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Heat production is a quantitative parameter for intracellular cell function [α]

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Abstract

Heat measurements were found to provide a quantitative assay of phagocytosis in which there is a dramatic increase in heat production. Glycyrrhizin, an extract from *Glycyrrhiza glabra*, has a beneficial effect on patients with viral infections including hepatitis and AIDS. Therefore the in vivo effect of glycyrrhizin on the phagocytic function of blood neutrophils in rats was investigated. Glycyrrhizin was injected in vivo into rats twice a day for 2 days and the blood was taken from the descending aorta. Neutrophils were isolated and the phagocytic function was determined both by heat production using a thermoactive cell analyser (ESCO 3000) and by the conventional assay system using *Saccharomyces cerevisiae*.

In vivo treatment of glycyrrhizin seemed to enhance heat production by 40% during phagocytosis, as compared with neutrophils from non-treated rats. However, it was not possible to detect this difference using the conventional assay system in which small functional changes cannot be determined.

Therefore microcalorimetry can provide clear evidence of a small change in cell function and may lead to a new field in cell biology.

INTRODUCTION

Calorimetry has been used mainly in the field of chemistry. Living things such as cells produce heat which can be measured quantitatively by a microcalorimeter [1]. This method has a great advantage over biochemical,

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microscopical and histochemical methods in that the cells need not be damaged, destroyed or fixed.

Neutrophils (polymorphonuclear cells, PMNs) are phagocytes present in circulating blood and are considered to be the first line of defence against infectious agents. Their functions include chemotaxis (movement towards a chemical gradient), phagocytosis (the engulfing of any foreign particle including microorganisms) and intracellular killing (the digestion or breakdown of foreign matter) [2]. The importance of neutrophils in vivo is clearly demonstrated in the clinical situation, where the absence of neutrophils will allow survival for only 3 or 4 days at most; death will occur due to infection.

The phagocytic function of neutrophils has been measured either by biochemical methods such as superoxide anion production [3] or by microbiological methods [4]. Recently, a microcalorimetric measurement was used by Hayatsu et al. to quantify phagocytosis [5].

Glycyrrhizin, an extract from *Glycyrrhiza glabra*, has been used for the treatment of hepatitis [6] and AIDS [7]. In this paper, the in vivo effect of glycyrrhizin on the phagocytic function of blood neutrophils is investigated.

MATERIALS AND METHODS

Animals

Wistar rats, 7–8 weeks old, weight 230–250 g, were used in the experiments. The experiments were carried out according to the Guideline for Animal Experimentation in the Tokai University School of Medicine.

The total number counting of white blood cells (WBC) and the differential counting of neutrophils and mononuclear cells were performed prior to the following WBC isolation procedure. Blood was diluted with 3% acetic acid to lyse the red blood cells. The total number count and the differential count of WBC were carried out microscopically using a haemocytometer. In this procedure, neutrophils (with two or more nuclei) were easily distinguished from mononuclear cells. The results was expressed as the number of cells per litre of blood.

WBC isolation procedure

WBC were isolated from blood by sedimenting red blood cells using plasmagel [8]. The WBC were washed at least three times with RPMI 1640 medium. In the isolated WBC suspension, several different types of cell were present: neutrophils, monocytes, lymphocytes and red blood cells. The WBC suspension was adjusted to 2×10^6 cells per millilitre according to the number of neutrophils, disregarding mononuclear cells. It should be stated

here that when the WBC suspension was made, the ratio of cell type was different and it was not possible to obtain a constant ratio of cell types under any experimental procedure.

The glycyrrhizin solution for intravenous injection (Stronger Neo-Minophagen C) was provided by Minophagen Pharmaceutical Company. Glycyrrhizin (200 μg per rat) was injected twice a day at 9:00 h and 17:00 h for 2 days. As a control, saline was injected twice a day at the same times for 2 days. The WBC were isolated from arterial blood taken under anaesthetic at 9:00 h on the third day.

Measurements

Phagocytosis and the intracellular killing ability of the neutrophils were measured by the combined methods of Yamamura et al. [9] and Bridges et al. [10] using live *Saccharomyces cerevisiae* (*S. cerevisiae*). The results were expressed as the number of *S. cerevisiae* phagocytosed and killed per neutrophil.

The heat production of the resting neutrophils was carried out in the absence of *S. cerevisiae*. The result was expressed in microwatts (μW) per 10^6 WBC.

