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Inhibition of arachidonic acid release from human peripheral mononuclear cells by heat shock treatment and geldanamycin

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Abstract

The objective of this study was to investigate the effects of heat shock (HS) treatment and geldanamycin (GA) on the release of arachidonic acid (AA) from human peripheral blood mononuclear cells (PBMC), monocytes and lymphocytes. Mononuclear cells prepared from blood of healthy subjects were preincubated with ^{3}H-AA. The release of ^{3}H-AA incorporated into the membrane was studied after pretreatment of cells by HS (43 °C, 1 h) and GA. The activation of AA producing enzymes was achieved by the addition of phorbol 12-myristate 13-acetate (PMA) or by the combination of PMA + calcium ionophore A-23187. Treatment of cells by HS inhibited the release of AA. Furthermore, the release of AA by PBMC was dose dependently inhibited by GA. The combination of treatments by HS and GA augmented the inhibition of AA release. The HS response involves a diminished release of AA from PBMC. The inhibitory effect of GA on the AA release is a new element in the antiinflammatory pharmacological ability of this drug.

Keywords: Arachidonic acid; Heat shock; Geldanamycin; Phospholipase A_2

1. Introduction

Arachidonic acid (AA) is not only the precursor of the potent inflammatory lipid mediators, leukotrienes, lipoxins, thromboxanes and prostaglandins but it also regulates a number of signal transducing elements, including phospholipase C, sphingomyelinase and certain protein kinase isoforms as PKC α, β and γ [1–3]. Furthermore, AA can modulate the nuclear translocation of NF-κB [4]. Thus, every pathway or substance leading to the inhibition of AA release from the cells may result in some antiinflammatory final effect. The actual level of AA mainly depends on the activity and interaction of three intracellular enzymes, cytosolic phospholipase A_2 (cPLA$_2$), Ca^{2+} independent phospholipase A_2 (iPLA$_2$) and diacylglycerol (DAG) lipase [5]. By the inhibition of the NF-κB dependent expression of cPLA$_2$ gene, glucocorticosteroids (GCSs) can reduce AA release in non-apoptotic cells [6].

A nearly universal cellular response to a variety of environmental stresses or unfavourable conditions is the rapid expression of heat shock proteins (HSPs). HSPs protect cells and tissues from the deleterious effects of numerous mediators of inflammation including reactive oxygen species and TNFα [7,8]. Heat shock (HS) inhibits the activation of NF-κB [9] required to the expression of cPLA$_2$ gene [6]. The production of inflammation related molecules like cytokines [10] is inhibited after HS. On the other hand, HSPs may exert also proinflammatory effects [11].

Geldanamycin (GA), a benzoquinoid ansamycin, specifically binds the N-terminal domain of the 90-kDa HSP [12] disrupting the glucocorticoid receptor function in intact cells [13]. Furthermore, GA inhibits the CD28 and kinase mediated signaling events of T cell activation [14–16]. These features show that GA may have some antiinflammatory and immunosuppressive pharmacologial effects.

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In this study, we investigated whether the HS treatment and GA can block the AA release of human peripheral blood mononuclear cells (PBMC).

2. Materials and methods

2.1. Materials

The sources of drugs were the following: GA: GIBCO-BRL (Gaithersburg, MD), phorbol 12-myristate 13-acetate (PMA) and calcium ionophore (A-23187): Sigma (St. Louis, MO), ^{3}H labelled AA: Amersham (UK).

2.2. Preparation of human peripheral blood mononuclear cells

Mononuclear cell suspensions (in average: 88–95% lymphocytes, 5–12% monocytes) were prepared from the peripheral blood samples of healthy volunteers according to the method of Boyum [17]. The averages of the various subsets were detected by flow cytometry: $CD3^{+}$: 69.4%, $CD19^{+}$: 13.5%, $CD56^{+}$: 8.8% and $CD14^{+}$: 8.3% (Coulter EPICS XL flow cytometer, Coulter, Hialeah, FL).

