Terlipressin/adrenaline is better than adrenaline alone in a porcine model of prolonged ventricular fibrillation
A randomized controlled study

ABSTRACT

Objectives. Vasopressors have been routinely used in cardiopulmonary resuscitation. Recent data show that terlipressin may restore blood pressure in asphyxial and prolonged arrests but its potential role in ventricular fibrillation (VF) remains unknown. The aim of this study was to compare coronary (CorPP) and cerebral (CPP) perfusion pressures achieved by terlipressin/adrenaline versus placebo/adrenaline in VF.

Methods. Fourteen domestic pigs were randomly assigned into group A and B. After 5 min of untreated VF, compression-only resuscitation was applied for 10 min, followed by advanced life support. Terlipressin in a single dose of 30 μg·kg⁻¹ was added to the first dose of adrenaline in group A, while placebo was given in group B. CorPP and CPP were calculated from right atrial, aortic and intracerebral pressures. Data were analyzed using repeated measurements ANOVA and a Fisher’s protected LSD post hoc test.

Results. Terlipressin/adrenaline maintained CorPP above 10 mmHg for 17.7 min longer than adrenaline alone (P=0.003) unable to prevent refractory hypotension. CorPP (mean±SD) measured at 35, 45, and 55 min after the onset of VF was 12 ± 4, 11 ± 6, and 10 ± 5 mmHg in the terlipressin group A; and 6 ± 4, 1 ± 5, and -1 ± 5 mmHg in placebo group B (P=0.03, <0.001, and <0.001). CPP measured at the same times was 23 ± 7, 20 ± 7, and 23 ± 7 mmHg in group A; and 13 ± 7, 6 ± 5, and 6 ± 7 mmHg in group B (P=0.01, <0.001, and <0.001).

Conclusion. The study showed that a single dose of terlipressin, when added to adrenaline, was effective for achievement of higher vital organ perfusion pressures compared to adrenaline alone.
Introduction
Cardiac arrest leads to rapid development of intense global myocardial ischemia, especially when ventricular fibrillation (VF) is the precipitating mechanism. (1) It is known that successful resuscitation relies on capability for generating coronary blood flow above critical threshold levels. (2) The achieved coronary perfusion pressure (CorPP) during cardiopulmonary resuscitation (CPR) predicts the probability of successful defibrillation and return of spontaneous circulation (ROSC), while cerebral perfusion pressure (CPP) determines the extent of ischaemic brain injury. (2,3) In order to increase vital organ perfusion pressures, vasopressor agents have been used since the early 1960s. (4) Both European Resuscitation Council (ERC) and American Heart Association (AHA) Guidelines recommend administration of adrenaline (epinephrine), although there is no evidence proving that its use in humans increases survival to hospital discharge. (5,6) It is important to find more effective treatment since the vital organ perfusion induced by chest compressions is not only critical but essential for survival. (6)

The non-adrenergic vasopressor, vasoressin (8-arginine vasopressin), has been the most promising alternative so-far studied, (7-10) but it is not routinely available in Europe. (11) Recent data show that use of terlipressin (triglycyl – lysine vasopressin), a vasoressin synthetic analogue, may also have a favourable effect in treatment of cardiac arrest caused by toxins, anaphylaxis, and in children. (12-17) Although terlipressin is known to be a strong vasoconstrictor, (18) its possible role in treatment of VF, the most frequent cause of cardiac arrest in adults, remains unknown.

Our study was designed to compare the effect on vital organ perfusion pressures of adjunct terlipressin with that of placebo when the drug was added to repeated doses of adrenaline. We expected that early addition of a synergically acting vasopressor would increase CorPP and CPP during prolonged CPR in a porcine model of VF with realistic time intervals to drug delivery derived from data on the out-of-hospital setting.

