm 7\%$ ,  $90 \pm 4\%$ ,  $88 \pm 4\%$  and  $94 \pm 5\%$  of the baseline value in the control, 20, 100 and 500  $\mu\text{M}$  landiolol group, respectively. There was no significant difference in the magnitudes of restoration in CF among the four groups.

### Discussion

The present study shows that landiolol hydrochloride, an ultra-short-acting cardioselective  $\beta$  blocker, as well as propranolol and esmolol, have cardioprotective effects on I/R injury in isolated guinea pig hearts. Low doses of propranolol (1  $\mu\text{M}$ ), esmolol (5  $\mu\text{M}$ ) and landiolol (20  $\mu\text{M}$ ) were not sufficient to elicit cardioprotective effects in this model. Pretreatment with 10  $\mu\text{M}$  propranolol, 50  $\mu\text{M}$  esmolol, and 100 and 500  $\mu\text{M}$  landiolol improved myocardial contractility after a transient period of ischemia, but the highest doses of propranolol (10  $\mu\text{M}$ ), esmolol (50  $\mu\text{M}$ ) and landiolol (500  $\mu\text{M}$ ) caused significant myocardial depression before ischemia. Landiolol at a dose of 100  $\mu\text{M}$  caused no significant myocardial depression before ischemia while improving postischemic functional recovery.

The effectiveness of  $\beta$ -adrenoceptor blockade in patients with ischemic heart disease has been demonstrated.<sup>5,6</sup> Wallace *et al.*<sup>6</sup> reported that perioperative administration of atenolol reduced the incidence of cardiac events in a randomized, double-blinded, placebo-controlled study. They found that perioperative atenolol given for one week after the operation to patients at high risk for coronary artery disease reduced the incidence of postoperative myocardial ischemia and the risk of death at two years. HR before an episode of ischemia and during the entire postoperative week were lower in the atenolol group. Such pre-ischemic energy sparing effects, due to depression of cardiodynamic function, have been suggested as mechanisms by which  $\beta$  blockers provide postischemic functional recovery of the myocardium.<sup>16</sup> However, the cardioprotective effects cannot only be explained by energy-sparing

mechanisms in the pre-ischemic period.<sup>13</sup> In fact, Wallace *et al.*<sup>6</sup> found that 15% or more of ischemic episodes are independent of HR. In the present study, landiolol (100  $\mu\text{M}$ ) had a protective effect on the myocardium subjected to I/R without causing a reduction in RPP in the pre-ischemic period. These findings suggest that  $\beta$ -adrenoceptor blockers may exert anti-ischemic effects independent of cardiac work.

Another possible mechanism underlying the anti-ischemic effects of  $\beta$ -adrenoceptor blockers is myocardial protection from ischemia-induced excess release of norepinephrine. Most cardiac  $\beta$ -adrenergic receptors are of the  $\beta_1$ -receptor subtype. Beta receptors are situated on the outer surface of the sarcolemma and are coupled to adenyl cyclase by the stimulatory guanine nucleotide-binding protein. The major intracellular effects of  $\beta$  agonists are elicited by formation of cyclic adenosine monophosphate (AMP), which increases the activity of cyclic AMP-dependent protein kinase A. The latter phosphorylates various proteins, including calcium channels. Acute myocardial ischemia leads to a gradual increase in  $\beta$ -adrenergic receptors. This increase occurs rapidly after the onset of global ischemia (15 min) and persists even after prolonged periods of global ischemia.<sup>17</sup> In normal hearts, high catecholamine levels induce receptor desensitization with internalization of  $\beta$ -adrenergic receptors. In acute myocardial ischemia, however, agonist-promoted internalization and functional uncoupling of  $\beta$ -adrenergic receptors are abolished. Consequently, the balance of internalization and externalization of receptors is shifted towards an increase in functionally coupled receptors at the cell surface.<sup>17</sup> The above-mentioned events lead to the overload of intracellular free calcium ions, which may contribute to myocyte injury.<sup>18</sup> If  $\beta$ -blockers exert their anti-ischemic effect mainly by blocking the  $\beta_1$ -receptor, postischemic contractile function should recover in order of  $\beta_1$  receptor blocking potency. However, in the present study, the three  $\beta$  blockers showed similar effects on postischemic recovery in spite of differences in  $\beta_1$ -blocking potency. Therefore, it is unlikely that their anti-ischemic effect depends only on  $\beta_1$ -receptor subtype blocking potency.

