

Received 1 December 2002
Accepted 27 February 2003

Short Communication

INDUCTION AND DECAY OF THERMOTOLERANCE IN HUMAN ERYTHROCYTES DETERMINED BY HEMOLYSIS

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Abstract: Hemolysis was used as an endpoint for the measurement of damage to the plasma membrane in human erythrocytes after a single or a double heat shock. The thermotolerance of erythrocytes is a transitional phenomenon, reaching its maximum at a 3-hour incubation at 37°C between the heat shocks.

Key Words: Thermotolerance, Erythrocytes, Single and Double Heat Shock

INTRODUCTION

Hyperthermia, heat treatment of cells or tissue above physiological temperature, has been used in cancer therapy [1–4]. There are many reports that using hyperthermia combined with ionizing radiation or chemical agents is an effective method applied in anticancer therapy [1, 5–8]. During the course of the therapy the patients undergo several repeated treatments with the above-mentioned agents, thus development of drug-resistance or their thermotolerance are often observed [1, 4, 8–10]. Thermotolerance is usually defined as the transient ability of the cells subjected to high temperature to survive subsequent lethal exposure to elevated temperature [1, 4, 11–13]. The mechanism of thermotolerance is not yet understood. Thermotolerance of nuclear cells appears at its maximum between 4–10 hours [1, 9, 11, 14, 15]. Our earlier experiments showed that enucleated erythrocytes subjected to a double heat shock become resistant to the second heat shock [16–21]. This was confirmed by studies on autohemolysis, osmotic fragility [17], internal microviscosity of cells [18], transport of anions [19], and vesiculation [20]. It is therefore possible that the plasma membrane is a target, probably an important one, in heat-induced damage cells, and that it plays some role in thermotolerance.

The aim of this work was to examine the change in the percent of thermotolerance depending on the incubation time at 37°C between two heat shocks.

MATERIALS AND METHODS

Blood was obtained from healthy donors and an anticoagulant solution (0.06 M trisodium citrate and 0.14 M glucose) was used. Erythrocytes were washed with phosphate-buffered saline at pH 7.4. They were suspended in the incubation medium (140 mM NaCl, 10 mM KCl, 1.5 mM MgCl₂, 10 mM glucose, 10 mM Hepes, 1 µg/ml antibiotic (penicillin), 0.005 M phosphate buffered at pH 7.4) at the hematocrit of 2%.

To examine the effect of hemolysis of erythrocytes induced by two consecutive heat shocks the following treatments were introduced:

- A. Erythrocytes were incubated at 37°C for X hours;
- B. Erythrocytes were incubated at 44°C for 15 minutes and at 37°C for X hours;
- C. Erythrocytes were incubated at 44°C for 15 minutes and at 37°C for X hours and at 48.5°C for 30 minutes;
- D. Erythrocytes were incubated at 37°C for X hours and at 48.5°C for 30 minutes.

In each experiment erythrocyte samples A, B, C, D were taken from the same individual. Several independent experiments were performed on the blood derived from different donors.

The measurements of erythrocyte hemolysis in isotonic solution were carried out following a single and a double heat shock with different incubation time at 37°C between the two heat shocks.

Absorbance of hemoglobin released from erythrocytes was measured at 540 nm.

The percentage of hemolysis was determined as [17]:

$$H = \frac{A_c - A_0}{A_{100}} \cdot 100\%$$

where:

A_c – the absorbance of supernatant for erythrocytes after a single or a double heat shock, A_{100} – the absorbance of supernatant for erythrocytes in distilled water, A_0 – the absorbance of supernatant for erythrocytes incubated all the time at 37°C.

The percentage of thermotolerance was calculated as follows [17]:

$$\% \text{ thermotolerance} = \left(1 - \frac{C - A}{D - A} \right) \cdot 100\% = \left(\frac{D - C}{D - A} \right) \cdot 100\%$$

where:

A – the values of the degree of hemolysis for erythrocytes kept all the time at 37°C, C – treated with a single heat shock, D – treated with a double heat shock.

We used the Student's test for paired data and the results were statistically significant.

RESULTS AND DISCUSSION

Figure 1 shows changes in hemolysis depending on the incubation time at 37°C. After incubation at 44°C the percent of hemolysis is very small. After a double heat shock the hemolysis is lower when the time between the shocks is 3 hours, and higher when the time between the heat shocks is 16 hours.

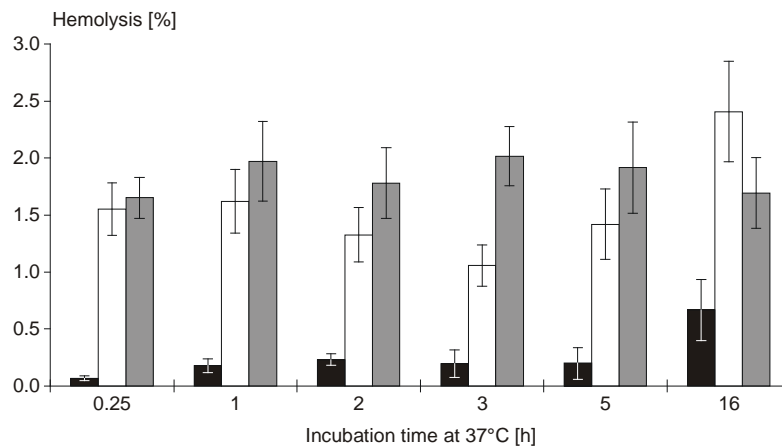


Fig. 1. Erythrocyte hemolysis depending on the incubation time at 37°C between the two heat shocks (**n** - 44°C - 37°C, **p** - 44°C - 37°C - 48.5°C, **p** - 37°C - 48.5°C).

