Cryobiology 69 (2014) 361–366

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

Milrinone ameliorates cardiac mechanical dysfunction after hypothermia in an intact rat model ☆

Erik Sveberg Dietrichs [b,a,*], Timofei Kondratiev [a], Torkjel Tveita [a,c]

[a] Anesthesia and Critical Care Research Group, Institute of Clinical Medicine, UiT, The Arctic University of Norway, 9037 Tromsø, Norway
[b] Department of Research and Education, Norwegian Air Ambulance Foundation, 1441 Drøbak, Norway
[c] Division of Surgical Medicine and Intensive Care, University Hospital of North Norway, 9019 Tromsø, Norway

ARTICLE INFO

Article history:
Received 19 June 2014
Accepted 2 September 2014
Available online 16 September 2014

Keywords:
Accidental hypothermia
Therapeutic hypothermia
Milrinone
Rewarming
Rewarming shock
PDE3 inhibitor
Cardiovascular support

ABSTRACT

Background: Rewarming from hypothermia is often complicated by cardiac dysfunction, characterized by substantial reduction in stroke volume. Previously we have reported that inotropic agents, working via cardiac β-receptor agonism may exert serious side effects when applied to treat cardiac contractile dysfunction during rewarming. In this study we tested whether Milrinone, a phosphodiesterase III inhibitor, is able to ameliorate such dysfunction when given during rewarming.
Methods: A rat model designed for circulatory studies during experimental hypothermia with cooling to a core temperature of 15 °C, stable hypothermia at this temperature for 3 h and subsequent rewarming was used, with a total of 3 groups: (1) a normothermic group receiving Milrinone, (2) a hypothermic group receiving Milrinone the last hour of hypothermia and during rewarming, and (3) a hypothermic saline control group. Hemodynamic function was monitored using a conductance catheter introduced to the left ventricle.
Results: After rewarming from 15 °C, stroke volume and cardiac output returned to within baseline values in Milrinone treated animals, while these variables were significantly reduced in saline controls.
Conclusions: Milrinone ameliorated cardiac dysfunction during rewarming from 15 °C. The present results suggest that at low core temperatures and during rewarming from such temperatures, pharmacologic efforts to support cardiovascular function is better achieved by substances preventing cyclic AMP breakdown rather than increasing its formation via β-receptor stimulation.

Introduction

Accidental hypothermia was the main cause of death among passengers of the Titanic, which sank in the North Atlantic Ocean in 1912 [16]. More recent case reports have stated that core temperatures down to 13.7 °C [7] and nearly 7 h of hypothermic cardiac arrest may be tolerated [15]. Upon this knowledge and the fact that the first responding ship arrived to aid the Titanic only 1 h and 50 min after the sinking, a similar catastrophe could possibly have had a better outcome today. Currently, ship traffic and activity in the search for gas and oil in the Arctic areas are increasing [1]. This is leaving large amounts of people exposed to a cold environment with low sea temperatures, far from hospitals capable of rewarming victims of accidental hypothermia. Successful resuscitation of several such victims was recently demonstrated after the Præstø Fjord accident [23]. However, rewarming these patients is often complicated by a potentially fatal cardiac dysfunction with gradual reduction in stroke volume (SV) [17]. The underlying pathological mechanisms are not fully understood, but experimental studies have found dysfunction mainly in the cardiovascular system [17,24].

In order to ameliorate hypothermia-induced cardiac dysfunction, cardioactive drug therapy aimed at elevating low SV seems advisable. The need for such inotropic support during hypothermia is established in surgical procedures on the aorta [3], where patients

Abbreviations: CO, cardiac output; TPR, total peripheral resistance; PDE3, phosphodiesterase 3; SV, stroke volume; G_p, parallel conductance; MAP, mean arterial pressure; LV, left ventricle; $LVdP/dt_{max}$, the maximum rate of LV pressure change; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; LVEDP, left ventricle end-diastolic pressure; CI, cardiac index; SW, stroke work.

☆ *Statement of funding:* The Norwegian Research Council supported this study.

* Corresponding author at: Anesthesia and Critical Care Research Group, Department of Clinical Medicine, Faculty of Health Sciences, UiT, The Arctic University of Norway, 9037 Tromsø, Norway.

E-mail addresses: erik.sveberg.dietrichs@norskluftambulanse.no (E.S. Dietrichs), timofey.kondratiev@uit.no (T. Kondratiev), torkjel.tveita@uit.no (T. Tveita).

http://dx.doi.org/10.1016/j.cryobiol.2014.09.002
0011-2240/

are cooled to temperatures down to 15 °C [14]. The effects of cardiovascular drugs used during hypothermia and rewarming are however not well described. This is reflected by the striking lack of consensus-based guidelines for use of such drugs during rewarming from accidental hypothermia [21]. Several studies in our *in vivo* rat model have shown that dose-dependent inotropic effects of cardiac β-receptor agonists are altered by hypothermia, resulting in lack of ability to elevate SV and cardiac output (CO) [8,10,11,19].

