Thermochimica Acta, 85 (1985) 489—492
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CALORIMETRIC AND CHEMILUMINESCENCE STUDIES ON HUMAN NEUTROPHILS

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ABSTRACT

Chemiluminescence and calorimetric studies were carried on human neutrophils activated with phorbol-12-myristate-13-acetate. A biphasic response to metabolic activation, less evident in the chemiluminescence curves, was detected by both methods of analysis. Superoxidedismutase and sodium azide strongly inhibited chemiluminescence while heat production was unaffected. It appears that both techniques give good information about the process of metabolic activation but only calorimetry can quantify the chemical energy expended during the process.

INTRODUCTION

The interaction between particulate material and human neutrophils results in activation of the cellular oxidative metabolism referred to as the "metabolic burst" (ref.1). This granulocytic activation can also be obtained with chemicals such as phorbol-12-myristate-13-acetate (PMA)(ref.2).

Neutrophils represent from the calorimetric point of view a unique model system since the metabolic burst they undergo produces very high heat effects and practically all of the enthalpic energy change derived from the oxidation of glucose is finally recovered as heat (ref.3,4). This allows a direct, continuous, and quantitative measurement of the granulocytic energy expenditure during the "burst". Using calorimetry we have recently shown that the phagocytosis of "pathogenic" *Staphylococcus aureus* strains produces heat effects three to four times higher than the effect produced by the saprophytic staphylococci, proving a good correlation between increased granulocytic energy expenditure and increased pathogenicity of the staphylococcal strains phagocytosed (ref.5).

During the metabolic burst, electronically excited molecules such as

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singlet oxygen and oxygen radicals including superoxide anion and hydroxyl radicals are formed (ref.6-8). Light is produced as a result of the relaxation of the electronically excited carbonyl chromophores generated as products of oxygenation (ref.9) and can be measured to evaluate the magnitude of the metabolic burst (ref.10).

The present studies were undertaken to compare the efficiency of the two methods, calorimetry and chemiluminescence, in the quantitave determination of the metabolic burst triggered by PMA.

METHODS

Granulocytes from whole blood drawn from healthy donors and collected into citrate-phosphate-dextrose (CPD) were prepared by a modification (ref. 3,5) of the method of Boyum (ref.11). The calorimeter employed in the experiments was the LKB 10700-1 flow calorimeter modified as previously described (ref.3). The temperature control of the instrument was set at 37°C and the voltage signal was amplified with a Keithly 150B microvolt ammeter. The cellular preparation ($5x10^6$ cells/ml of Gey's saline solution) was placed in a constantly stirred culture flask thermostatted at 37°C. The neutrophils were pumped continuosly from the culture flask into the calorimeter by a peristaltic pump (LKB varioperpex, flow rate 60 ml/h), located at the outlet connection of the instrument. After passage through the pump the cells were discarded.

All chemiluminescence measurements were performed at ambient temperature with a Packard Tri-Carb 460 C liquid scintillation counter set in the out of coincidence mode. Dark adapted glass scintillation vials with a total reaction volume of 5ml were used. The cellular concentration was $5x10^6$ neutrophils/ml. The experiment was performed in presence of 10^{-6}M of the chemilumigenic probe luminol. Chemiluminescence measurements were made automatically at 25s intervals over a period of 90 min. The granulocytic metabolic burst was triggered by the addition of 100ug/ml of PMA (ref.3).

Oxygen consumption was measured polarographycally with a Clark electrode (Yellow Spring Co.)

RESULTS AND DISCUSSION

Fig. 1 shows the comparative results of calorimetric and chemiluminescence determinations. A biphasic response to metabolic activation, less evident in

the chemiluminescence curves, was detected by both methods of analysis. In the previously reported calorimetric experiments (ref.3), we did show that the biphasic behaviour of metabolic response is the result of two different non-mitochondrial oxygen reduction pathways.

By integrating the areas of the power-time curves recorded, a value of 23.4 $\pm$ 1.8 nJ/neutrophil was calculated, corresponding to a glucose oxidation (to H_2O and CO_2) equivalent to 8.0 $\pm$ 0.6 fmoles/cell (ΔH = -488 kJ/mole O_2 in aq. sol.)(ref.3).

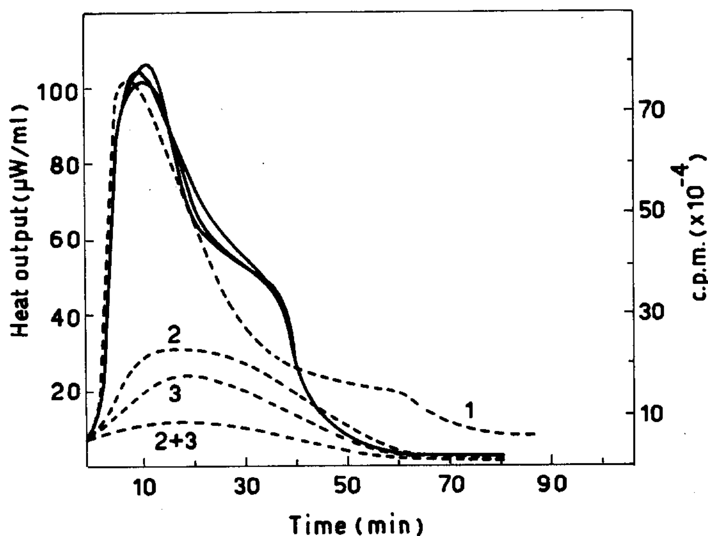

Fig.1. Calorimetric power-time curves (——) and chemiluminescence curves (----) of human neutrophils activated with 100 ug/ml PMA in absence or in presence of 1mM sodium azide (2) or 100 ug/ml SOD (3).

Fig.1. shows also the effect of the two inhibitors of granulocytic chemiluminescence, superoxidedismutase (SOD) and sodium azide (ref.12). SOD forces superoxide anion to dismutate to H_2O_2 while sodium azide is a strong inhibitor of heme containing enzymes such as catalase and myeloperoxidase. It has been suggested that granulocytic chemiluminescence could be dependent on both superoxide anion and singlet oxigen, the former derived from the univalent reduction of O_2 and the latter from the interactions of the various products of oxygen reduction or from reactions catalyzed by myeloperoxidase (ref.1). No effect is evident in case of the power-time curves, while the light production is strongly affected (see Fig.1). O_2 consumption measured

both in presence and absence of the inhibitors did not show any significant differences. The calorimetric results indicate that the light production represents only a negligible fraction of the total amount consumed during the burst. Only a minor part of the O_2^- formed during the burst (responsible of chemiluminescence) acts physiologically as a reductant at these experimental conditions. The rest of O_2^- produced dismutates spontaneously to H_2O_2 and is finally recovered as H_2O (ref.3).

The same considerations can be applied to the azide inhibitable, singlet oxygen dependent chemiluminescence.

These experiments indicate that chemiluminescence is correlated with very specific reactions occurring during cellular activation and gives only a qualitative estimate of the "burst" while heat production does not provide any information on the intermediate reactions but allows a quantification of the energy expenditure involved in granulocytic activation.

ACKNOWLEDGMENTS

This work has been supported by CNR Progetti Finalizzati per la Chimica Fine e Secondaria grant 104520/95/8302906

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