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Therapeutic hypothermia — a powerful tool in preventing ischemia-reperfusion-injury in rat hearts

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Therapeutic hypothermia — a powerful tool in preventing ischemia-reperfusion-injury in rat hearts

Hipotermia terapeutyczna — potężne narzędzie w zapobieganiu uszkodzeniu niedokrwienno-reperfuzyjnemu w sercach szczurów

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Abstract

Introduction. The aim of the experiment was to find the relationship between the extent of myocardial infarction and the internal body temperature of the rats. Our second goal was to investigate if mild hypothermia applied during myocardial ischemia and reperfusion brings cardioprotective effects.

Materials and methods. The study was performed in vivo on rat hearts. Myocardial infarction was induced by 30 minutes of the left anterior descending artery occlusion followed by 60 min reperfusion. Thirteen rats were assigned to a group depending on the internal body temperature (t) $\leq$ 35.5°C (n = 5), 35.6 $\geq$ t $\geq$ 37.5°C (n = 4) and t $\geq$ 37.6°C (n = 4). The desired temperature was achieved during pre-ischemic procedures. The presence of the infarction scar and its size were assessed in planimetry. Infraction size was calculated as the infarct area relative to the area at risk (IA/RA).

Results. The incidence of infarction scar in the groups of rats with body temperature $\geq$ 35.6°C was significantly higher ($p < 0.01$) compared to the group with the temperature $\leq$ 35.5°C.

There was statistically significant positive correlation ($r = 0.787$, $p < 0.01$) between IA/RA and the internal body temperature of the rats.

Conclusions. Any reduction in the body temperature during myocardial ischemia and reperfusion seems to be beneficial for the rat's myocardium. Moreover, hypothermia ≤ 35.5ºC applied during ischemia and reperfusion fully prevents from myocardial infarction. We believe that there is a great need to intensify research on therapeutic hypothermia in humans, so that this method of treatment could be finally used in the course of myocardial infarction.

Key words: ST-elevation myocardial infarction, internal body temperature, cardioprotection

Introduction

Percutaneous coronary intervention is a treatment of choice in patients with ST-elevation myocardial infraction (STEMI). Unfortunately, the reperfusion itself leads to further myocardial damage and the final area of infarction is the result of ischemia and reperfusion injury (IRI). Many methods for diminishing IRI have been tested; one of them is therapeutic hypothermia (TH) [1]. TH is widely used for myocardial protection during cardiac surgery and also for neurological protection after sudden cardiac arrest, but still not established in the course of myocardial infarction (MI).

The aim of this study was to investigate the influence of internal body temperature of the rats during myocardial ischemia and reperfusion on the infarct size. Our second goal was to determine if mild hypothermia applied during myocardial ischemia and reperfusion is cardioprotective. In the article we also present data from clinical trials and shortly discuss the discrepancy in effectiveness of TH between experimental studies and clinical trials.

Materials and methods

The study was performed in 13 female Sprague Dawley rats. All the experiments were conducted in compliance with National Research Council‘s guidelines for the care and use of laboratory animals. All procedures were also approved by the Local Ethical Committee. Animals were anaesthetized by intraperitoneal administration of ketamine (100 mg/kg) and xylazine (10 mg/kg). If needed, half a dose of anesthetics was repeated every 30–60 min

Surgical procedures

Rats were connected to a rodent ventilator (Harvard Apparatus — VentElite) and ventilated with room air at the breathing rate of 100/min and the tidal volume of 1 mL/100 g body mass. The electrocardiographic monitoring was performed continuously (limb leads). In each animal, the chest was opened through the fifth intercostal space. Left anterior descending artery (LAD) was ligated at the bottom of the left atrium. Ischemia was confirmed by electrocardiographic (ECG) changes (Figure 1). After 30 min of ischemia, the ligation was resolved.

