ACTIVATION OF THE EARLY CHEMILUMINESCENCE RESPONSE OF HUMAN NEUTROPHILS BY COMBINED TREATMENT WITH TUMOR NECROSIS FACTOR (TNF-α) AND CALCIUM IONOPHORE A23187

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Activation of neutrophils by tumor necrosis factor (TNF) is one evident way of realization of the antitumor action of TNF in vivo [6]. The possible mechanism of potentiation of the tumor-toxicity of neutrophils may be the activating action of TNF on the production of active forms of oxygen (AFO) by neutrophils [2]. AFO generation in neutrophils is carried out by NADPH-oxidase, whose activity depends on two main factors: the intracellular Ca^{2+} concentration and phosphorylation of proteins by protein kinases [3, 4]. In the investigation described below the effect of TNF-α, the calcium ionophore A23187, and the protein kinase activator phorbol-12-myristate-13-acetate (PMA) on chemiluminescence of human neutrophils was studied.

EXPERIMENTAL METHOD

Neutrophils were isolated from heparinized venous blood of patients with precancerous diseases of the stomach, under investigation at the Research Institute of Oncology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. The cells were isolated on a double Ficoll-Verografin density gradient ($d_1 = 1.007$, $d_2 = 1.119$), suspended in medium RPMI 1640, with fetal serum (10%) and gentamicin. Chemiluminescence was recorded on a 1251 luminometer (LKB, Sweden). The neutrophils were activated by TNF-α (15,000 U/5 $\cdot 10^5$ cells), ionophore A23187 (1 μM), or PMA (1 nM). Hanks' solution was added to the control vials. As chemiluminescence amplifiers we used luminol (25 μl) or lucigenin (67 μM). The experimental results were recorded and processed on an IBM-PC computer (USA). Coefficients of synergism were calculated by the formula

$$\frac{\int_0^t I(\text{TNF} + \text{A23187})}{\int_0^t I(\text{TNF}) + \int_0^t I(\text{A23187}) - \int_0^t I(\text{control})}$$

where $\int_0^t$ is the light sum of the chemiluminescence response of the neutrophils during time t from the moment of addition of the activators (t = 0): TNF-α, A23187, or TNF-α + A23187.

The following reagents were used: recombinant human TNF-α ("Ferment" Research and Production Combine, Vilnius), PMA and A23187 ("Sigma, USA), luminol ("Serva," West & Germany), and lucigenin ("Reanal," Hungary).

EXPERIMENTAL RESULTS

Addition of TNF-α to the neutrophils in the presence of lucigenin led to the development of a chemiluminescence response. The absolute magnitude of the chemiluminescence signal varied considerably in the different patients. Meanwhile lu-

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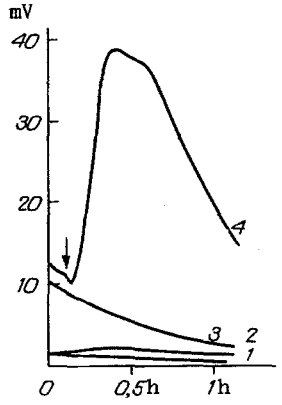

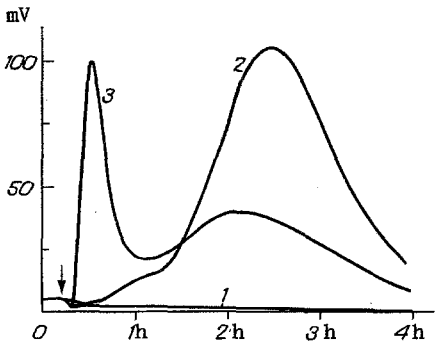

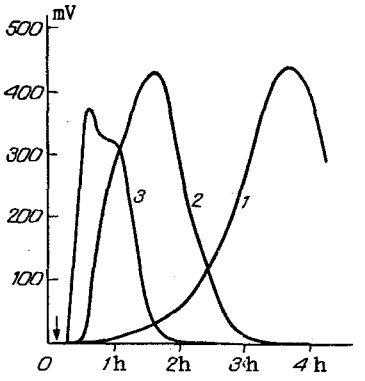

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Kinetics of chemiluminescent response of neutrophils in presence of luminol (1, 2) and lucigenin (3, 4). Neutrophils treated (↓): with TNF-α (2,4) or Hanks' solution (1, 3).

