Cardiopulmonary resuscitation in the mouse

LEI SONG,1 MAX HARRY WEIL,1,2 WANCHUN TANG,1,2 SHIJIE SUN,1,2 AND TOMMASO PELLIS1
1Institute of Critical Care Medicine, Palm Springs, 92262; and 2Keck School of Medicine of the University of Southern California, Los Angeles, California 90033

Received 29 October 2001; accepted in final form 21 February 2002

Shijie Sun,1,2 and Tommaso Pellis. Cardiopulmonary resuscitation in the mouse. J Appl Physiol 93: 1222–1226, 2002. First published July 5, 2002; 10.1152/japplphysiol.01079.2001.—We sought to develop a model of cardiac arrest and resuscitation on mice that would be comparable to that of large mammals and would allow for more fundamental investigations on cardiopulmonary arrest and cardiac resuscitation. A model of cardiopulmonary resuscitation previously developed by our group on rats was adapted to anesthetized, mechanically ventilated adult male Institute of Cancer Research mice that weighed 46 ± 3 g. The trachea was intubated through the mouth, and end-tidal PCO2 (PETCO2) was measured with a microcapnometer. Catheters were advanced into the aorta and into the right atrium, and coronary perfusion pressure (CPP) was computed. A 1.5-mA alternating current was delivered to the right ventricular endocardium, which produced ventricular fibrillation or a pulseless rhythm. Precedrial compression was begun 4 min later. Ten sequential studies were performed, during which five animals were successfully resuscitated and five failed resuscitation efforts. Successful resuscitation was contingent on the restoration of threshold levels of CPP and PETCO2 during chest compression. As in rats, swine, and human patients, threshold levels of mean aortic pressure, CPP, and PETCO2 were critical determinates of resuscitability in this murine model of threshold level of cardiac arrest and resuscitation.

Herefore, studies on both mechanisms and therapy of cardiac arrest have generally been confined to larger experimental animals and especially dogs and swine (6, 10, 18, 21). Research in this field, as has recently been recognized by the Postresuscitative and Initial Utility in Life Saving Efforts Coalition (23), has been very limited. The reality that <5% of human victims of cardiac arrest survive when cardiac arrest occurs outside of the hospital prompts vigorous experimental pursuit of a better understanding of mechanisms and management. Our group (19, 22) had established a rodent model of cardiac arrest and cardiopulmonary resuscitation (CPR). Other species, including cats (4, 20) and mice (1), served as models of circulatory arrest but for the primary purpose of investigating cerebral injury. We were prompted to develop a model of CPR in the mouse to determine whether the hemodynamic and respiratory changes were comparable to those of large mammals. As an initial effort, we sought to develop measurement under controlled conditions of ventilation and chest compression. We specifically sought to define threshold levels of mean aortic pressure (MAP), coronary perfusion pressure (CPP), and end-tidal PCO2 (PETCO2) during CPR for comparison with the same measurements established for large mammals.

Methods

This study was approved by the Institute’s Animal Use and Care Committee. The experiments were in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources. Our Institute is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Animal preparation. Ten Institute of Cancer Research adult male mice were supplied by Harlan Sprague Dawley of San Diego, California. Animals weighed 46 ± 3 g. Mice were fasted overnight but had free access to water. Pentobarbital sodium was injected intraperitoneally in a dose of 45 μg/g and supplemented by additional doses of 10 μg/g at hourly intervals. The last dose was administered at least 30 min before cardiac arrest was induced so as to minimize cardiodepressor effects of the anesthetic agent. Hair was clipped from the neck and ventral thorax. Anesthetized animals were immobilized in a supine posture on a surgical board. The proximal trachea was orally intubated with an 18-gauge cannula (Abbocath-T, Abbott Hospital Products, Chicago, IL) mounted on a blunt needle with a 145° angled tip, as previously described (8). The tracheal tube was secured at the mouth. Mechanical ventilation was maintained with a pressure-controlled ventilator as previously described for the rat (22) but modified to deliver a tidal volume of 9 μl at a frequency of 130 breaths/min. PETCO2 was measured by a microcapnometer monitor (Columbus Instruments, Columbus, OH) adapted with a T cannula attached to the tracheal tube. The precision of the CO2 monitor was confirmed before and after each experiment with room air and certified 5.35% CO2 gas.

