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Original Contribution

Addition of glucagon to adrenaline improves hemodynamics in a porcine model of prolonged ventricular fibrillation ☆,☆☆

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ABSTRACT

Objective: Cardiac arrest is a daunting medical emergency. The aim of the present study was to assess whether the combination of adrenaline and glucagon would improve initial resuscitation success, 48-hour survival, and neurologic outcome compared with adrenaline alone in a porcine model of ventricular fibrillation.
Methods: Ventricular fibrillation was induced in 20 healthy Landrace/Large White piglets, which were subsequently left untreated for 8 minutes. The animals were randomized to receive adrenaline alone (n = 10, group C) and adrenaline plus glucagon (n = 10, group G). All animals were resuscitated according to the 2010 European Resuscitation Council guidelines. Hemodynamic variables were measured before arrest, during arrest and resuscitation, and during the first 60 minutes after return of spontaneous circulation. Survival and a neurologic alertness score were measured at 48 hours after return of spontaneous circulation.
Results: Return of spontaneous circulation was achieved in 8 animals (80%) from group C and 10 animals (100%) from group G ($P = .198$). A significant gradual increase in coronary perfusion pressure and diastolic aortic pressure over time, which started 1 minute after the onset of cardiopulmonary resuscitation, was observed. Three animals (30%) from group C and 9 animals (90%) from group G survived after 48 hours ($P = .006$), whereas neurologic examination was significantly better in the animals of group G ($P < .001$).
Conclusions: In this porcine model of prolonged ventricular fibrillation, the addition of glucagon to adrenaline improves hemodynamics during resuscitation and early postresuscitation period and may increase survival.

1. Introduction

Cardiac arrest is a daunting medical emergency as approximately 300 000 arrested people are treated annually by medical personnel [1,2]. Although both survival and neurologic outcomes after in-hospital cardiac arrest have slightly improved during the past decade in the United States [3], approximately one-third of patients admitted to an intensive care unit survive to hospital discharge worldwide [2,4].

Vasopressors used in cardiopulmonary resuscitation (CPR) have a limited effect on long-term outcomes after cardiac arrest. Both adrenaline and vasopressin are associated only with increased chance of return of spontaneous circulation (ROSC) and improved short-term survival, but no long-term survival benefit has been demonstrated so far. Indeed, until now, there is no placebo-controlled study showing that routine use of any vasopressor at any stage during human cardiac arrest increases neurologically intact survival to hospital discharge [5-8].

Glucagon is a 29-amino acid peptide hormone secreted by the pancreatic alpha cells of the islets of Langerhans. Although it is usually administered for the treatment of hypoglycemia [9], it exerts additional cardiovascular effects that may favor its use in selected patients. Glucagon can restore heart rate, cardiac output, and blood pressure rapidly after an overdose of β-blockers [10], and it may also be used as an adjunctive therapy in the management of patients with shock who are on therapeutic doses of β-blockers [11-14]. Interestingly, in the late 60s, several clinical studies showed the positive inotropic effects of glucagon in normal subjects and in patients with variable degrees of cardiac disease [15]. In 1970, Murtagh et al [16] characterized the hemodynamic effects after an intravenous injection

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of a single dose of glucagon as immediate and dramatic, while in the same decade, 3 other studies reported that glucagon produced an increase in cardiac output, stroke volume, heart rate, and systolic index in patients with acute and chronic heart failure [17-19].

Based on the aforementioned observations, the aim of the present study was to assess whether the combination of adrenaline and glucagon would improve initial resuscitation success, 48-hour survival, and neurologic outcome compared with adrenaline alone in a swine model of ventricular fibrillation (VF).

2. Methods

The experimental protocol was approved by the General Directorate of Veterinary Services (permit no. 351/22-01-2013) according to Greek legislation regarding ethical and experimental procedures. The experiment was carried out in ELPEN Experimental-Research Center, Athens, Greece. Twenty healthy male Landrace/Large White piglets, all supplied by the same breeder (Validakis, Athens, Greece), aged 10 to 15 weeks, with an average weight of 19 ± 2 kg comprised the study population. The animals were fasted overnight but had free access to water.

Animals were premedicated with intramuscular injection of 10 mg/kg ketamine hydrochloride, 0.5 mg/kg midazolam, and 0.05 mg/kg atropine sulfate, as previously described [20]. The marginal auricular vein was catheterized, and anesthesia was induced with an intravenous bolus dose of 2 mg/kg propofol. They were then intubated with a 4.5-mm endotracheal tube (Portex, 4.5 mm ID; Mallinckrodt Medical, Athlone, Ireland). Animals were immobilized in the supine position on a surgical table. Additional 1 mg/kg propofol, 0.15 mg/kg cis-atracurium, and 4 μg/kg fentanyl were administered immediately before connecting the animals to a ventilator (Alpha Delta lung ventilator; Siare, Bologna, Italy) in 21% oxygen. Propofol infusion of 0.1 mg/(kg min) and additional doses of cis-atracurium at 20 μg/(kg min) and fentanyl at 0.6 μg/(kg min) were administered to maintain adequate anesthetic depth.

