Thermochimica Acta, 151 (1989) 63–68
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

MICROCALORIMETRIC INVESTIGATIONS ON DIPLOID AND VIRUSTRANSFORMED HUMAN FIBROBLASTS TREATED WITH PENTOXIFYLLINE

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SUMMARY

The effect of treatment with pentoxifylline (10^{-4}M and 10^{-7}M) on the heat production (HP) of diploid (WI 38) and virustransformed (WI 38 VA 13) human embryo lung fibroblasts, growing in monolayer, was studied by microcalorimetry. HP rates were related to cell growth, glucose consumption, lactate production and cellular ATP-content. Addition of pentoxifylline did not affect HP in WI 38 fibroblasts essentially, whilst in the transformed subline a reduction of HP and glucose consumption is apparent. The present results demonstrate the usefulness of microcalorimetry as an analytical tool for monitoring alterations in metabolism of cells, caused by agents.

INTRODUCTION

The overall metabolic life process in organisms is accompanied by the consumption, transfer and production of energy and finally dissipation of energy in form of heat. The measurement of heat production (HP) of organisms by Microcalorimetry, a very sensitive technique, enables the determination of their metabolic situation. This direct and continuous calorimetric assay can be performed with as less as 10^4 cells "in vitro". In previous reports, microcalorimetry was successfully applied to detect the effect of agents like antibiotics (1) and cytostatics (2) on the thermogenesis of microorganisms or cells, respectively.

The aim of the present study was to examine, whether the metabolic activity of pentoxifylline, a derivate of xanthine, can be ascertained by microcalorimetry. Pentoxifylline is therapeutically used in the treatment of vascular diseases. The investigations were performed with human embryo lung fibroblasts, line WI 38 and a SV 40 virus-transformed subline WI 38 VA 13 over a culture period of 72 hours. HP was correlated with the following parameters: cell growth, glucose consumption, lactate production and cellular ATP-content.

METHODS

Microcalorimetry

Heat production (HP) was measured with the LKB Thermal Activity Monitor (TAM) 2277 (Pharmacia-LKB, Sweden), a thermopile heat conduction calorimeter, fitted with four ampoule measuring cylinders and operated at 37°C (3). Thermal power

004-6031/89/$03.50

(μw), generated per culture, is recorded per time as a power-time-curve (p-t-curve).

Cells and culture conditions

Human embryo lung fibroblasts of line WI 38 (4) and of transformed subline WI 38 VA 13 (5) are a gift from Prof. Bayreuther, Institute of Genetics, University of Hohenheim, FRG. We used WI 38 cells with a cumulative population doubling of 40 and WI 38 VA 13 cells with passage number 286. Cells were grown in Dulbecco's MEM (Gibco/BRL, Eggenstein, FRG) containing 1 g glucose/l, supplemented with 10% foetal calf serum (Biochrom, Berlin, FRG) and 15 mg achromycine/l (Lederle, München, FRG). Cell counts were performed with a Fuchs-Rosenthal-hemocytometer.

Cells were grown as monolayers in glass bottles under a gas phase of 95% air/5% CO_2 and incubated at 37°C. Cells of dense monolayers were suspended by trypsinization. For investigations of HP and other parameters, 1 ml of a suspension with 2.5 x 10^5 cells/ml was seeded in 3 ml glass ampoules (Machery & Nagel, Düren, FRG). The ampoules were sealed with rubber stoppers, placed in holes of a roller cylinder with a diameter of 30 cm and rolled continuously for 3-4 hours at 4.3 rph. With this method a plating efficiency of 70-80% was achieved. Thereafter, the medium was sucked off and replaced by 2.5 ml fresh growth medium (37°C) with or without the agent. Ampoules were hermetically sealed with metal caps and inserted into the TAM, where they were transfered to the measuring position in three steps. This transfer took 30 minutes. Reference cultures for the determination of noncalorimetric parameters were prepared in the same manner and kept in upright position in an incubator at 37°C. In each experiment, treated and untreated cells were derived from the identical suspension.

