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ORIGINAL ARTICLE

Landiolol, an ultra short acting β_1-blocker, improves pulmonary edema after cardiopulmonary resuscitation with epinephrine in rats

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Abstract
Purpose Epinephrine is frequently administered as an essential drug for cardiopulmonary resuscitation (CPR) in clinical situations. Unfortunately, epinephrine elicits unfavorable effects, for example pulmonary edema, both during and after CPR. We hypothesized that administration of landiolol during CPR with epinephrine would reduce the degree of pulmonary edema and improve survival. Therefore using a rat CPR model, we investigated the effect of landiolol with epinephrine on pulmonary and cardiac injury following CPR.
Methods Twelve male Sprague–Dawley rats were allocated to Group-E (Gr.-E: 0.02 mg/kg epinephrine) and thirteen animals to Group-EL (Gr.-EL: 0.02 mg/kg epinephrine with 0.5 mg/kg landiolol). After tracheotomy, cardiac arrest was induced by obstructing the endotracheal tube. We measured the lung wet-to-dry (*W/D*) weight ratio to evaluate the degree of pulmonary edema 2 h after CPR. The hematocrit (Hct) difference between before and after CPR (Hct-D) was calculated. We measured the plasma levels of troponin-I (T-I) to evaluate the degree of cardiac injury.
Results The lung *W/D* weight ratio in Gr.-E (6.4 ± 1.06, mean ± SD) was significantly higher than that for Gr.-EL (4.9 ± 0.80, $p < 0.01$). Hct-D was significantly higher in Gr.-E (10.2 ± 3.1%) than in Gr.-EL (5.2 ± 3.5%, $p < 0.01$). We observed no difference in survival or difference of T-I. (Gr.-E: 2.62 ± 0.51 ng/ml, Gr.-EL: 3.43 ± 2.72 ng/ml).
Conclusion Administration of landiolol during CPR with epinephrine prevented the development of pulmonary edema and the increase in Hct during and after CPR.

Keywords Landiolol · Epinephrine · Cardiopulmonary resuscitation · Pulmonary edema

Introduction

Epinephrine is frequently administered as an essential drug for cardiopulmonary resuscitation (CPR) in clinical situations [1]. Unfortunately, epinephrine elicits unfavorable effects, for example hypertension, tachycardia, myocyte injury, and pulmonary edema, both during and after CPR [2]. These beneficial and unfavorable physiological effects of epinephrine related to CPR have been shown in both animal and human studies [1–4].

Recently, several studies have reported that β-blockers may exhibit cardioprotective effects in some clinical and experimental settings [5, 6]. Menegazzi et al. [7] reported that administration of propranolol with epinephrine and vasopressin improved survival after CPR. Some researchers have mentioned that esmolol, another ultra-short acting β_1-blocker, improved the survival after CPR [8, 9].

Although most studies of β-blockers during CPR have referred to the effects on cardiac performance and myocardial protection, few reports have mentioned the pulmonary perspective.

Landiolol is an ultra-short acting β_1-blocker that has been widely used in Japan since 2002 [10–12]. This drug is a highly cardioselective β_1-blocker with a potency ratio (β_1/β_2) of 255, compared with 33 for esmolol and 0.68 for

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propranolol [13]. Its rates of onset and elimination are faster, and it exhibits a less potent negative inotropic effect than esmolol [10]. Moreover, the antioxidative effect of landiolol has been described in some papers [5, 14, 15].

We hypothesized that administration of landiolol during CPR with epinephrine might be more beneficial than administration of other β-blockers, for example propranolol or esmolol. And concomitant administration of these drugs might improve survival and reduce the extent of epinephrine's unfavorable effects, for example pulmonary edema.

Therefore using a rat CPR model, we investigated the effect of landiolol with epinephrine on pulmonary and cardiac injury following CPR.