Beta-adrenoceptor blockers are believed to have some direct effects on the myocardial membrane,<sup>13,19</sup> antiperoxidative activity,<sup>10</sup> antiradical effect,<sup>20</sup> sarcoplasmic reticular (SR) function,<sup>14</sup> and cardiac SR gene expression.<sup>14</sup> Propranolol, a non-specific  $\beta$ -adrenoceptor blocker, has been reported to have a cardioprotective effect via several actions, such as membrane-stabilizing activity<sup>13</sup> and inhibition of the enzyme NADH oxidase,<sup>21</sup> and/or higher lipophilic

property.<sup>22</sup> Propranolol induced better LVP recovery in rat ischemic-reperfused hearts than did atenolol, a  $\beta$ 1-specific blocker.<sup>14</sup> Propranolol caused marked myocardial depression in our model, whereas the same dose of propranolol had no effect on cardiac performance in the rat.<sup>14</sup> This may be due to differences in experimental models and species. Esmolol, a  $\beta$ 1-specific blocker, has also been reported to exert a cardioprotective effect during myocardial I/R. Several studies have suggested that esmolol could be used effectively as an adjunct to cardioplegic solution for induction and maintenance of cardiac arrest during cardiac surgery.<sup>7</sup> Modulation of free-radical-mediated reaction and arachidonic acid metabolism<sup>11</sup> and inhibition of neutrophil superoxide generation and platelet aggregation<sup>12</sup> have been postulated as cellular mechanisms underlying esmolol-induced myocardial protection. While this could be true *in vivo*, it does not apply to the present study, which was performed in buffer-perfused hearts. Further investigation is needed to compare these cellular mechanisms of  $\beta$ -adrenoceptor blockers underlying myocardial protection.

We investigated the effect of landiolol on cardiac performance during I/R. Yasuda *et al.*<sup>8</sup> reported that landiolol has the potential to enhance postischemic cardiac function after warm cardioplegic arrest in isolated rat hearts. They infused a crystalloid cardioplegic solution (37°C) with landiolol (1, 2.5, 5 or 10 mmol·L<sup>-1</sup>) to the coronary artery and then subjected the heart to 30 min of ischemic arrest followed by 40 min of reperfusion. They observed a significant improvement in postischemic recovery of aortic flow only when 2.5 mmol·L<sup>-1</sup> of landiolol was used. Although their results are in agreement with ours on the cardioprotective effect of landiolol, the effective doses in their study are more than tenfold higher than those used in our study. This may be due to differences in experimental models and species. In the present study, landiolol (100  $\mu$ M) had a protective effect on the myocardium subjected to I/R without causing a reduction in RPP in the pre-ischemic period. These findings suggest that the cardioprotective effect of landiolol cannot be explained only by energy-sparing mechanisms in the pre-ischemic period. However, landiolol has neither intrinsic sympathomimetic activity nor significant membrane-stabilizing activity.<sup>1</sup> In *in vitro* experiments, landiolol and esmolol failed to inhibit human neutrophil function, including reactive oxygen species production.<sup>23</sup> It is not known to what extent the cardioprotective effect of landiolol is due to antioxidation such as that seen with propranolol; further investigation is needed.

A limitation of this study is that the doses of  $\beta$  blockers were not equipotent with  $\beta$ 1-receptor sub-

type blocking potency. Although landiolol is reported to be more potent than esmolol as a  $\beta$ -blocker,<sup>1,2</sup> it is also reported that the cardiodepressant effect of landiolol is less than that of esmolol.<sup>3</sup> It is most likely that differences in pre-ischemic hemodynamics lead to differences in postischemic recovery. We chose the doses of  $\beta$  blockers based on their cardiodepressant effects instead of their  $\beta$ 1-adrenoceptor blocking potency. Because landiolol is not approved for clinical use, the clinical relevance of our study remains hypothetical. Our study was carried out only to clarify whether landiolol might have cardioprotective effects. Based on our findings, further research should determine if landiolol is advantageous in reducing myocardial damage after I/R injury in the perioperative period.

In conclusion, the present study shows that landiolol hydrochloride, an ultra-short-acting cardioselective  $\beta$  blocker, as well as propranolol and esmolol, have cardioprotective effects on I/R injury in isolated guinea pig hearts. The cardioprotective effect of landiolol cannot be explained only by a reduction in cardiac work in the pre-ischemic period. The mechanisms responsible for the cardioprotective effect of landiolol against I/R injury remain to be determined.

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