A **B**

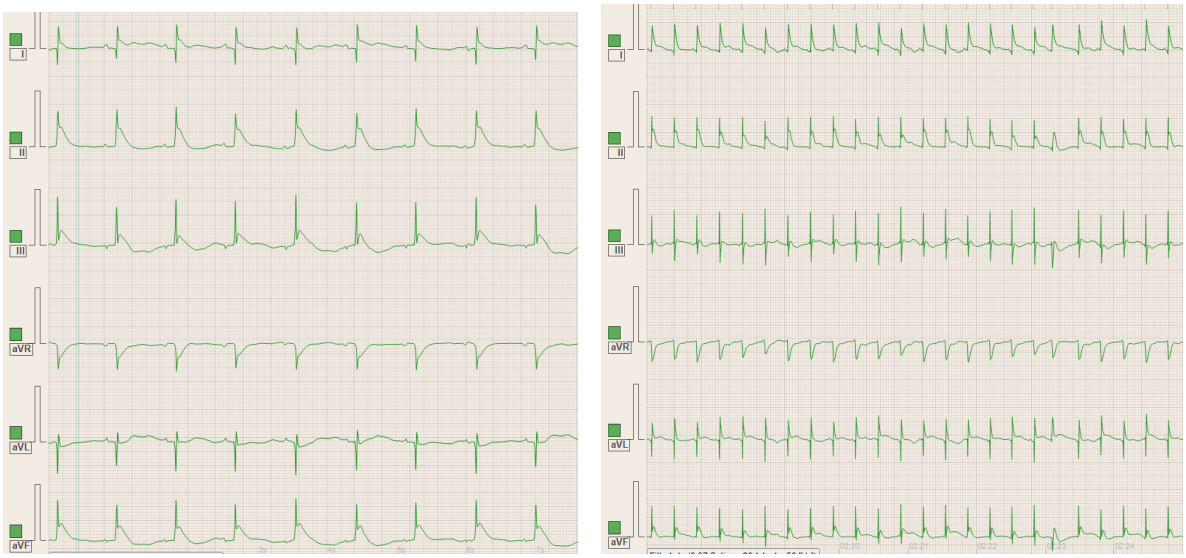

Figure 1. ST-elevation after left anterior descending (LAD) occlusion: **A.** Sinus bradycardia 75/min in rat with internal temperature 35.1°C; **B.** Normal sinus rhythm 170/min with single ventricular extra beat in rat with internal body temperature 38.0°C

Detection of the myocardial infarction

After 60 min of reperfusion, the LAD was occluded again and 2 ml of 5% Evans Blue was administered intravenously. All perfused tissues tinted dark blue and the myocardium supplied by the occluded artery remained pink [area at risk (RA)]. The assessment of the infarct area (IA) was made by tetrazolium staining with the use of planimetric method (ImageJ) [2].

To measure IA independently from anatomical variability of vascularization, IA as a percentage of RA was calculated.

Temperature monitoring and perioperative hypothermia

Our goal was to achieve the internal body temperatures from 35.0 to 38.5°C. After anesthesia and shaving, the animals were put on an operating table, which was covered with a temperature controlled heating pad. The temperature of the heating pad was regulated and the desired internal body temperature was achieved before the start of ischemia. The temperature was recorded by a rodent rectal thermometer and from the beginning of ischemia to the end of reperfusion it was kept close to the intended target.

Statistical analysis

The animals were assigned to a group depending on the internal body temperature (t) ≤ 35.5°C (n = 5), 35.6 ≥ t ≥ 37.5°C (n = 4) and t ≥ 37.6°C (n = 4). Differences in the incidence of infarction scar between the groups (assessed by the presence of IA or not), were tested with the Chi-square test.

The Spearman correlation was used to test the correlation between the body temperature of the rats and the infarct area relative to the area at risk (IA/RA). Due to the lack of infarcts in the animals with the temperature ≤ 35.5°C, the mean value of the temperature in this group was used to calculate correlation coefficient.

Results

The internal body temperatures of the rats ranged from 34.8 to 38.6°C (Table 1). In the groups of rats with body temperature 35.6 ≥ t ≥ 37.5°C (n = 4) and ≥ 37.6°C (n = 4) in all animals IA was clearly visible (Figure 2A, B). In the group with body temperature ≤ 35.5°C (n = 5), IA has not been detected at all in any of the rats (Figure 2C). The frequency of IA detected in planimetry in groups of rats with body temperature ≥ 35.6°C was significantly higher ($p < 0.01$) compared to the group with the temperature ≤ 35,5ºC (Table 2). There was statistically significant positive correlation ($r = 0.787$; $p < 0.01$) between IA/RA and the body temperature of the rats (Figure 3).