Determinations of glucose, lactate and ATP contents

Samples of culture medium were taken every 24 hours. The growth medium was sucked off, centrifuged (1500 rpm, 10 min.) and deproteinized with 1.2% perchloric acid. The extract was neutralized by K_3PO_4. Glucose and lactate contents were determined by enzymatic methods (6,7). To analyze ATP, cells were washed with PBS^- and trypsinized. After detachment of cells, enzyme activity was stopped with ice-cold medium, containing 10% foetal calf serum. Cells were suspended rigorously. An aliquot of this suspension was used for cell counts. The rest of the suspension was centrifuged (1500 rpm, 10 min., 4°C), the liquid phase was removed and the pellet was washed twice with ice-cold PBS^-. The final pellet was suspended in 300 μl sterile, ice-cold H_2O bidest. 200 μl of this suspension were stored at -20°C until analyzed for protein content by the method of Lowry (8). The residual 100 μl were added to the same volume of an ice-cold, aquous solution of trichloracetic acid (10% w/v) and stored in liquid nitrogen in order to

disrupt cell structures. The assay of ATP was performed by the luciferin-luciferase-system (9) in a LKB-Wallac luminometer 1251 (Pharmacia-LKB, Sweden).

Pentoxifylline

(Trental ^{R}; 3,7-dimethyl-1-(5-oxo-hexyl)-xanthine) is a gift from Hoechst AG, Werk Albert (Wiesbaden, FRG) and was used in concentrations of 10^{-7} and 10^{-4} M.

RESULTS

Cell growth of WI 38 and WI 38 VA 13 was not affected by pentoxifylline in both concentrations used (Fig.1 A,C).

Diploid cells WI 38 exhibited a p-t-curve, which is about 10% lower than that of control cultures, when treated with pentoxifylline at 10^{-4} M (Fig.1 B). The lower concentration of the substance did not alter HP of this cell line. Addition of pentoxifylline to virustransformed cells WI 38 VA 13 (Fig.1 D) caused a decrease in HP, already at 10^{-7} M. Moreover, pentoxifylline at 10^{-4} M lowered

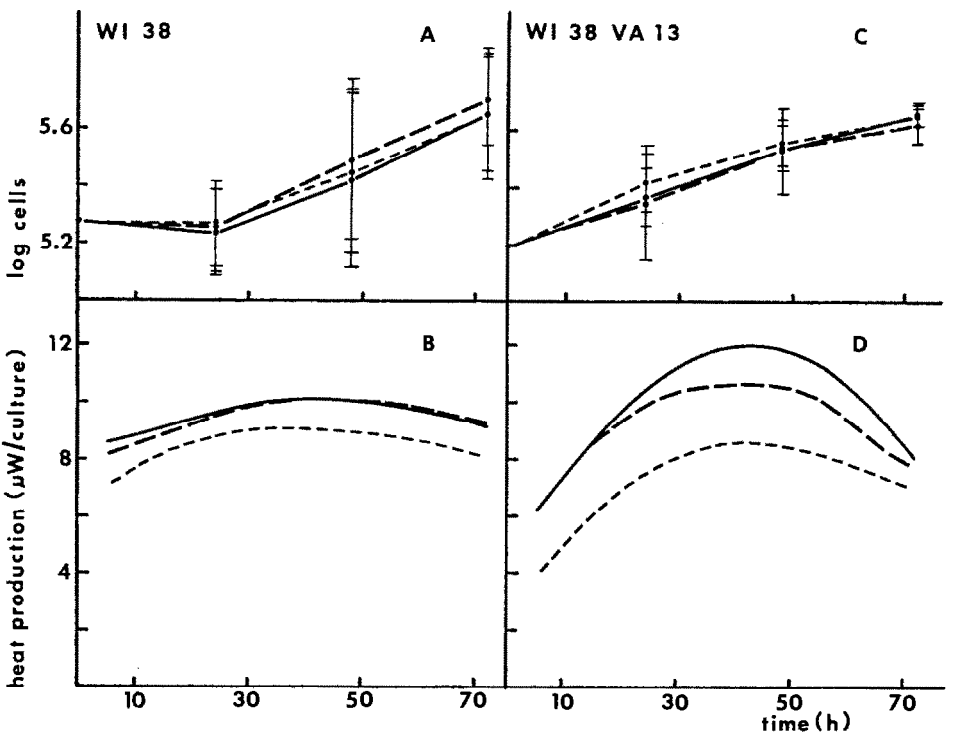

Fig. 1. Growth (panel A,C) and heat production (panel B,D) of WI 38 cells and transformed WI 38 VA 13 cells treated by pentoxifylline at 10^{-7} M (— — —) and 10^{-4} M (- - - -), compared with untreated control (———). Values shown indicate means of three individual experiments.

p-t-curves considerably and through the whole culturing. During the period of 15-55 hours after the start of the experiment this reduction of HP was about 30% in mean, if compared with the p-t-curve of the control.