Materials and methods

Animal preparation

This investigation conformed to the guidelines for the care and use of laboratory animals published by the National Institutes of Health and was approved by our institutional animal-care committee. Following intraperitoneal injection of pentobarbital (Nembutal; Abbott Laboratories, IL, USA; 30 mg/kg), 25 male Sprague–Dawley rats (13–15 weeks, body weight 410–480 g) underwent tracheotomy. After tracheotomy, all animals were mechanically ventilated (FiO_2 0.21, 50 breaths/min, tidal volume 10 ml/kg; Model 683; Harvard, Holliston, Massachusetts, USA). The right-femoral artery and vein were cannulated (polyethylene tube SP31; Natsume, Japan) for mean arterial blood pressure (MAP) monitoring and drug administration, respectively. In order to avoid the increase of intrathoracic negative pressure after cessation of ventilation, the muscle relaxant, pancuronium bromide (Mioblock; Schering-Plough Corporation, NJ, USA) 0.5 mg/kg, was administered as an induction dose, followed by lactate Ringer's solution at 10 ml/kg/h containing pancuronium bromide 0.05 mg/ml. Catheterization (SP31) of the left external jugular vein was performed to monitor the right atrial pressure (RAP). Lead II electrocardiograms, heart rate (HR), MAP, RAP, and rectal temperature were monitored (Power Lab; AD Instruments, Australia). Rectal temperature was maintained between 36.5 and 37.5°C by use of a heating pad throughout the experiment. The coronary perfusion pressure (CPP) was calculated as follows:

$$\mathrm{CPP} = \mathrm{BP/dia.} - \mathrm{RAP(BP/dia: \ diastolic \ blood \ pressure)}$$

We observed the animals over the subsequent 15 min interval and arterial blood samples (0.2 ml) were withdrawn for blood gas analysis and hematocrit (Hct) evaluation (ABL 77; Radiometer Medical, Copenhagen, Denmark). The animals were then observed for an additional 15 min.

Experimental protocol

Twelve animals were allocated to Group-E (Gr.-E: 0.02 mg/kg epinephrine) and thirteen animals to Group-EL (Gr.-EL: 0.02 mg/kg epinephrine with 0.5 mg/kg landiolol). First, we administered 0.1, 0.2, 0.5, and 1.0 mg/kg of landiolol. We could not find significant decrease in HR or MAP. There have been no previous reports of the administration of landiolol in CPR. Tsunekawa et al. [16] reported changes in plasma concentration after a bolus injection of landiolol in male Sprague–Dawley rats. According to their report, we assumed that the 0.5 mg/kg injection would reach the landiolol clinical concentration. In Gr.-E and Gr.-EL, cardiac arrest was induced by obstructing the endotracheal tube. Cardiac arrest was defined as the onset of a decline in MAP to 10 mmHg. One min after the confirmation of cardiac arrest, we initiated CPR with mechanical ventilation (FiO_2 1.0, 80 breaths/min, tidal volume 10 ml/kg), administration of the drug(s), and chest compression (180/min) using an instrument made specifically for rat cardiac massage [17, 18]. Successful resuscitation (i.e., return of spontaneous circulation, ROSC) was defined as an MAP above 50 mmHg. CPR was discontinued when ROSC did not occur within 5 min of cardiac massage. Arterial blood gas samples (0.2 ml) were drawn at 10, 30, 60, and 120 min after resuscitation. The Hct difference between before and after CPR (Hct-D) was calculated as follows:

$$\mathrm{Hct\text{-}D} = (\mathrm{Hct \ 10 \ min \ after \ CPR} - \mathrm{Hct \ before \ CPR})/\mathrm{Hct \ before \ CPR} \times 100$$

We defined death as when the MAP decreased to <10 mmHg after ROSC. In the surviving rats, a blood sample (2 ml) was withdrawn 2 h after CPR to measure plasma levels of troponin-I (T-I). We then performed bilateral pneumonectomy and measured the lung wet-to-dry (*W/D*) weight ratio to evaluate the degree of pulmonary edema. McCaul et al. [2] assessed the lung edema using the lung *W/D* weight ratio 2 h after CPR in a rat asphyxia model. Therefore, in this study we also measured lung *W/D* weight ratio 2 h after CPR.

In order to obtain control data of T-I and lung *W/D* weight ratio with no procedures, eight animals were allocated to the Reference group (Gr.-R).

Statistical analysis

Our objective was to detect a lung *W/D* weight ratio difference of 1.7 between Gr.-E and Gr.-EL with reference to the standard deviation in a previous study [2] and our

Table 1 Results of CPR in the two groups

	Group-E	Group-EL
Survival	8/12	9/13
Vent-A time[a] (s)	288.3 ± 23.3	301.2 ± 21.1
CPR time[b] (s)	64.0 ± 16.5	83.7 ± 65.3

Data are expressed as the mean ± SD

[a] Ventilation arrest time

[b] The time from the start of cardiac massage to ROSC

preliminary investigation. A sample size calculation based on an α value of 0.05 and a β value of 0.2 gave an estimated required sample size of eight animals. In our preliminary study, ROSC did not occur in all animals. Therefore, 12 animals were actually included in the study to compensate for those that did not complete the study. As a result, this study was performed on 33 animals. We determined the sample size using StatMate (2.00 for Windows; GraphPad Software, San Diego, CA, USA).