The heat production during phagocytosis was measured by the method described by Hayatsu et al. [5] using an ESCO 3000 thermoactive cell analyser. *S. cerevisiae*, killed by autoclaving, were used as the material to be phagocytosed. The ratio of *S. cerevisiae* to neutrophils was ten to one. The net heat increase in neutrophil phagocytosis was calculated as the heat production during phagocytosis minus the heat production of the resting neutrophils, i.e. not phagocytosing. The equation is

Net heat increase of neutrophil phagocytosis	=	Heat production of neutrophils during phagocytosis	− Heat production of resting neutrophils

The results were expressed in microwatts (μW) per 10^6 neutrophils.

RESULTS

The effect of glycyrrhizin on the total number of WBC, and on the differential counting of neutrophils and mononuclear cells in blood was determined. There was no statistically noticeable change in the number and differential counting of cells following glycyrrhizin treatment (see Table 1).

The effect of glycyrrhizin on phagocytosis and on the intracellular killing function of neutrophils is shown in Table 2. The number of *S. cerevisiae*

TABLE 1

The effect of glycyrrhizin on the number of neutrophils, mononuclear cells and total WBC in rat whole blood

	Neutrophils ($\times 10^9\ l^{-1}$)	Mononuclear cells ($\times 10^9\ l^{-1}$)	Total WBC ($\times 10^9\ l^{-1}$)
Control ($n = 6$) [a]	1.6 ± 0.7	6.8 ± 3.2	8.4 ± 4.0
Treated ($n = 6$) [b]	0.9 ± 0.2	6.3 ± 2.4	7.2 ± 2.6

[a] Normal saline was injected.
[b] Glycyrrhizin was injected.

TABLE 2

The effect of glycyrrhizin on phagocytosis and intracellular killing of neutrophils [a]

	Phagocytosis	Intracellular killing
Control ($n = 4$)	4.2 ± 1.0	4.2 ± 0.6
Treated ($n = 4$)	4.1 ± 0.8	3.6 ± 0.6

[a] The number of *S. cerevisiae* phagocytosed and killed intracellularly per neutrophil are indicated. The assay system was described in the text. Control and treated are defined in Table 1. The difference is not statistically significant.

phagocytosed and killed intracellularly were not statistically different in either group.

The effect of glycyrrhizin on the heat production of WBC without phagocytosis and during phagocytosis indicates that although no significant change in heat production of WBC (resting neutrophils) was observed, the net heat increase of neutrophils isolated from glycyrrhizin-injected rats was 40% higher than in the non-treated control neutrophils (see Table 3).

TABLE 3

The effect of glycyrrhizin on the heat production of resting neutrophils (no phagocytosis) and neutrophils during phagocytosis [a]

	Heat production of resting neutrophils ($\mu W\ 10^{-6}$ WBC)	Net heat increase during phagocytosis ($\mu W\ 10^{-6}$ neutrophils)
Control ($n = 6$)	1.7 ± 0.4	17.8 ± 1.9
Treated ($n = 6$)	1.4 ± 0.4	25.6 ± 2.1

[a] No statistically significant difference was found in the heat production of treated and untreated resting neutrophils, but a statistical significance ($p < 0.05$) was found in the net heat increase.

DISCUSSION

The results shown above indicate that although no detectable effect of glycyrrhizin on neutrophil phagocytic function, including phagocytosis and intracellular killing, was demonstrated by the method described by Yamamura et al. [9] and Bridges et al. [10], there was a significant increase in the net heat increase in neutrophil phagocytosis. It can therefore be concluded that the injection of glycyrrhizin into rats activated the phagocytic function of the neutrophils equivalent to the net heat increase. This activation of the phagocytic function may be important for the clearance of virus from a host infected with HB and AIDS virus.

It should be stated here that the net heat increase described is due totally to the phagocytic function of the phagocytes, i.e. the neutrophils. This statement is very important because any viable cells produce heat and different types of cell cannot be distinguished by this measurement. However, if only one type of cell is activated, as described in this paper, the measurement is due totally to the type of cell one wishes to investigate. When this is achieved, the isolation of one particular type of cell is not needed. It is also possible to state from the above result that as this activation of neutrophils is only detected by heat measurement and not by any other method, heat measurement is the most sensitive method of detecting slight changes in cell function. Therefore it may have great potential in the clinical field for the detection of even small abnormalities in phagocytic functions.

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