For measurement of MAP, a saline-filled microcannula with an internal diameter of 0.2 mm and an external diameter of 0.4 mm (BioTime, Berkeley, CA) was surgically in-
serted into the right carotid artery with the aid of a ×4 magnifier lens and advanced into the descending aorta. An identical microcannula was inserted into the left jugular vein and advanced through the cranial vena cava into the right atrium for measurement of right atrial pressure. Pressures were measured with a TRANSPAC IV transducer (Abbott Critical Care Systems, Abbott Laboratories, Chicago, IL). A guide wire was then advanced from the surgically exposed right jugular vein into the right ventricle for inducing cardiac arrest. Endocardial contact of the tip of the guide wire was electrocardiographically confirmed. Core temperature was measured continuously through a rectal microprobe (Yellow Springs Instruments, Yellow Springs, OH). Rectal temperature was maintained at 37.0 ± 0.5°C with the use of infrared thermolamps. The conventional lead II electrocardiogram was recorded with the aid of subcutaneous needles.

**Hemodynamic and blood chemistry measurements.** Aliquots of 150 μl of arterial blood were withdrawn for baseline measurement of pH, arterial PCO2, arterial Po2, arterial O2 saturation, HCO3, hemoglobin, and hematocrit, and were delivered to a NOVA analyzer (PHOX Stat Profile, Waltham, MA). Lactic acid concentration was measured in 30 μl of blood with a lactimeter (YSI 2300, Yellow Springs Instruments). Arterial blood in the amount of 200 μl was replaced immediately after blood sampling with blood from a donor mouse of the same colony. In resuscitated animals, the inventory of measurements was repeated after 4 h. Aortic and right atrial pressures were continuously recorded together with lead II of the scalar electrocardiogram and PETCO2. Pressure recordings had a damped frequency response of 22 Hz. CPP was calculated as the difference between minimal aortic diastolic pressure and the simultaneously measured right atrial pressure.

**Experimental protocol.** Before cardiac arrest was induced, mechanical ventilation was begun with room air and a tidal volume of 9 μl/g body weight with a frequency of 130 breaths/min. Mechanical ventilation was stopped coincident with the onset of cardiac arrest. Ventricular fibrillation (VF) was induced with a 60-Hz, 1.5-mA alternating current delivered to the right ventricular endocardium. Current flow was reduced to 0.75 mA after the onset of cardiac arrest and continued for 1.5 min, which prevented spontaneous defibrillation. As soon as the current was stopped, VF or electromechanical dissociation was confirmed (Fig. 1). Precordial compression and coincident mechanical ventilation with an inspired O2 fraction of 1.0 were begun after 4 min of untreated cardiac arrest, as previously implemented in studies in larger mammals (6, 16, 19, 22). Our laboratory had established in larger animals that the increased inspired oxygen concentrations favor survival during mechanical ventilation (6, 22). A pneumatically driven mechanical chest compressor with a miniaturized thumper was utilized with 260 compressions/min. The chest compressor represented an adaptation of the device developed for the rat as previously described (22). Compressions were adjusted to decrease the ventro-dorsa chest diameter by ~30% with equal compression-relaxation durations. Adjustments in depth of compression were made to secure a MAP that exceeded 30 mmHg. Preliminary studies had identified this level of aortic pressure as minimal for successful resuscitation. Successful resuscitation was defined as the return of spontaneous rhythm with a MAP of 60 mmHg for a minimum of 5 min. Resuscitated animals were invasively monitored for an additional 4 h. A total of 1.6 ml of physiological salt solution was administrated during that 4-h interval during which animals were allowed to recover from anesthesia. All catheters were then removed, and wounds were surgically closed. Animals were subsequently returned to their cages and continuously observed. Necropsy was routinely performed after death. Thoracic and abdominal organs were examined for gross evidence of traumatic injuries that

Table 1. Baseline measurements

<table>
<thead>
<tr>
<th>Animals</th>
<th>n</th>
<th>BW, g</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Dias AP, mmHg</th>
<th>PETCO2, Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresusc</td>
<td>5</td>
<td>46 ± 3</td>
<td>597 ± 63</td>
<td>90 ± 10</td>
<td>81 ± 9</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>Resusc</td>
<td>5</td>
<td>47 ± 3</td>
<td>559 ± 68</td>
<td>95 ± 4</td>
<td>88 ± 5</td>
<td>29 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. Nonresusc, nonresuscitated animals; Resusc, resuscitated animals; BW, body weight; HR, heart rate; MAP, mean aortic pressure; Dias AP, diastolic aortic pressure; PETCO2, end-tidal PCO2.