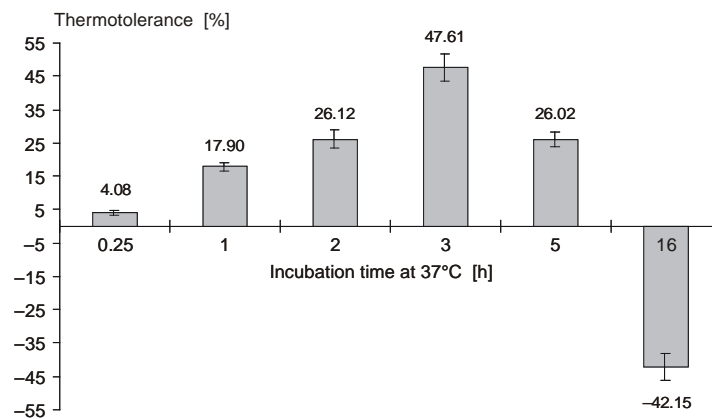


Fig. 2. Percentage of thermotolerance depending on the incubation time at 37°C between the two heat shocks.

Figure 2 shows the percent of thermotolerance depending on the incubation time between the two shocks. This phenomenon increases with the incubation time reaching its maximum at 3 hours, then it vanishes.

The results may indicate that the resistance of erythrocytes to heat is transitional similarly to that of nuclear cells [1, 9, 11, 14, 15]. This could confirm an essential role of the plasma membrane in heat induced damage of cells [1, 11, 12, 16, 22] and in thermotolerance [1, 11, 12, 16, 22].

REFERENCES

1. Hahn, G.M. **Hyperthermia and cancer**. Plenum Press, New York-London (1982).
2. Ohno, S., Sumiyoshi, Y., Mori, M. and Sugimachi, K. Hyperthermia for rectal cancer. **Surgey** 131 (2002) 121–127.
3. Reinbold, H.S. and Overgaard, J. Hyperthermia in clinical oncology. **Eur. J. Cancer** 26 (1990) 915–916.
4. Field, S.B. Hyperthermia in the treatment of cancer. **Phys. Med. Biol.** 32 (1987) 789–811.
5. Nozoe, T., Saeki, H., Ito, S., Ohna, T. and Kitamura, K. Preoperative hyperthermochemoradiotherapy for esophageal carcinoma. **Surgey** 131 (2002) 35–38.
6. Takahashi, I., Emi, Y., Hasuda, S., Kakeji, Y., Maehara, Y. and Sugimachi, K. Clinical application of hyperthermia combined with anticancer drugs for the treatment of solid tumors. **Surgey** 131 (2002) 78–84.
7. Lindegaard, J.C. Thermosensitization induced by step-down heating – A review on heat-induced sensitization to hyperthermia alone or hyperthermia combined with radiation. **Int. J. Hyper.** 8 (1992) 561–586.
8. Nielsen, J., Overgaard, J. and Kamura, T. Influence of thermotolerance on the interaction between hyperthermia and radiation in solid tumor *n vivo*. **Brit. J. Radiology** 56 (1983) 267–273.
9. Henle, K.J. and Dethlefsen, L.A. Heat fractionation and thermotolerance: A review. **Cancer Res.** 38 (1978) 1843–1851.
10. Sapareto, S.A. Thermal isoeffect, addressing the problem of thermotolerance. **Int. J. Hyper.** 13 (1987) 297–305.
11. Field, S.B. and Anderson, R.L. Thermotolerance: a review of observations and possible mechanism. **Natl. Cancer Inst. Monoger.** 61 (1982) 193–201.
12. Józwiak, Z. and Leyko, W. Role of membrane components in thermal injury of cells and development of thermotolerance. **Int. J. Radiat. Biol.** 62 (1992) 743–756.
13. Laszlo, A. Evidence for two states of thermotolerance in mammalian cells. **Int. J. Hyper.** 4 (1988) 513–526.
14. Majima, H. and Gerweck, Z.E. Kinetics of thermotolerance decay in Chinese hamster ovary cells. **Cancer Res.** 43 (1983) 2673–2677.

15. Boon-Niermeijer, E.K., Soveren, J.E., de Waal, A.M. and van Wijk, R. Thermotolerance induced by heat and ethanol. **Int. J. Hyper.** 4 (1988) 211–222.
16. Koter, M. Termoczułość błony plazmatycznej. **Praca habilitacyjna** Uniwersytet Łódzki, Łódź (1994).
17. Koter, M. and Łaski, J. Does thermotolerance occur in human red blood cells? **Int. J. Radiat. Biol.** 56 (1989) 339–349.
18. Koter, M. Effect of hyperthermia on internal microviscosity and lymphocytes. A spin label study. **Int. J. Radiat. Biol.** 58 (1990) 157–164.
19. Koter, M. Effect of hyperthermia on transport of spin-labelled electrolyte and non-electrolyte across the erythrocyte membranes. **Med. Sci. Res.** 20 (1992) 417–418.
20. Koter, M. Erythrocyte “vesiculation” after single or double heat shock. **Med. Sci. Res.** 20 (1992) 633–685.
21. Koter, M. Changes in erythrocyte membranes after fractioned hyperthermia. **Cell Biol. Int.** 17 (1993) 665–670.
22. Laszlo, A. The effects of hyperthermia on mammalian cell structure and function. **Cell Prolif.** 25 (1992) 59–87.