Table 1. Internal body temperatures of the rats (n = 13) and the corresponding infarct area relative to the area at risk (IA/RA) ratios

Internal body temperature of the rats [°C]	**IA/RA [%]**
38.6	38.7
38.3	37.7
38.2	39.1
38.0	34.8
37.5	38.8
37.5	30.4
36.5	25.2
36.0	16.0
≤ 35.5 (n = 5)	0

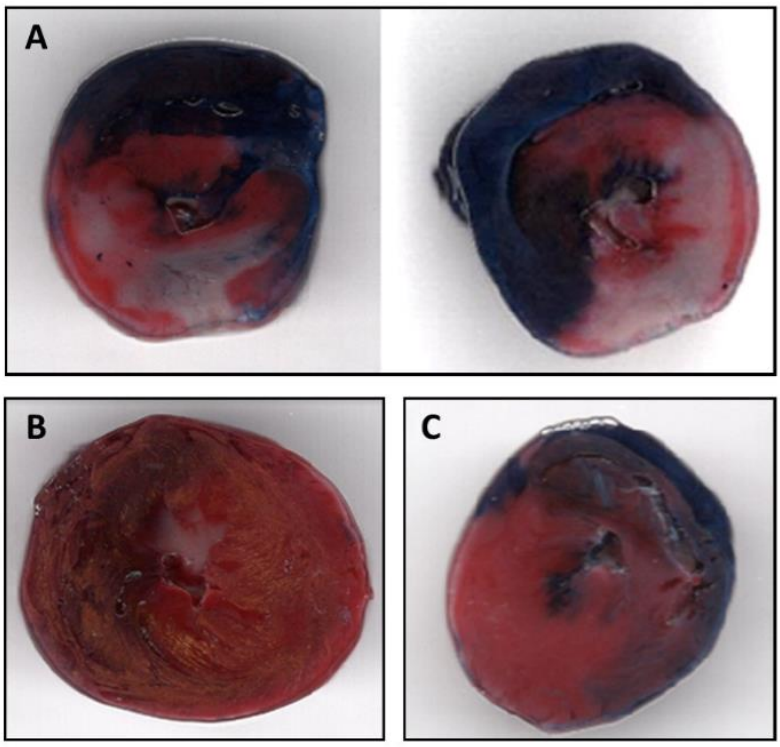

Figure 2. Transverse cross-sections of the hearts (examples). White color — infarct area (IA), red— myocardium at risk (RA), blue— area not supplied by left anterior descending: **A.** Temperature 38.0°C, clearly visible IA; **B.** Temperature 36.0°C, small IA in subendocardium; **C.** Temperature 35.1°C, no visible IA

Table 2. The incidence of infarction scar depending on internal body temperature (Chi^2 Pearson: 13.00, df = 2, $p < 0.01$)

Temperature range [°C]	**Presence of infarction scar (IA, infarct area)**	
	Yes	**No**
$t \leq 35.5$	0	5 (100%)
$35.6 \leq t \leq 37.5$	4 (100%)	0
$t \leq 37.6$	4 (100%)	0