All animals were volume controlled, ventilated with a total tidal volume of 15 mL/kg. End-tidal CO_2 pressure ($ETCO_2$) was monitored (Tonocap-TC200; Datex Engstrom, Helsinki, Finland), and the respiratory rate was adjusted to maintain an $ETCO_2$ of 35 to 40 mm Hg. Noninvasive monitoring (Datascope Expert DS-5300 W ECG; Fukuda Denshi, Tokyo, Japan) also included electrocardiogram and pulse oximetry. For measurement of aortic pressures, an arterial catheter (model 6523, USCI CR; Bart Inc, Papapostolou, Athens, Greece) was inserted into the aorta via the right common carotid artery.

Mean arterial pressure (MAP) was determined by the electronic integration of the aortic blood pressure waveform and was calculated electronically. The internal jugular vein was surgically prepared, and a Swan-Ganz catheter (Opticath 5.5 F, 75 cm; Abbott, Ladakis, Athens, Greece) was inserted into the right atrium for continuous measurement of right atrial pressure. Coronary perfusion pressure (CPP) was electronically calculated as the difference between minimal aortic diastolic pressure (DAP) and the simultaneously measured right atrial diastolic pressure. The second internal jugular vein was also surgically prepared, and a 5F flow-directed pacing catheter (Pacel, 100 cm; St Jude Medical, Ladakis, Athens, Greece) was advanced into the apex of the right ventricle.

Before the experimental procedure, the piglets were randomly assigned to 2 different groups of 10 subjects each, according to the agents used, by means of a sealed envelope. The control group (group C) received saline as placebo (10-mL dilution, bolus) plus adrenaline 0.02 mg/kg, whereas the adrenaline-glucagon group (group G) received adrenaline 0.02 mg/kg plus glucagon 1 IU per 1-mL dilution (total 1 mL), bolus (glucagon 1 mg (1 IU)/vial, Novo Nordisk Hellas) at the onset of CPR. All animals were resuscitated according to the European Resuscitation Council guidelines on resuscitation with 10 breaths delivered asynchronously with 100 chest compressions. The investigators involved in data recording, data entry, and data analysis were blinded to each animal's allocation.

Ventricular fibrillation was induced with a 9-V ordinary cadmium battery, as previously described [20]. Arrhythmia was recognized electrocardiographically and confirmed by a sudden drop in MAP. After VF induction, mechanical ventilation was discontinued, and the animals were left untreated for 8 minutes. At the end of the eigthth minute of VF, resuscitation was immediately initiated with ventilation in 100% oxygen and chest compressions at a rate of 100/min (LUCAS; Jolife, Lund, Sweden) [21,22]. A 20-mL flush of isotonic sodium chloride solution was followed after each drug administration. Defibrillation was attempted with a 4 J/kg monophasic waveform shock delivered between the right infraclavicular area and the cardiac apex (Primedic Defi-B Defibrillator; Metrax GmbH, Rottweil, Germany).

Successful resuscitation was defined as ROSC with an MAP of at least 60 mm Hg for a minimum of 5 minutes. When ROSC was achieved, intensive care was provided, and the animals were monitored for 60 more minutes. Subsequently, anesthesia was discontinued, all catheters were removed as previously described, and manual ventilation was initiated [23]. Atropine 0.2 mg/kg followed by neostigmine 0.05 mg/kg was administered when spontaneous swallowing reflex was detected, whereas extubation was performed after adequate inspiration depth was confirmed. Each animal was then transferred to the animal house for observation for 48 hours.

The primary end point of the experiment was ROSC. Secondary outcomes were 48-hour survival rate and 48-hour neurologic outcome. A quantitative neurologic alertness score was used for the evaluation of neurologic recovery 48 hours later [24]. Alertness was scored from 0 (coma) to 100 (fully alert). The investigator who assessed the pigs neurologically was blinded as to the allocation of each animal.

Finally, these animals that survived were humanely euthanized by an intravenous overdose of pentobarbital and underwent necropsy [25]. Thoracic and abdominal organs were examined for gross evidence of traumatic injuries or other pathology.

2.1. Statistical analysis

The data analyses were performed with SPSS for Windows (version 16.0; SPSS, Chicago, IL) software. Kolmogorov-Smirnov test was used for assessing the normality of data distribution. Data are presented as mean ± SD for continuous variables if normally distributed or as median and interquartile range and as frequencies for the discrete variables. We also used repeated-measures analysis of variance to test the equality of means between different measurements. The categorical variables were analyzed using a χ^2 test, whereas continuous variables were analyzed using an unpaired t test. The P values were 2 sided and were considered to be significant for $P < .05$.

3. Results

No significant difference was observed in ROSC between the 2 groups, as 8 animals (80%) from group C and 10 animals (100%) from group G achieved ROSC ($P = .198$). More specifically, after the first shock, 8 animals (80%) from group C and 9 animals (90%) from group G restored ROSC ($P = .258$). After the second shock, 1 animal from group G restored ROSC. The remaining 2 animals from group C had asystole after 4 and 6 cycles, respectively.

No statistically significant differences were observed in baseline and 8-minute untreated VF hemodynamic parameters between the 2 groups (Table 1). Significant hemodynamic differences were observed between groups after 1 minute of CPR and before attempting the first defibrillation (Table 2). Coronary perfusion pressure values in group G were significantly higher compared with those in group C during the first cycle of CPR. During the second cycle of CPR, CPP was significantly