Data were expressed as the mean ± SD. Fisher's exact test was performed to compare survival between Gr.-E and Gr.-EL. Student's *t* test was performed to compare the duration of ventilatory arrest, CPR time, and the extent of Hct-D. Physiological values, lung *W/D* weight ratio, and plasma levels of T-I were analyzed by ANOVA and post hoc tests. Data were analyzed using Statview (version 5.0; SAS Institute, SAS Campus Drive, Cary, NC, USA). A p value of <0.05 was considered statistically significant.

Results

We observed ROSC in 8 of 12 animals in Gr.-E and 9 of 13 animals in Gr.-EL. We collected data for eight animals in Gr.-E and nine animals in Gr.-EL. There were no differences in survival, ventilatory arrest time, or CPR time between Gr.-E and Gr.-EL (Table 1). We did not observe any differences among the experimental groups in terms of the baseline characteristics (Table 2). We did not find any intergroup differences in HR, MAP, CPP, or oxygenation index (PaO_2/FiO_2) during and after CPR (Table 3; Fig. 1).

The lung *W/D* weight ratio in Gr.-E (6.4 ± 1.06, mean ± SD) was significantly higher than those of Gr.-EL (4.9 ± 0.80, $p < 0.01$) and Gr.-R (4.6 ± 0.35, $p < 0.01$) (Fig. 2). There was no difference in the lung *W/D* weight ratio between Gr.-EL and Gr.-R.

Hct-D was significantly higher in Gr.-E (10.2 ± 3.1%) compared with Gr.-EL (5.2 ± 3.5%, $p < 0.01$) (Fig. 3).

Two animals in Gr.-E and one animal in Gr.-EL died within 1 h due to severe pulmonary edema, so the number of rats used for plasma T-I data collection were 6 in Gr.-E and 8 in Gr.-EL.

Table 2 Baseline data

	Group-E	Group-EL	Group-R
Body weight (g)	430.4 ± 13.7	432.5 ± 9.1	438.8 ± 22.6
pH	7.48 ± 0.03	7.50 ± 0.03	7.49 ± 0.02
$PaCO_2$ (mmHg)	36.2 ± 3.9	34.0 ± 3.3	34.8 ± 2.1
PaO_2 (mmHg)	65.4 ± 8.5	72.5 ± 6.1	72.5 ± 7.7

Data are expressed as the mean ± SD

The plasma T-I levels in Gr.-E (2.62 ± 0.51 ng/ml) and Gr.-EL (3.43 ± 2.72 ng/ml) were significantly higher than in Gr.-R (0.11 ± 0.01 ng/ml, $p < 0.01$) (Fig. 4). Because the data for two animals that survived after VF or VT in Gr.-EL were remarkably high (6.74, 8.55 ng/ml), the difference of plasma T-I levels between Gr.-E and Gr.-EL was not significant. However, when these two animals were excluded from analysis, the plasma T-I levels in Gr.-EL were significantly lower than in Gr.-E (2.02 ± 0.75 vs. 2.62 ± 0.51, respectively; $p < 0.05$).

Discussion

This study demonstrated that landiolol administration with epinephrine during CPR improved lung *W/D* weight ratio and suppressed the elevation in Hct after CPR. We can easily speculate that it was brought about by a decrease of water diapedesis from the pulmonary vessels to the interstitial tissue.

Systemic vasoconstriction through α_1-adrenoceptor stimulation by epinephrine is one of the mechanisms of deterioration and severe systemic vasoconstriction can cause pulmonary edema. On the other hand, these factors were important in maintaining adequate CPP during CPR [1, 19, 20]. Therefore, it may be recommended to avoid an inadequately high dose of epinephrine or the combined use of some drugs to prevent pulmonary edema. In this study, we used landiolol for the latter purpose.