Material and methods
This was a prospective, randomized, blinded, placebo-controlled experimental study in fourteen healthy, 15- to 17-week-old, female domestic pigs (Sus Scrofa f. Domestica) weighing 30 to 35 kg (32.0 ± 1.8 kg). The experiment was realized in the Animal Research Laboratory of the University of Defence, Faculty of Military Health Sciences. The study protocol was approved by the Animal Investigation Committee of the University of Defence Brno, Faculty of Military Health Sciences Hradec Králové, Czech Republic and the Departmental Commission for the Protection of Animals of the Ministry of Defence, Prague, Czech Republic. All the animals received humane care in compliance with the institutional guidelines. Animals were fasted overnight, but had free access to water. Anaesthesia was used in all surgical interventions.

Table 1. Selected baseline characteristics of the experimental animals. A two-sample t-test was used to determine statistical significance between groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>A = terlipressin (n = 7)</th>
<th>B = placebo (n = 7)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight [kg]</td>
<td>32.0 ± 1.9</td>
<td>32.1 ± 1.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Thorax diameter [cm]</td>
<td>66.4 ± 3.4</td>
<td>67.1 ± 2.9</td>
<td>0.68</td>
</tr>
<tr>
<td>Heart rate [min⁻¹]</td>
<td>114 ± 11</td>
<td>111 ± 14</td>
<td>0.67</td>
</tr>
<tr>
<td>Mean arterial pressure [mmHg]</td>
<td>68 ± 12</td>
<td>69 ± 11</td>
<td>0.92</td>
</tr>
<tr>
<td>Central venous pressure [mmHg]</td>
<td>5 ± 2</td>
<td>7 ± 5</td>
<td>0.42</td>
</tr>
<tr>
<td>Intracerebral pressure [mmHg]</td>
<td>6 ± 4</td>
<td>8 ± 4</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Figure 1. Flowchart of the study protocol. The white arrows indicate administrations of adrenaline [epinephrine] at a dose of 0.03 mg·kg⁻¹ IV repeated every 3 min; a black arrow indicates administration of a single dose of terlipressin 0.03 mg·kg⁻¹ IV (group A) or placebo (group B); a flash sign indicates induction of VF.
Animal preparation

Anaesthesia was initiated by intramuscular injection of azaperone (neuroleptic agent: 2 mg·kg⁻¹), atropine (0.02 mg·kg⁻¹) and ketamine (20 mg·kg⁻¹) 30 minutes before surgery, and completed by ear vein injection of thiopental (1 – 3 mg·kg⁻¹) in the operating room. The pigs were placed onto the backboard of the resuscitation system AutoPulse Model 100 (Zoll Medical Corp., Chelmsford, MA, USA) and fixed in the right lateral position. Into each ear vein was placed an 18G peripheral venous catheter for drug administration. Continuous electrocardiographic (ECG) monitoring was achieved using four adhesive electrodes applied to the proximal ends of the limbs. A cuffed endotracheal tube (size 7.0 ID; SIMS Portex Ltd., Kent, UK) was advanced into the trachea during spontaneous respiration, and the animals were mechanically ventilated with a volume-controlled ventilator Siemens-Elema, Model SV 900C (Siemens-Elema AB, Solna, Sweden). The ventilator delivered 19 breaths per minute with a FiO₂ of 0.4. Tidal volumes were adjusted to maintain normocapnia (end tidal CO₂ - EtCO₂ 35 to 40 mmHg).

Anaesthesia was maintained with a continuous infusion of midazolam (0.3 mg·kg⁻¹·h⁻¹), fentanyl (5 – 20 μg·kg⁻¹·h⁻¹) and pancuronium (muscle relaxant: 0.5 mg·kg⁻¹·h⁻¹). The depth of anaesthesia was assessed from heart rate and blood pressure. Ringerfundin solution (B. Braun, Melsungen, Germany; 10 mL·kg⁻¹·h⁻¹) was given continuously to replace fluid loss during instrumentation. Vital signs were continuously monitored with two Datex-Ohmeda Type S/5 monitors (Datex-Ohmeda Instrumentarium Corp., Helsinki, Finland) and a Zoll M Series defibrillator (Zoll Medical Corp., Chelmsford, MA, USA). A warming unit Warm Touch Model 5100 (Mallinckrodt Inc., Cincinnati, OH, USA) was used to maintain body temperature at the physiological level between 37.5 and 38.5 °C, measured in the oesophagus.

