Bioelectrochemistry 125 (2019) 58–69

Contents lists available at ScienceDirect

Bioelectrochemistry

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

The efficiency of heart protection with HTK or HTK-N depending on the type of ischemia

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ARTICLE INFO

Article history:
Received 11 July 2018
Received in revised form 23 August 2018
Accepted 17 September 2018
Available online 20 September 2018

Keywords:
Heart protection
Cardioplegia
Ischemia
Reperfusion
Gap junctions
Calcium

ABSTRACT

We investigated isolated guinea pig hearts (n = 121) in an ischemia/ reperfusion model with the aim to compare the efficiency of the cardioplegic solution HTK with its novel replacement HTK—N.
Following consolidation with Tyrode's solution, ischemia started either immediately or after preceding cardioplegia with HTK, HTK—N, or modified HTK enriched with Ca. Ischemia lasted either 80 min at 30 °C, or 360 min at 5 °C, or 81 min at 30 °C with intermittent cardioplegic perfusion. During ischemia we measured intracellular calcium (iCa^{++}) and the time of gap junction uncoupling (t-in). During reperfusion we measured the re-establishment of cell coupling (t-ret), left ventricular developed pressure (LVDP), and heart rhythm (VC-RR).
In 5 °C groups, iCa^{++} at t-in was significantly higher than before ischemia, and longest t-in, shortest t-ret, and best VC-RR were observed after HTK-protection. Of all 30 °C groups, the intermittent group with modified HTK showed shortest t-ret, best VC-RR, and the highest LVDP. At 5 °C, HTK groups had higher LVDP than HTK-N groups, but not at 30 °C.
The data suggest that the higher calcium level in the HTK-N solution improves reperfusion after short ischemia at 30 °C but for long lasting ischemia at 5 °C it is beneficial to use the HTK solution.
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1. Introduction

The cardioplegic solution HTK (Histidine-Tryptophane-Ketogluterate, Custodiol®, Koehler Chemie, Alsbach Haehnlein, Germany) was developed in 1964 by Bretschneider and has been used as an alternative to other preservation methods in clinical cardiac operations for several decades [1]. The HTK solution is used, for example, in transplants to protect the heart for long periods of cold temperature ischemia as well as for short periods during heart surgery as an alternative to intermittent blood perfusion [2–4]. For heart transplants, the maximum recommended ischemia duration is between 6 and 8 h [5] and even for heart operations on children, ischemia times of up to 4 h do occur [6]. The ischemia conditions for the clinical use of the HTK solution for heart protection thus vary from shorter ischemia periods at possibly warm tissue temperatures to ischemia durations of >6 h at cold temperature. In the above-mentioned pediatric operations, the protection with HTK solution is used as an alternative to the classic method with intermittently administered protection with St. Thomas solution [6]. For intra-operative protection by intermittent perfusion, clinical solutions such as the St. Thomas solution are used, whose composition resembles the extracellular environment. To our knowledge, intermittent perfusion of solutions such as the HTK solution, whose ionic composition is more similar to that of the intracellular environment, is not used in clinical applications but it is conceivable.

A few years ago, the company Koehler developed a new protection solution called HTK—N, which showed a faster and better post-ischemic recovery of hearts in ischemia/ reperfusion experiments compared to hearts protected with HTK solution [7–9]. In these ischemia/ reperfusion experiments the ischemia duration was 60 min. To our knowledge, the application of HTK-N for cardiac protection in ischemia/ reperfusion experiments has not yet been investigated under other ischemia conditions.

In this work we investigated the protective effect of HTK and HTK-N under different ischemia conditions, as they can occur during heart surgery or transplantation. We chose three different conditions: 80 min

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https://doi.org/10.1016/j.bioelechem.2018.09.005
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ischemia at 30 °C, 81 min ischemia at 30 °C with short intermittent perfusions of the cold cardioplegic solution, and 360 min ischemia at 5 °C. We measured gap junction coupling, intracellular calcium, heart rhythm, diastolic and developed pressure to obtain information about electrical, chemical and mechanical changes during ischemia/ reperfusion.

2. Materials and methods

2.1. Animals and heart preparation

All animals were treated humanely in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85–23, revised 1985) and the German Animal Protection Law (Tierschutzgesetz 1998). All experiments were approved by the animal protection committee.

The experiments were carried out on hearts of 121 female guinea pigs weighing between 150 g and 300 g. The guinea pigs were anaesthetized with pentobarbital (60 mg/kg body weight) by i. p. injection, decapitated and the heart was then exposed by opening the abdominal cavity. The hearts were excised and incubated in a Petri dish filled with ice cooled modified Tyrode's solution (mmol/L: NaCL 120, KCL 5.5, $CaCL_2$ 2.5, $MgCL_2$ 0.5, NaH_2PO_4 0.5, glucose 5, HEPES 5, HEPES-Na 5.6, $NaHCO_3$ 27). A cannula was inserted into the aorta and the heart was flushed with the Tyrode's solution for 2 min. Afterwards, the hearts were connected to a Langendorff apparatus and were perfused with 37 °C warm Tyrode's solution. The Tyrode's solution was aerated with carbogen gas consisting of 95% O_2 and 5% CO_2.