Fig. 2. Kinetics of luminol-dependent chemiluminscence of neutrophils under the influence of (↓): TNF-α (1), A23187 (2), and TNF-α + A23187 (3).

Fig. 3. Kinetics of luminol-dependent chemiluminescence of neutrophils under the influence of (↓): PMA (1), A23187 (2), and PMA + A23187 (3).

TABLE 1. Coefficient of Synergism of TNF-α and Ionophore A23187 in Chemiluminscent Response of Neurophils

Patient	30 min		3h	
	luminol	lucigenin	luminol	lucigenin
1	4,1	4,8	1,0	0,59
2	15,9	1,2	0,33	0,58
3	20,5	2,1	0,73	1,2
4	3,7	2,0	0,46	0,77
5	1,8	—	0,50	—

Legend. Relative error of determination of coefficient does not exceed 10%.

minol-dependent chemiluminescence of the neutrophils did not differ significantly from the control (Fig. 1). To record chemiluminescence in the presence of lucigenin, all that is required is for the phagocytic cells to produce the superoxide-radical ($O_2^{\cdot}$), whereas luminol-dependent chemiluminescence requires, in addition, the working of an H_2O_2-myeloperoxidase system [1]. The results are in agreement with data on the direct activating effect of TNF-α on O_2 production by neutrophils [2, 8]. The absence of a significant response to TNF-α in the presence of luminol can be taken as evidence of insufficient activation of the H_2O_2-myeloperoxidase system. Addition of the Ca^{2+} ionophore A23187 to neutrophils treated with TNF-α, both simultaneously with (Fig. 2) and 30 min before the ionophore, led to the appearance of a peak of luminol-dependent chemiluminescence at 5-10 min. An increase of the intracellular calcium concentration under the influence of the ionophore can evidently cause activation of the H_2O_2-myeloperoxidase system, due to labilization of lysosomal enzymes [7].

The fact will be noted that combined treatment of neutrophils with the Ca^{2+} ionophore and either TNF-α or PMA causes a rapid increase in chemiluminescence, not present under the influence of only one of the activators (Figs. 2 and 3). The kinetic curve of chemiluminescence thus assumes a complex character, and in the case of the combined use of TNF- and A23187 there was a second well-marked maximum, coinciding in time with the maximum of chemiluminescence of the neutrophils in response to the ionophore alone, but 1.5-2 times lower than that response (Fig. 2).

As a result of the biphasic nature of the kinetic curve, the light sum of chemiluminescence of the neutrophils under the influence of two activators during different time intervals was nonadditive. During the first 30 min of activation of the neutrophils the effect of the combined action of TNF-α and the ionophore, in the presence of luminol or lucigenin, was synergic, whereas during 3 h of incubation it was less than additive (Table 1). Thus the integrative parameter (the light sum of chemiluminescence), without allowing for the kinetics of the process, gives no idea about synergism of action of activators. From this point of view data on the synergic action of PMA and the ionophore on O_2 production by neutrophils in the course of 10 min [3] cannot be extended to later times of activation of the cells.

The appearance of the early chemiluminescence response under the influence of TNF-α or PMA, jointly with ionophore A23187 (Figs. 2 and 3), and also depression of neutrophil activation by the preparation H7, a specific inhibitor of protein kinase C [5], can be taken as evidence that the latter is involved in activation of neutrophils by tumor necrosis factor. Consequently, the cause of the effect of synergic action of the Ca^{2+} ionophore and TNF-α on chemiluminescence observed in these experiments may be stimulation of different pathways of activation of NADPH-oxidase of the neutrophils by them: an increase in the intracellular calcium concentration and activation of protein kinases.

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AGE DIFFERENCES IN THE EFFECT OF OSMOTIC PRESSURE ON RESTORATION OF RAT LIVER MITOCHONDRIA

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During aging changes take place in the properties of the mitochondria, and they are most marked in relation to the structural state of a membrane: the "osmotic" strength of the outer mitochondrial membrane is reduced in the liver of male rats, and sensitivity of these organelles to freezing and thawing is altered [2, 3]. It has been shown that after short-term hypotonic shock, the respiratory control (RC) of the liver mitochondria is reduced by a greater degree in old rats than in young animals [4]. Data also have been obtained to show that mitochondria and tissues of old animals contain less water than those of young, a fact attributable to age changes in the structural state of the membranes [11]. This fact may perhaps be of physiological importance know that the osmotic pressure of the medium has a significant influence on respiration and oxidative phosphoryla-

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