Measurements

After induction of anaesthesia, the thoracic aorta was cannulated via the caro-
tid artery with a 7F 200 mm catheter Certofix Duo (B. Braun Melsungen AG, Melsungen, Germany) for monitoring of aortic blood pressure and withdrawal of arterial blood samples. An 8SF percutaneous sheath introducer Intro-Flex (Edwards Lifesciences LLC, Irvine, CA, USA) was inserted via the internal jugular vein into the superior vena cava to measure right atrial pressure (RAP). All catheters were flushed with isotonic saline containing heparin (5 IU ml⁻¹) at a rate of 3 mL h⁻¹ to prevent obstruction, and attached to electronic pressure transducers Gabarith PMSET (Becton Dickinson, Franklin Lakes, NJ, USA) aligned at the level of the right atrium. CorPP of the left ventricle is defined as the pressure difference between aorta and right atrium during the decompression phase. It was calculated from diastolic aortic pressure (DAP) and RAP according to the formula: \( \text{CorPP} = \text{DAP} - \text{RAP} \).

A 5-mm diameter burr-hole craniotomy was created on the left side to place an intracranial pressure-monitoring device. The craniotomy was performed at the upper part of the frontal bone and 10 mm paramedian of the sagittal suture, which runs in the rostrocaudal axis in the midline of the skull. (19) A parenchymal probe Codman MicroSensor ICP Transducer (Codman, Johnson & Johnson, Raynham, MA, USA) was tunneled subcutaneously with a Tuohy needle, and inserted approximately 20 mm into the frontal lobe. The pressure transducer was connected to the monitor via Codman Interface Control Unit. CPP was calculated from mean aortic pressure (MAP), intracranial pressure (ICP) and central venous pressure (CVP) according to the formula: \( \text{CPP} = \text{MAP} - (\text{ICP} + \text{CVP}) \). (20)

The preparation phase, including all surgical procedures and instrumentati-on, was followed with a period of animal stabilisation lasting 45 min.

Arterial blood gases (ABG) were obtained every 15 min to analyse pH, partial pressures of oxygen and carbon dioxide (PaO₂ and PaCO₂), oxygen saturation (SaO₂) and base excess (BE). ABG were measured with a blood-gas analyser AVL Omni 6 (Roche Diagnostica GmbH, Graz, Austria) and corrected to body temperature.

**Experimental Protocol**

The time line of the study protocol is presented in figure 1. Five minutes before induction of VF (\( T = -5 \)), all anaesthetic drugs were withdrawn, fraction of inspired oxygen (\( \text{FiO}_2 \)) was decreased to 0.21 and heparin (5000 IU) was given to prevent intra-cardiac clot formation. Baseline (BL) haemodynamic variables and ICP were recorded.

VF was induced with an alternating current of 5–10 V using 5F intra-cardiac bipolar pacing lead VascoStim (Arrow Int. Europe, Tongeren, Belgium) introduced via the Intro-Flex sheath into the right atrium. Circulatory arrest (\( T = 0 \)) was defined as that point at which both systolic and diastolic aortic pressures dropped down to equal values and the ECG showed VF. At that time, artificial ventilation was stopped. After 5 minutes of untreated cardiac arrest (\( T = +5 \)), mechanical chest compressions without ventilation were started using the AutoPulse. Its backboard contains a motor to retract a load-distributing band (LDB) which adjusts lengthwise to the size of the thorax. The microprocessor is programmed to provide a constant 20% reduction in the anterior—posterior dimension of the individual chest during compressions at a rate of 80 min⁻¹. (21) At the time of 15 minutes after onset of VF (\( T = +15 \)), non-synchronized mechanical ventilation (\( \text{FiO}_2 = 1.0, \text{Fi} \% 8 \text{mL kg}^{-1} \)) at a rate of 10 breaths per minute was started and continued for 45 minutes. The AutoPulse-generated chest compressions were then paused every 5 minutes for 10 seconds (for rhythm analysis and to check for pulse).