With the endotracheal tube clamp model of asphyxia, there is generally production of negative intrathoracic pressure induced by spontaneous breathing. This pressure can easily enhance lung edema fluid accumulation resulting in pulmonary edema [21]. However, in this model of respiratory arrest we could not observe any spontaneous breathing, because of the administration of a muscle relaxant. Thus, there was no generation of negative intrathoracic pressure in our model. The lung *W/D* weight ratio did not exhibit any significant difference between Gr.-EL (with stress of apnea, cardiac arrest, drug intervention, and CPR) and Gr.-R (without these stresses). We estimate that landiolol administration might reduce the degree of pulmonary edema during CPR.

Table 3 HR, MAP, and oxygenation index after CPR

Time after CPR (min)	10	30	60	120
HR (beats/min)				
Gr.-E	377.5 ± 34.1	409.3 ± 50.2	389.8 ± 36.5	391.5 ± 21.3
Gr.-EL	379.7 ± 13.4	406.6 ± 33.3	382.9 ± 37.0	388.6 ± 24.0
MAP (mmHg)				
Gr.-E	115.0 ± 31.3	83.9 ± 40.0	96.1 ± 19.8	109.3 ± 7.4
Gr.-EL	131.7 ± 30.0	101.3 ± 31.1	111.5 ± 15.2	116.1 ± 18.1
Oxygenation index				
Gr.-E	333.9 ± 187.7	371.6 ± 174.5	490.2 ± 28.8	521.2 ± 31.2
Gr.-EL	425.1 ± 186.0	452.3 ± 170.6	526.6 ± 72.4	544.3 ± 95.8

Oxygenation index was calculated as PaO_2/FiO_2. Data are expressed as the mean ± SD

HR, Heart rate; MAP, mean arterial blood pressure; CPR, cardiopulmonary resuscitation

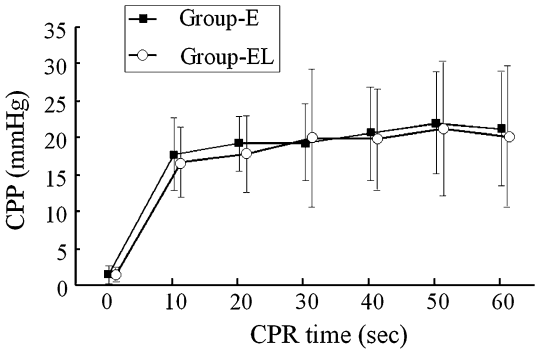

Fig. 1 Change of CPP during CPR. *CPP*, coronary perfusion pressure; *CPR*, cardiopulmonary resuscitation. Data are expressed as the mean ± SD

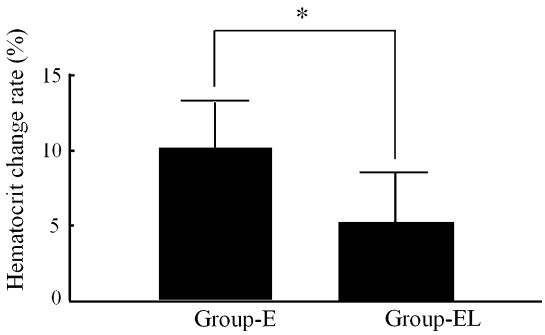

Fig. 3 Hematocrit change rate 10 min after CPR. *CPR*, cardiopulmonary resuscitation. Data are expressed as the mean ± SD; *$p < 0.01$ Gr.-E vs. Gr.-EL

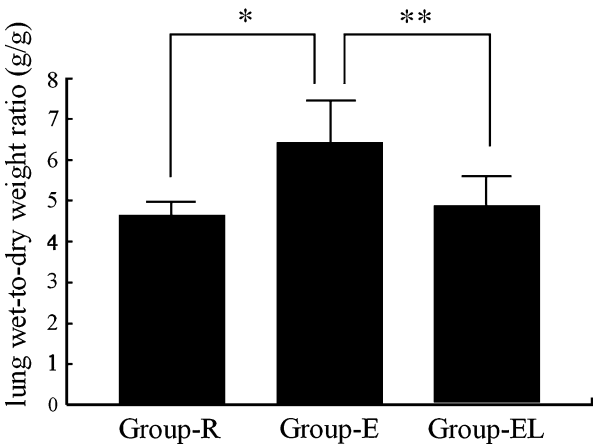

Fig. 2 Lung wet-to-dry weight ratio. Data are expressed as the mean ± SD; *$p < 0.01$ Gr.-E vs. Gr.-R; **$p < 0.01$ Gr.-E vs. Gr.-EL