2.3. Measurement of AA release in non-stimulated cells

Cells were distributed into tissue culture plates of 24 wells (GIBCO) (0.5×10^{6} cells/well) and were incubated in RPMI medium (GIBCO, completed with 1% fetal calf serum, antibiotics and glutamine) at 37 °C in sterile 5% CO_2 milieu (ASSAB, Sweden). 0.1 μCi of ^{3}H arachidonate (^{3}H-AA) was added to the 0.5×10^{6} cells for 20 h. After labeling, the cells were washed for three times in phosphate-buffered saline and were further incubated without any stimuli for 4 h. These cells were regarded as the 'non-stimulated' controls.

2.4. Heat shock treatment

PBMC were incubated at 43 °C in a water bath for 1 h. The labeling of cells with ^{3}H-AA took place immediately after HS (taking 20 h), similarly as it was carried out with the cells not treated by HS. HS did not alter the efficacy of ^{3}H-AA labeling. The viability of HS treated cells determined by trypane blue exclusion test and lactate dehydrogenase release assay did not decrease more than 5% compared to the non-treated controls.

2.5. Treatment of PBMC by geldanamycin

Different concentrations of GA ($0–10^{-4}$ M) were added to PBMC for 1 h before the activations by PMA or PMA+calcium ionophore.

2.6. Measurement of AA release in activated PBMC

Activation of cells preincubated for 20 h with ^{3}H-AA was achieved by the addition of 50 ng/ml of PMA and 0.5 μM calcium ionophore (A-23187) at 37 °C for 4 h. The samples were centrifuged for 10 min (1200 rpm) and the supernatants were added to 5 ml of TRITOSOL scintillator and were counted by a scintillation counter (Packard 2200CA). ^{3}H-AA and metabolites released into the medium were determined by measurement of the total ^{3}H radioactivity released without separating the various metabolites. The values of isotope measurements were given in desintegration per minute (dpm) [18].

2.7. Statistical analysis

The statistical means and S.D. values of data were calculated. The statistical significance of the differences was determined by Student's paired and unpaired *t*-tests. At least five independent experiments were performed and all samples were used in triplicates. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effects of PMA and PMA+calcium ionophore combination on the AA release of PBMC

PMA significantly increased the AA release (B: 4653±412 dpm vs. A: 2414±123 dpm, $P < 0.01$) as shown in Fig. 1. When PMA and calcium ionophore were administered concurrently, the increase in AA release was even more significant (C: 6020±511 vs. A: 2414±123 dpm, $P < 0.001$, C: 6020±511 vs. B: 4653±412, $P < 0.05$). This protocol was used in our earlier work already [18].

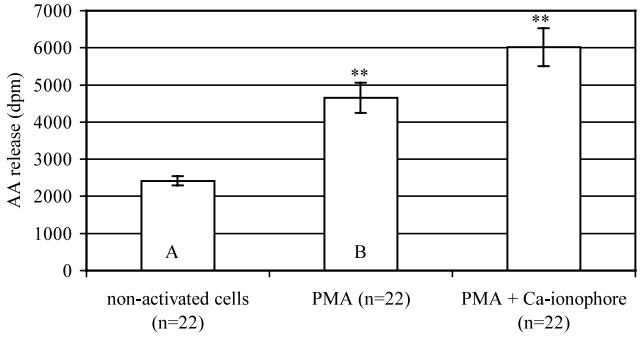

Fig. 1. Release of AA by PBMC stimulated by PMA and PMA+calcium ionophore. Data represent the mean of 22 independent experiments and the bar shows the standard deviation (S.D.). $^{**}P < 0.01$.