The pigs were randomly assigned into 2 groups to receive either terlipressin (group A: \( n = 7 \)) or placebo (group B: \( n = 7 \)) together with the first dose of adrenaline. The randomization scheme was generated from the website ‘Randomization.com’ (available at http://www.randomization.com) using a method of randomly permuted blocks with a size of 6 and 8. All investigators involved in the study were blinded to both the randomization scheme and the drugs, which were diluted in isotonic saline and distributed in numbered 10 mL syringes by someone not involved in the trial. The randomization plan was not available to the staff before statistical analysis was completed.

The first drug was given in both groups 19 min after induction of VF (\( T = +19 \)). Terlipressin (Ferring-Leciva, Jesenice/Praque, Czech Republic) together with adrenaline (Zentiva a. s., Prague, Czech Republic), both at a dose of 30 μg kg⁻¹, were administered in group A; group B received placebo (10 mL of isotonic saline: NaCl 0.9%) together with adrenaline at a dose of 30 μg kg⁻¹. All drugs were administered IV via peripheral ear vein and flushed with Ringerfundin solution (20 mL). In both study groups, equal doses of adrenaline were repeated every 3 min thereafter as recommended. (5,6) Haemodynamic variables and ICP were recorded every minute. Blood samples for ABG analysis were taken at times –1 min (pre-arrest BL), +15 min (after the 5 min period of untreated VF followed by a 10 min period of continuous chest compressions without ventilation), +30 min and +45 min (after 15 min and 30 min of advanced life support (ALS), respectively).

CPR was terminated 60 min after induction of VF. All animals were autopsied to verify correct positioning of catheters and to check for damage to the rib cage.

**Statistical Analysis**

Statistics were performed using commercial software Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA). The tested null hypothesis was that adjunct terlipressin would not change CorPP and CPP compared to placebo given in treatment of VF. A Kolmogorov-Smirnov test was used to verify Gaussian distribution. Data were analyzed using a two-way repeated measures analysis of variance (ANOVA) factoring for time and treatment effects. Fisher’s protected least significant difference
(LSD) was used for post hoc testing to identify the differences. The baseline data and time intervals were analyzed with a two-sample t-test for continuous variables. Comparisons of proportions were analyzed with a Fisher’s exact test. All data are expressed as mean ± standard deviation (S.D.). Statistical significance was considered at P-value < 0.05.

Results

There were no differences in pre-arrest baseline characteristics between groups (table 1). At the end of the basic life support (BLS) period, there were also no significant differences in received medication, intracerebral, arterial or venous pressures, or ABG analysis between groups.

The mean responses of CorPP and CPP were different for terlipressin and placebo (P = 0.018, and 0.003 respectively). CorPP increased immediately after vasopressors were given, but remained higher in group A compared to group B until the end of the experiment. CorPP (mean ± S.D.) measured 35, 45, and 55 min after the onset of VF was 12 ± 4, 11 ± 6, and 10 ± 5 mmHg in the terlipressin group A; and 6 ± 4, 1 ± 5, and -1 ± 5 mmHg in the placebo group B (P = 0.03, < 0.001, and < 0.001 respectively). Adrenaline with added terlipressin was able to maintain CorPP above critical threshold level of 10 mmHg for longer compared to adrenaline alone: 27.9 ± 11.0 vs. 10.1 ± 5.8 min (P = 0.003). At a time of 20 min after the first drug administration, terlipressin maintained CorPP > 10 mmHg in 6 out of 7 animals (85.7%) compared to none in group B (P = 0.005) (figure 2).