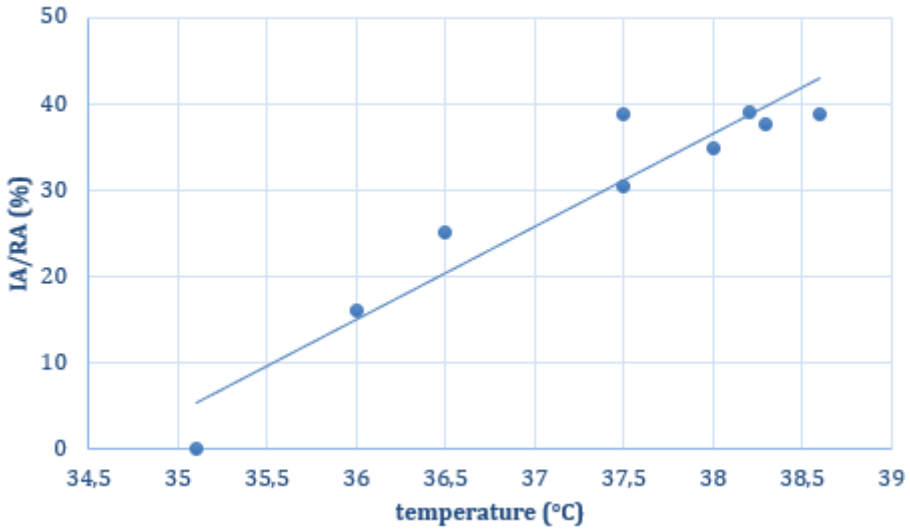

Figure 3. Correlation between infarct area relative to the area at risk (IA/RA) and the internal body temperature ($r = 0.787$, $p < 0.01$)

Discussion

TH in experimental studies

Cardioprotective effects of TH have been demonstrated in different animal models of IRI [3–7]; however, only few of them have been performed on rats [5, 7]. Due to little coronary collateral circulation, reliable infarct production and relatively high survival rate, rats have become commonly used models in translational research for IRI experiments [8].

The aim of our study was to investigate the dependence of the infarct size on internal temperature of the animals during ischemia and reperfusion. As the applied hypothermia varies between the experiments in a range of 32–36ºC [3–7] our second goal was to determine if mild hypothermia brings cardioprotective effects.

The most important finding of our experiment is that in the course MI induced by 30 min of ischemia and 60 min of reperfusion, the internal body temperature in the range of 34.8–38.6ºC is positively correlated with the IA/RA ratio. Interestingly, this relationship is also visible within the normal range (37.5–38.5ºC), which means that any reduction in body temperature during ischemia and reperfusion is beneficial for the myocardium [9]. On the other hand, it also means that inadequate temperature control may be a source of noise in infarct size studies [2].

In the group of rats with body temperature ≤ 35.5ºC infarction has not been detected at all and it can be generally deduced, that the temperature point, below which cardioprotective effects of hypothermia become evidently visible is 35.5ºC. As hypothermia depresses all physiological functions, including cardiac (e.g. through bradycardia), it seems that energy demands of the cardiomyocytes ≤ 35.5ºC are so reduced, that 30 min of ischemia is too short to lead to cell death.

Our results indicate that mild hypothermia is cardioprotective [4, 6] and there is no need to lower the temperature to 32–34ºC. Similar results were obtained with the neuroprotection [10]. It seems important, since the higher the target temperature, the easier it is to reach it; moreover, the risk of possible complications decreases.

TH in clinical trials

The results of clinical trials on TH and MI are inconclusive [11]. A small analysis carried out in humans demonstrated that the area of infraction in comparison with the myocardium at risk after TH was significantly reduced [12]. On the other hand, CHILL-MI trial, which was performed on 120 patients with STEMI, demonstrated that TH did not significantly reduce the area of MI [13]. Another multi-center randomized trial on 101 patients showed that the TH had no impact on myocardial salvage assessed in magnetic resonance [14]. However, systemic review and meta-analyses [15, 16], same as detailed analysis of negative studies mentioned above: CHILL-MI trial and the study of Testori et al. [13, 14] showed that patients with STEMI of anterior wall was the subgroup that significantly benefited from TH. Also a study of Noc et al. [17] showed absolute 30% reduction of infarct size in anterior STEMI patients.