Fig. 1 shows the heart in the Langendorff apparatus and the arrangement of the probes.

Through an opening in the left atrium, a cannula with a balloon made of plastic foil was pushed into the left ventricle. The cannula was connected to a pressure transducer (Edward Lifesciences, Irvine, CA) and the balloon could be filled with water via a syringe. The pressure in the balloon was read into a customized computer monitoring program via a customized measuring bridge with preamplifier and AD converter.

Two thin wires were attached to the atrium and to the tip of the heart for measurement of the electrocardiogram (ECG) using a customized preamplifier, a customized analog to digital interface, and the same customized computer monitoring program.

A surface probe with 4 electrodes was elastically pressed against the right ventricle to measure the electrical tissue impedance. The left ventricle was touched by an optical reflection measuring probe (FCR-7UV200-2-1,5 × 100, Avantes, Apeldoorn, Netherlands). This rigid reflection probe was attached to a force transducer (FORT100, WPI, Berlin, Germany). The force transducer could be moved linearly with a stepper motor. The force was measured with an analog measuring bridge (TBM4M, WPI, Berlin, Germany) and the electrical force signals were read with an AD converter into the above-mentioned customized computer monitoring program. Via a customized interface, the stepper motor was controlled in such a way that the contact pressure of the reflection probe to the heart remained constant during the ischemia/ reperfusion experiments.

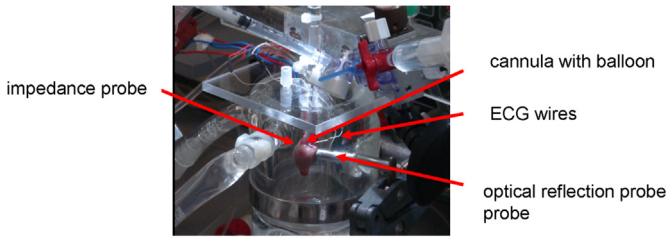

Fig. 1. Guinea pig heart with the attached measuring probes.

A double-walled glass container was placed over the heart from below for temperature control. The glass container was rinsed with water at a suitable temperature. The temperature was measured at two points with temperature sensors (NTC resistor 10K3MCD1 with a diameter of 0.5 mm, Delta Regeltechnik GmbH, Muenchen, Germany). One temperature sensor was located in the right ventricle and the second sensor was located at a distance of 5 mm from the heart surface. Because of the small heart dimensions and the short diffusion distances, a 100% nitrogen atmosphere was created around the heart during ischemia. The nitrogen gas fed into the glass container was heated and humidified to prevent the heart tissue from drying out during ischemia.

2.2. Experimental groups

In our ischemia/ reperfusion experiments we compared untreated hearts (group UI) with hearts that received a pre-ischemic protection with HTK (group HTK) or HTK-N (group HTK—N) solution. Table 1 shows the components of the HTK and HTK-N solution [7].

Three different ischemic situations were investigated: 80 min ischemia at 30 °C (Isch80), 81 min ischemia with intermittent perfusion of cold perfusion solution (i-Isch80, for reasons of uniformity, the additional minute was not included in the name), and 360 min ischemia at 5 °C (Isch360). An overview of the groups and their sizes is given in Table 2.

The groups iHTKCa80 (HTK with a total calcium of 0.05 mmol/L) and iHTKCaN80 (HTK with a total calcium of 0.03 mmol/L) are HTK solutions with an addition of calcium. As described above, protection with HTK solution is similar to intermittent blood perfusion [2–4]. For our comparison between HTK and HTK—N, we have omitted the iUI80 group in our ischemia/ reperfusion model.

In untreated hearts, ischemia was initiated by switching off the perfusion with Tyrode's solution. At the same time, the temperature of the water in the double-walled glass container was changed from 37 °C to the respective temperature during ischemia and the flow of the nitrogen gas was started.

For the pre-ischemic protection with HTK, HTKCa, HTKCaN, or HTK-N solution, the perfusion with Tyrode's solution was stopped and the hearts were perfused for 10 min with approx. 5 °C cold protection solution. The perfusion was flow-controlled and started at 6.3 mL/min during the first minute. From the second to the eighth minute the flow rate was 3.2 mL/min and was reduced to 1.6 mL/min for the ninth and tenth minute. During the ten-minute protection period, the water temperature in the double wall of the glass container had been already changed and the nitrogen gas flow had started. Ischemia started with the end of perfusion.

In the group of intermittently perfused hearts, three perfusions were performed during ischemia for 4 min each. The flow of the 5 °C cold

Table 1
Components of the cardioplegic solutions [7].

	HTK/mmol/L	HTK-N/mmol/L
Na^+	15	16
K^+	10	10
Mg^{2+}	4	8
Ca^{2+}	0.015	0.02
Cl^-	50	30
L-histidine	198	124
N-α-acethyl-L-histidine	–	57
Tryptophan	2	2
A-ketogluterate	1	2
Aspartate	–	5
Aginine	–	3
Alaniane	–	5
Glycine	–	10
Mannitol	30	–
Sucrose	–	33
Defoxamine	–	0.025
LK-614	–	0.0075

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