**Fig. 1.** Electrocardiographic (ECG), aortic pressure (Aorta), right atrial pressure (RA), and end-tidal CO2 (PETCO2) recorded from a representative mouse before onset of cardiac arrest and after successful resuscitation. BL, baseline ECG; VF, ventricular fibrillation; EMD, electromechanical dissociation; PC, precordial compression; PR10, 10 min post-resuscitation; PR240, 240 min postresuscitation.
followed vascular cannulation, intraperitoneal injection of anesthesia, or precordial compression.

Statistical analyses. Measurements are reported as means ± SE. Comparisons between time-based measurements on the same animals and differences between resuscitated and nonresuscitated animals were analyzed with ANOVA multiple measurements. A P value of <0.05 was considered significant.

RESULTS

Survival. Five of ten animals were successfully resuscitated. Spontaneous circulation was restored during minute 1 of precordial compression in three of the five survivors and within 3 min in the remaining two animals. There were no significant differences in weight between resuscitated (47 ± 3 g) and nonresuscitated (46 ± 3 g) animals. Resuscitated animals survived between 7 and 57 h. Only two animals survived for >24 h.

Hemodynamic observations. No significant differences in baseline hemodynamic measurements were observed between resuscitated and nonresuscitated animals (Table 1). MAP before cardiac arrest was induced ranged from 90 to 98 mmHg, and no significant differences were observed between baseline values in survivors and nonsurvivors. MAP over the initial 3 min of CPR in survivors was 31 ± 4 mmHg and was 8 ± 5 mmHg in nonsurvivors (P < 0.05). CPP achieved with precordial compression averaged 23 ± 7 mmHg in survivors but only 7 ± 5 mmHg in nonsurvivors. These differences were highly significant (P < 0.01).

$P_{ETCO_2}$ decreased from a baseline level of 30 ± 6 to 0.5 Torr during cardiac arrest. It increased as anticipated to 13 ± 4 Torr during the first 3 min of chest compression in resuscitated animals but only to 4 ± 2 Torr in nonresuscitated animals (P < 0.05) as shown in Fig. 2.

At the end of 1.5 min when current flow to the endocardium was discontinued, a ≤3-s interval of VF was documented, followed by pulseless electrical activity (Fig. 1).

Acid-base and respiratory measurements. Metabolic and blood-gas measurements obtained at baseline and at 4 h after successful resuscitation are shown in Table 2. Mild metabolic acidosis was documented at 4 h after successful resuscitation. As anticipated, $P_{ETCO_2}$ correlated with arterial $P_{CO_2}$ at baseline and 4 h after resuscitation in all experimental animals (Fig. 3).

Necropsy. No evidence of injury to the bony thorax or abdomen was identified. Laryngotracheal edema was observed in four of five survivors. Minor lung contusions were observed in three animals in which 15 min of chest compression failed to return spontaneous circulation.

DISCUSSION

A resuscitation model in the mouse was previously reported by Böttiger et al. (1) but in the context of cerebral resuscitation. The intent of the present study was to measure cardiocirculatory function with the focus on CPP and forward blood flow as reflected in $P_{ETCO_2}$. Earlier studies in larger animals, and especially the pig and the rat, had defined critical levels of both CPP and $P_{ETCO_2}$ for successful resuscitation (6, 14–17, 19, 22). Because our studies were focused on the heart itself, we also sought a more physiological level of externally generated heart rates. The intrinsic heart rate of the mouse exceeds 500 beats/min. To approach these rates, we utilized a mechanical chest compressor in lieu of manual compression. Arterial blood was sam-