Failure to transfer TH from animal models to the clinic

Despite the very good results obtained in animals, the results in humans are not so spectacular. Too late implementation of TH may be the reason, as in most clinical studies hypothermia was induced only before reperfusion therapy [13, 15, 17], which in some cases additionally delayed invasive treatment. In contrast, in most animal studies TH was applied at the beginning of ischemia [3, 6, 7]. In one of the study performed on rabbits it was shown that the later TH was induced during the ischemia-reperfusion period, the worse the benefits were; however, the temperature at the time of reperfusion most strongly correlated with the degree of IRI [4]. Therefore, we believe that one of the methods to improve the benefits of TH in clinical trials is to initiate TH before hospital, preferably at the time of first medical contact. Conventional method of inducing TH, which is cold saline infusion or surface cooling systems like cooling blankets or pads, can be easily used. And it is not about pre-hospital achieving the desired temperature, but any reduction of the temperature seems to be beneficial and speeds up getting the target with more advanced method.

Another aspect that may be important is the type of MI. In animal studies MI is usually induced by the LAD occlusion [5–8], which in electrocardiography causes ST segment elevation. This may suggest that clinical trials should focus on patients with STEMI of anterior wall, which is partly evidenced by the encouraging effects of hypothermia in this group, which was discussed above.

There are many other questions concerning TH in humans, like the best cooling method (intravascular? intracoronary?), the desired temperature, the duration of hypothermia during reperfusion and heating rate. All of these are important and necessary to establish.

Limitations of the study

Some consider the 60 min of reperfusion is too short for tetrazolium staining. However, based on our previous experience and other studies [18–20], this reperfusion was long enough for the relevant areas to be easily outlined.

Also the method of induction and maintenance of hypothermia may be a limitation of the study. Surface methods are not precise; however, they are used commonly [3, 4, 6, 21].

Conclusions

Hypothermia ≤ 35.5ºC applied during 30 min of ischemia and 60 min reperfusion is a powerful tool to decrease the infarction area. Moreover, any reduction in body temperature during ischemia and reperfusion, also within the normal range, is beneficial for the rat's myocardium. We conclude that further research on hypothermia is necessary to transfer this powerful tool into clinical practice, so that TH could be used in the course of MI in humans.

Conflict of interest

None declared

Streszczenie

Wstęp. Celem doświadczenia było zbadanie zależności obszaru zawału (IA) od temperatury wewnętrznej szczurów oraz określenie, czy łagodna hipotermia zastosowana podczas niedokrwienia i reperfuzji działa kardioprotekcyjne.

Materiały i metody. Badanie przeprowadzono *in vivo* na sercach szczurzych. Zawał serca wywoływano poprzez 30-minutowe zamknięcie gałęzi przedniej zstępującej (LAD), po czym następowała 60-minutowa reperfuzja. Trzynaście szczurów przydzielono do grup zależnie od wewnętrznej temperatury ciała (t) ≤ 35,5°C (n = 5), 35,6 ≥ t ≥ 37,5°C (n = 4) oraz t ≥ 37,6°C (n = 4). Pożądaną temperaturę wewnętrzną osiągano podczas czynności poprzedzających rozpoczęcie niedokrwienia. Obecność zawału i jego rozmiar oceniano planimetrycznie. Obszar blizny zawałowej określano jako procent obszaru zagrożonego martwicą (RA).

Wyniki. Występowanie blizny zawałowej w ocenie planimetrycznej w grupach zwierząt z temperaturą ≥ 35,6°C było znacząco wyższe ($p < 0,01$) niż w grupie z temperaturą ≤ 35,5ºC. Wykazano istotną statystycznie pozytywną korelację ($r = 0,787$; $p < 0,01$) między IA/RA a wewnętrzną temperaturą ciała szczurów.

Wnioski. Nawet niewielkie obniżenie temperatury ciała podczas niedokrwienia i reperfuzji wydaje się korzystne dla zmniejszenia uszkodzenia miokardium. Co więcej, zastosowanie hipotermii ≤ 35,5ºC podczas niedokrwienia i reperfuzji pozwala w pełni zapobiec wystąpieniu obszaru martwicy w sercach szczurzych. Autorzy niniejszej pracy wierzą, że istnieje duża potrzeba poszerzenia badań na temat hipotermii, tak aby metoda ta mogła ostatecznie znaleźć zastosowanie u chorych z ostrym zespołem wieńcowym.

Słowa kluczowe: zawał serca w uniesieniem odcinka ST, temperatura ciała, kardioprotekcja

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