CPP at time 35, 45, and 55 min was 23 ± 7, 20 ± 7, and 23 ± 7 mmHg in group A; and 13 ± 7, 6 ± 5, and 6 ± 7 mmHg in group B (P = 0.01, < 0.001, and < 0.001 respectively). CPP was significantly higher in group A compared to placebo group B, and was maintained above the level of 20 mmHg until the late period of CPR (figure 2).

Pre-arrest ABG analysis showed no statistical differences between study groups. The pH was less than 7.2 after no-CPR and compression-only CPR periods, and increased in both groups after ALS was initiated (P = 0.01 and P = 0.02 for T = +30 versus T = +15). There were no differences in pH between groups at T = +30 and T = +45 (7.30 vs. 7.24, P = 0.26, and 7.25 vs. 7.25, P = 0.92 respectively). In pigs treated with terlipressin, there was higher PaO2 measured 10 min after administration of the drug when compared to animals treated with adrenaline alone (341 vs. 238 mmHg, P = 0.03) (table 2). ROSC was not achieved in any pig. In all animals, VF converted into asystole before termination of the experiment. However, VF lasted 47.4 ± 8.9 min in group A but only 40.3 ± 11.4 min in group B with lower coronary perfusion (P = 0.22).

Autopsy confirmed correct positioning of all catheters and revealed minor injuries in 2 of 14 animals after 45 min of mechanical CPR (2x rib fractures; 1x haemothorax < 100 mL).

Discussion

In our time-realistic model of ventricular fibrillation, adjunct terlipressin added to the first dose of repeated adrenaline increased both CorPP and CPP, and maintained perfusion pressures higher until the very end of prolonged 60 min duration cardiac arrest. Current Guidelines recommend continuation of CPR until VF is present, but maintaining blood pressure over a prolonged period may be difficult. (5) Some ambulance services also transport their patients to hospital with ongoing CPR. (5,22) This approach increases demands on maintaining sufficient blood pressure for a longer time. There is a strong relationship between CorPP and initial resuscitation rates. (2) Different critical threshold levels for CorPP predictive of outcome have been identified in various cardiac arrest models, corresponding to 20 mm Hg in dogs, 15 mm Hg in humans, and 10 mm Hg in pigs. (2,23,24) In order to increase CorPP above these resuscitability thresholds, administration of exogenous vasopressors is often necessary.