The rates of glucose utilization and lactate production are outlined in Table 1. Dealing with the diploid cell line WI 38, alterations in glycolytic behaviour could not be seen between treated and untreated cells. Transformed cells WI 38 VA 13, however, exhibited less glucose consumption as well as lactate production according to the amount of pentoxifylline. Cellular ATP-contents dropped during the 72 hour culture period (Table 1). This reduction was slighter when cells were treated with pentoxifylline at 10^{-4} M.

TABLE 1

Effect of pentoxifylline on glycolysis and cellular ATP-content.
Values after a culture period of 72 hours.

		glucose consumption (u mol) n = 3	% of control	lactate production (u mol) n = 3	% of control	Reduction of ATP in cells (% of initial value) n = 2
WI 38	control	7.9 ± 0.8	100	13.4 ± 2.4	100	41.0 ± 2.8
	+ 10^{-7} M pentoxif.	7.0 ± 1.5	89	13.2 ± 3.0	99	52.5 ± 12.8
	+ 10^{-4} M pentoxif.	7.4 ± 2.0	94	13.4 ± 3.0	100	57.2 ± 9.5
WI 38 VA 13	control	8.7 ± 1.0	100	15.5 ± 0.7	100	35.9 ± 0.7
	+ 10^{-7} M pentoxif.	8.1 ± 1.4	93	14.6 ± 1.0	92	33.2 ± 11.1
	+ 10^{-4} M pentoxif.	6.8 ± 1.1	78	13.4 ± 1.6	87	51.2 ± 0.5

DISCUSSION

The results demonstrate that the number of cells, inoculated in our experiments, was suitable for appropriate microcalorimetric measurements of heat produced during the whole culture period. Although treatment of both cell lines by pentoxifylline did not result in any inhibition of cellular growth, the heat output was partially reduced. The quantity of this reduction depended on the cell type used and reflected a dose-response relationship for pentoxifylline, particularly in regard to transformed fibroblasts WI 38 VA 13. The fact that the effect of substances can be manifested by various thermogenesis was reported for other agents (10,11,12). Whereas those studies were performed for some hours, our intention was to study the effect of a substance on the HP over a whole

growth cycle. Actually this might be important for detecting an effect becoming evident later in time of culturing, as can be seen in Fig.1 D. Furthermore, a correlation between changed HP, glucose utilization and possible cellular ATP-content could be detected.

It can be stated that consumption of glucose and production of lactate reflected nearly stoichiometric conversions. Therefore HP measured can be principally deduced from glycolysis and not from oxydative phosphorylation, certainly due to the "Crabtree effect" (13). Moreover, a decreased rate of HP, as strikingly shown in the case of transformed cells treated with 10^{-4} M pentoxifylline (Table 1), correlates with a lowered rate of glucose consumption without an important change in its ratio to lactate production. The obviously lower reduction rate of the ATP-content in cells treated with pentoxifylline at 10^{-4} M - associated with the lower glycolytic rate - does not agree with determinations of an enhanced glucose consumption and higher ATP levels as reported for erythrocytes, treated with the same substance (14). Since oxygen consumption is not altered in our experiments (unpublished results), we suppose that treatment with pentoxifylline did not enhance ATP production in our cells, but lowers ATP consumption. The latter fact might possibly reduce glycolytic rate by a feedback mechanism.

We conclude that microcalorimetry can serve as an analytical tool for monitoring alterations in metabolism of cells, caused by agents, and might possibly be developed further as a screening method.

ACKNOWLEDGEMENTS

We wish to thank Ms Angela Harmsen for her excellent assistance. These investigations were supported by grants of the Bundesminister für Forschung und Technologie (FV 0318792A7).

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