Through binding of sarcolemmal β-receptors in cardiomyocytes, β-receptor agonists exert inotropic effects by increasing intracellular cAMP concentration. The phosphodiesterase III (PDE3) inhibitor Milrinone differs from β-receptor agonists as it increases cAMP through inhibiting the cytosolic enzyme breaking it down. A recent experiment demonstrated that Milrinone elevates SV and CO during cooling to 15 °C [18]. Further, Milrinone also possess vasodilating properties in normothermic conditions [4] and has been used for inotropic support during successful resuscitation from hypothermic cardiac arrest [15]. Use of Milrinone is however also associated with side effects in normothermic acute heart failure patients [5]. Based upon knowledge of altered effects of β-receptor agonists [8,10,11,19] during rewarming in our rat model, it is therefore important to establish whether good inotropic support can be provided through cytosolic strategies like PDE3 inhibition.

To assess whether Milrinone has positive effects on SV and CO during rewarming from stable hypothermia at 15 °C (core temperature), we used our experimental model where spontaneous cardiovascular activity is maintained throughout the temperature protocol.

Materials and methods

Male Wistar rats (233–365 g) were provided by Charles River (Sulzfeld, Germany) and used in the experiments. The rats had a microbiological status according to the recommendation of the Federation of European Laboratory Animal Science Associations. The animals were quarantined for 1 week on arrival. During experiments, housing was provided in accordance with guidelines for accommodation and care of animals (article 5 of European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 18.III.1986). Housing conditions for the rats were controlled with temperature and humidity maintained at 21 ± 1 °C and 55 ± 5%, respectively. The ambient temperature in the surgical theatre was also kept at 21 ± 1 °C. The animals were allowed free access to food and water. The experimental protocol was approved by the Norwegian Animal Research Authority and conducted accordingly.

Anesthesia

Anesthesia was induced intraperitoneally by pentobarbital sodium (55 mg/kg) and fentanyl (50 μg/kg), followed by a continuous infusion of 7.5 mg kg^{-1} h^{-1} pentobarbital sodium and 50 mg kg^{-1} h^{-1} fentanyl through an intravenous line in the right jugular vein, extended to the right auricle. The infusion was maintained at all hours in normothermic animals. Infusion in hypothermic animals was terminated at 30 °C during cooling and restarted at the same temperature during rewarming, due to hypothermia-induced anesthesia and reduced drug metabolism. The animals were monitored by toe-pinch for any sign of discomfort so that additional anesthesia could be provided if necessary.

Respiratory support

Animals were placed on the operating table in a supine position. The trachea was opened, and a tracheal tube inserted. All animals had spontaneous and sufficient ventilation at core temperatures >20 °C. Below 20 °C, ventilation was achieved by a volume-controlled small-animal respirator (New England rodent ventilator, model 141, New England Instruments, Medway, MA) using room air.

Core cooling and rewarming

Animals were core cooled and rewarmed by circulating cold or warm water (Thermo stated water bath type RTE-110, Neslab Instruments, Newington, NH) through an U-shaped polyethylene tube placed in the lower bowel. The tube was inserted gently to avoid harm of the intestine. In addition, the double-layered operating table made of hollow aluminum was circulated by temperature-adjusted water. Core temperature was continuously monitored using a thermocouple wire positioned in the lowest part of esophagus, connected to a thermocouple controller (Thermalert Th-5, Bailey Instruments). The hypothermic period (15 °C) lasted 3 h, while cooling and rewarming each lasted 2 h. The rate of core rewarming was chosen based on clinical practice in our university hospital, where fast rewarming has proven successful in hypothermic patients after nearly 7 h of hypothermic cardiac arrest [15] and with core temperatures down to 13.7 °C [7].

Experimental protocol

After surgery, animals were allowed to rest for 45 min before start of experiments. Animals in hypothermic groups were cooled to a core temperature of 15 °C and maintained at this temperature for 3 h, before rewarming to 37 °C. In the normothermic group, animals were held at 37 °C for 5 h. Milrinone (Corotrop, Sanofi-Aventis, Paris, France) or saline was administrated through an intravenous line in the femoral vein, extended to the inferior caval vein. Doses were chosen according to a previous study in the present rat model, using Milrinone during cooling [18].

Normothermic Milrinone group (group 1, n = 6)

Animals received a bolus dose of 0.25 ml Milrinone (0.1 mg/ml) after 3 h of normothermia. This was followed by a continuous infusion of 1.2 ml/h during the last two hours of experiments.

Hypothermic Milrinone group (group 2, n = 7)

Animals were given a 0.25 ml (0.1 mg/ml) Milrinone bolus after 2 h of hypothermia. This was followed by a continuous infusion of 1.2 ml/h given during the last hour of stable hypothermia (15 °C) and during rewarming.

Hypothermic saline control group (group 3, n = 7)

Animals were given a 0.25 ml bolus dose of isotonic NaCl after 2 h of hypothermia. This was followed by continuous infusion of 1.2 ml/h during the last hour of stable hypothermia (15 °C) and during rewarming.

Hemodynamic measurements

Hemodynamic variables were obtained using a Millar pressure–volume conductance catheter (SPR-838, Millar Instruments Inc., Texas). The miniaturized 2.0 French pressure–volume conductance catheter allowed for the assessment of *in vivo* left ventricular (LV) mechanical function in rats [2]. A constant sinusoidal alternating current (0.02 mA root means square at 20 kHz) was applied to drive the conductance catheter, through which changing conductance was used for the measurement of blood volume. Volume measurements in this study included parallel conductance (G_p). Further description of this method and calibration of the catheter is described in detail in a previous report [8]. In addition, mean arterial pressure (MAP) was measured using a pressure transducer connected to a fluid-filled catheter (22G) inserted into the left