Adrenaline is routinely used in the treatment of cardiac arrest. However, there is no evidence that adrenaline improves long-term survival in humans. (5,6,25,26) The failure of many experimental studies to translate their results into clinical practice was caused by their inappropriate design that did not reflect realistic conditions. (27,28) Based on available literature, the mean time to first drug administration was only 9.5 min in experiments while in the out-of-hospital setting, the first drug is usually given 19.4 minutes after suspected onset of VF. (27) Thus, the flowchart of our study was designed to respect realistic timing of OHCA. (27) Initially, compression-only CPR followed the first 5 minutes of untreated VF. (29) The first drug was given only 19 minutes after induction of VF. It was during the late metabolic phase of cardiac arrest, when adrenaline is considered ineffective because of desensitization of adrenergic receptors as a result of severe hypercapnic acidosis and global hypoxia. (30,31) Results from our study support this. The average pH was 7.15 ± 0.09 after 15 min of VF, and in those conditions adrenaline alone was unable to restore blood pressure despite mechanical chest compressions. Another vasopressor, which use in cardiac arrest was already accepted in the AHA Guidelines 2000, is vasopressin. (32) It is known that deficiency of endogenous vasopressin causes hypotension in septic shock and cardiac surgery patients. (18,33) It can potentiate the effect of adrenergic agents via stimulation of V1 receptors located on vascular smooth muscle. (30,34) However, as compared with adrenaline alone, a combination of vasopressin and adrenaline in cardiac arrest did not improve outcome in a French clinical multicentre trial published in 2008. (9) The European Resuscitation Council (ERC) has never classified vasopressin as a superior drug and does not recommend its use. (5) Furthermore, vasopressin is not commonly available in most European countries. (11) We decided to add a single dose of terlipressin instead, because of its ava-
Terlipressin acts on three subtypes of receptors: $V_1$ receptors are found on various cells, including vascular smooth muscle cells, causing vasoconstriction, $V_2$ receptors are expressed by kidney collecting duct cells and mediate water retention, and $V_3$ receptors are found on cells within the central nervous system and modulate corticotrophin secretion. The vascular selectivity ($V_1/V_2$) of terlipressin is 2.2/1.0 compared with 1.0/1.0 for vasopressin. Its half-life is 6 hours compared with 20 minutes for vasopressin. (11,18) Until now, there have been only a few papers published on its beneficial use in cardiac arrests refractory to standard catecholamine therapy. (12-17) Addition of terlipressin to adrenaline restored blood pressure despite severe acidosis (pH 7.03) during prolonged CPR. (12) In a paediatric model of post-asphyxial cardiac arrest, it increased rate of ROSC. (16) Several limitations to our study should be noted. Firstly, defibrillation efforts, neurological outcome and long-term survival were not investigated because it was necessary to observe the drug effects during the late phase of cardiac arrest. In order to maintain VF as long as possible, neither defibrillation nor anti-arrhythmics were used. This was the reason why ROSC was not achieved in any pig. Secondly, different vasopressin receptors in pigs (lysine vasopressin) and humans (arginine vasopressin) may result in different conclusions in humans. Thirdly, pressure variables were measured in the study, not flow variables. However, animal CPR experiments have shown that microcirculatory blood flow, responsible for delivery of oxygen to cardiomyocytes, is highly correlated with CorPP. (36) Fourthly, the dose–response relationship was not investigated. There is no recommended dose for terlipressin in CPR but up to two doses of 10-20 µg·kg$^{-1}$ have been previously used in humans. (17) Fifthly, an automated device was used instead of manual CPR to achieve uniform chest compressions in all animals. The device was not scaled down from that designed for human use. The method might have influenced circulation by increases of intrathoracic pressure as its band was tightened around the chest. (37) Pressure calculations would probably be different if manual chest compressions were used. However, the aim was not to determine the efficacy of the AutoPulse but to compare different treatment algorithms in identically resuscitated animals. The values of CorPP and CPP themselves could not therefore be transferred into human practice wherein the AutoPulse would cause a different response. It is known that CorPP typically deteriorates over time. (38) The calculated CorPP in adrenaline/placebo group was extremely low after 45 minutes of arrest, but its values were even higher than those measured at comparable times in a similar study using the AutoPulse and repeated doses of adrenaline in pigs. (30) Furthermore, it is not common to maintain cardiac arrest for such a long time in experimental research as we did. In addition, usage of anaesthetics may have impaired cardiovascular function and vascular tone as well.

Searching for alternative pharmacological support is a great challenge for today’s resuscitation medicine. Terlipressin is a potent vasopressor beneficial in treatment of oesophageal variceal haemorrhage, (39) catecholamine-resistant septic shock, (34,40-43) and probably cardiac arrest. (12-17) The main advantage of terlipressin is maintenance of a long-lasting vasoconstrictive effect despite abnormal metabolic conditions. (26,30,34) In contrast to vasopressin, terlipressin is widely available in most countries. (11) Possible worsening of post-cardiac arrest myocardial dysfunction (44) and other terlipressin-related ischaemic adverse effects, (43) when given during CPR, are still unknown. Only further research focused on long-term survival may confirm the future role of vasopressin-analogues in CPR.

Conclusion
The study showed that a single-dose of terlipressin – when added early to adrenaline in a time-realistic animal model of ventricular fibrillation – is effective for maintaining higher vital organ perfusion pressures until the very late phase of CPR while adrenaline alone was unable to prevent severe refractory hypotension.
REFERENCES


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