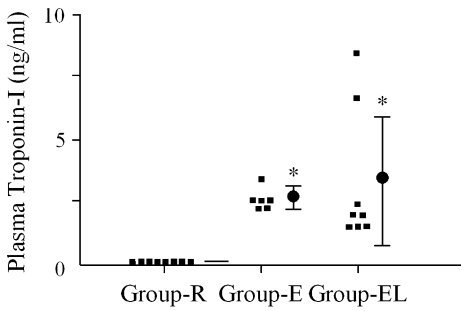

Fig. 4 Plasma level of troponin-I. Data are expressed as the mean ± SD; *$p < 0.01$ vs. Gr.-R

Reoxygenation after ischemia produces a large amount of hydroxyl radicals, which causes lipid-peroxidation of the biomembrane, DNA damage, and disruption of enzyme protein function. Reoxygenation also disturbs the balance between hydroxyl radical production and radical scavengers. These undesirable responses cause injury to vascular endothelial cells and the alveolo-capillary membranes. When these responses become too severe, pulmonary edema can occur because of enhanced permeability of the pulmonary blood vessel [22, 23].

Koga et al. [14] reported that measurement of the radical spectrum by electron spin resonance revealed landiolol exhibited hydroxyl radical scavenging activity in vitro. The antioxidative effect of landiolol has been described in some papers [14, 15]. Kimura-Kurosawa et al. [5] demonstrated that landiolol exhibited a lipid peroxidation-reducing effect

in isolated guinea pig hearts subjected to ischemia–reperfusion. In this study we investigated whether landiolol might reduce lung injury and prevent pulmonary edema because of its hydroxyl radical-scavenging activity and antioxidative effect.

We did not observe any difference of T-I between Gr.-E and Gr.-EL. However, when we excluded data for two animals that exhibited VF or VT, T-I in Gr.-EL was significantly lower than that of Gr.-E. Although we found significant difference of Hct-D between Gr.-E and Gr.-EL, Hct at 120 min showed the same value (39% in both groups). So, we estimated the effect of hemoconcentration on the value of T-I might be small. We speculate that the use of landiolol during CPR with epinephrine could have exhibited myocardial protection. The same cytoprotective mechanism noted above, reduction of lipid peroxidation and hydroxyl radical-scavenging activity, might contribute to these findings. It has been said that the cardioprotective effects of β-blockers are mediated by reducing myocardial oxygen consumption through a decrease in both HR and myocardial contractility [5]. In this study, however, 0.5 mg/kg landiolol did not cause any significant decrease of HR. So, we considered that 0.5 mg/kg landiolol was insufficient to cause the beta-blocking effect. Therefore, we estimated that the antioxidative effect might be more important than the beta-blocking effect for this amount of landiolol. Landiolol at a dose sufficient to reduce HR might have exhibited a more beneficial cardioprotective effect. However, it is not certain whether or not a larger dose of landiolol, which could reduce the HR, would be desirable in a model of CPR.

In this study, we were not able to demonstrate that Gr.-EL improved survival compared with Gr.-E. There have been controversial reports on survival after administration of β-blockers [3, 7–9, 19]. Hilwig et al. [19] reported that administration of propranolol with epinephrine did not improve survival 1 h after ROSC. On the other hand, some papers reported that concomitant administration of esmolol with epinephrine in a rat VF model improved survival after ROSC [3, 8, 9]. These controversial results might be because of differences in β_1-receptor selectivity or in the half-life of each of the β-blockers. These findings may also be because of differences in the methodology in each experiment. In order to evaluate the lung *W/D* weight ratio, we terminated the observation 2 h after CPR. If we had utilized a longer observation period, we might have been able to detect a difference in survival after ROSC.

The current study has some limitations. First, because this experiment was performed in young, healthy rats, the results of this study cannot be directly applied to a general human population. Second, because blood esterase activity is higher in rats than in humans, the blood level of landiolol could be different from that in humans [15]. Third, the PaO_2/FiO_2 ratio showed a tendency of improvement even though the differences were not significant. The main topic of this study is occurrence of pulmonary edema. Although we ended the study 2 h after CPR, longer observation might be necessary in future.

In summary, administration of landiolol during CPR with epinephrine prevented the development of pulmonary edema and the increase in Hct during and after CPR. Further studies are needed to elucidate the mechanism by which landiolol suppresses pulmonary edema.

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