Table 2. Metabolic and arterial blood-gas measurements

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Postresuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R (n = 5)</td>
<td>NR (n = 5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.37 ± 0.04</td>
<td>7.36 ± 0.03</td>
</tr>
<tr>
<td>$P_{CO_2}$, Torr</td>
<td>33 ± 6</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>$P_{O_2}$, Torr</td>
<td>99 ± 3</td>
<td>96 ± 12</td>
</tr>
<tr>
<td>$S_O_2$, %</td>
<td>97 ± 0.6</td>
<td>97 ± 0.9</td>
</tr>
<tr>
<td>HCO$_3$, mmol/l</td>
<td>19 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Hct, mmol/l</td>
<td>34 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Hb, mmol/l</td>
<td>11 ± 0.5</td>
<td>10 ± 0.9</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>1.2 ± 0.6</td>
<td>1.2 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. $S_O_2$, oxyhemoglobin saturation; Hct, hematocrit; R, resuscitated; NR, not resuscitated. *Inspired $O_2$ fraction = 1.0.
plied for blood-gas analyses and lactic acid measurement, and the blood removed was replaced with fresh donor blood to maintain stability of intravascular volume and red cell mass.

Because the mouse model has a high resting heart rate, the refractory period is correspondingly short (2, 5, 12). In addition, the heart itself is physically of small size. It is therefore difficult to secure reentrant rhythms with which to sustain VF. Accordingly, VF reverts spontaneously during the initial 1.5 min of electrically induced cardiac arrest as it does in rats (5, 13, 24, 25). The continuous delivery of a low-intensity electric current maintains VF. After the current is stopped, a pulseless rhythm is documented. In contrast to large mammals, cardiac arrest in both the rat and the mouse is, therefore, more often associated with pulseless electrical activity or asystole. Nevertheless, whether in settings of VF or pulseless rhythm, the ultimate effect is a failure of the heart to maintain forward blood flow, including coronary, systemic, and pulmonary blood flows. As in rats, pigs, and humans, PETCO₂ decreased to near zero during cardiac arrest and returned to between 30% and 40% of baseline values during precordial compression. Return of spontaneous rhythm was heralded by a prominent and progressive increase in PETCO₂ coincident with the reappearance of arterial pulsations and a rise in arterial pressure (7). These observations on PETCO₂ during CPR were indistinguishable from those previously observed in large mammals.

Aortic pressure and, more specifically, CPP (3, 9, 14) produced by precordial compression were shown to be critical determinants of resuscitability as they were in each of the species previously studied. Prolonged failure of myocardial perfusion during cardiac arrest is followed by global myocardial ischemic injury and postresuscitation myocardial dysfunction, which accounts for the fatal progression after successful resuscitation (6, 16, 19).

Different anesthetics have been employed for studies on mice. Our primary purpose was to develop a model that would be closely related to the mammalian models previously investigated in our laboratory, all of which were performed with pentobarbital anesthesia and subsequently validated with an alternative anesthetic (6, 10, 19, 22). Pentobarbital had the advantages of a comfortabe safety margin and both circulatory and respiratory stability.

With respect to blood volume, we estimate that the total blood volume of our mice was ~3.2 ml. Accordingly, in an effort to maintain more appropriate O₂ delivery, we utilized donor blood like we had done with rats (15, 19, 22). Like rats from the same colony, mice are universal donors (11).

In view of the potential trauma to the chest in these small animals, we performed routine necropsy to exclude traumatic injuries to the chest wall, lungs, or heart. We were able to exclude significant injuries in the present studies. Each animal in which threshold levels of CPP were exceeded was successfully resuscitated and survived for ≥7 h.

We have not as yet exercised studies in genetically engineered mice, which was admittedly the incentive for developing a mouse model. As an initial effect, we were committed to the development of a valid model of cardiac arrest and resuscitation in mice with measurements confirmatory of those established in large mammals. The present model has the advantages of providing a standardized insult and hemodynamic and respiratory measurements that are pertinent to outcome. We further sought a mouse model that would support continuing research on the role of adrenergic receptors on the heart and peripheral vessels during cardiac arrest (16, 17, 19). For instance, we presently attribute the diminution of vasopressor effects to downregulation of α₁-receptors, and confirming this would provide an important asset in the pursuit of our studies. We further hope that this model will allow other investigators to advance to studies on genetically engineered mice with opportunities to better define mechanisms of circulatory arrest, including the effects of reperfusion, postresuscitation myocardial function, and response to treatment.

This study was supported, in part, by National Heart, Lung and Blood Institute Grant HL-54322, the Weil Family Foundation, and the Rosse Family Foundation.

REFERENCES