3.2. Effects of heat shock treatment on the release of AA by PBMC

HS treatment resulted in a significant decrease in the release of AA by the cells stimulated by PMA and calcium ionophore (4530±426 vs. 6020±712 $P < 0.05$). There was no change, however, in the AA release of non-stimulated cells (2457±392 dpm vs. 2414±123 dpm) (Fig. 2). Cells tested for AA release 1 and 4 h later than the HS treatment, did not show any significant change compared to the non-treated controls (data not shown). It could be concluded that the inhibitory effect of HS was a time dependent process on the AA release of PBMC. It was only detectable at least after 4 h as the treatment was finished. This observation also confirmed the data of the viability tests on the negligible damage of HS treated cells. Since, there was no significant difference in the AA release of stimulated cells treated or not treated by HS in the first 4 h.

3.3. Inhibition of AA release by geldanamycin from human PBMC stimulated by PMA and calcium ionophore

2×10^{-6} M of GA significantly decreased the release of AA in PBMCs stimulated by PMA and calcium ionophore (4586±395 dpm vs. 6020±712 dpm $P < 0.05$), whereas, it had no significant influence on the release of AA in non-stimulated cells (2362±368 dpm vs. 2414±123 dpm) (Fig. 3).

3.4. Dose dependent inhibitory effect of geldanamycin on the release of AA in PBMC stimulated by PMA and PMA+calcium ionophore

The inhibitory effect of GA was dose dependent on the AA release in the suspensions of PBMC stimulated by PMA or by the combination of PMA+calcium ionophore. It was a difference, however, that already 10^{-6} M of GA caused a significant decrease in AA release in the cells stimulated by PMA. On the other hand, 2×10^{-6} M of GA was required to reach the same extent of inhibition if the cells were stimulated by the combination of PMA+calcium ionophore, where the amounts of released AA were higher than in the cell cultures stimulated by PMA alone (Fig. 4).

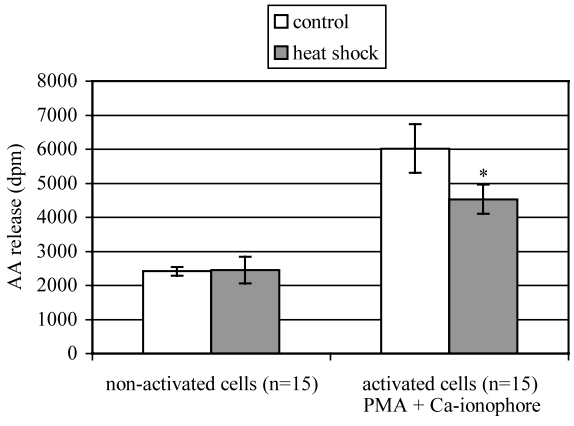

Fig. 2. Effect of HS treatment on the release of AA by PBMC stimulated by PMA+calcium ionophore. Cells were incubated for 1 h at 43 °C. Data represent the mean of 15 independent experiments and the bar shows the S.D. *$P < 0.05$. P value was calculated by the data points after various treatment with the respective control value.

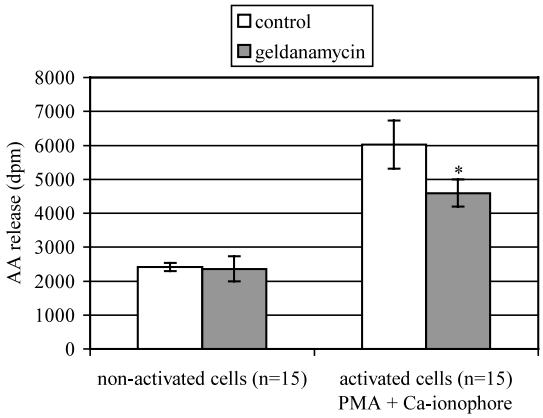

Fig. 3. Inhibitory effect of GA on the AA release of PBMC stimulated by PMA+calcium ionophore. Cells were incubated with 2×10^{-6} M of GA for 1 h before the activation. Data represent the mean of 15 independent experiments and the bar shows the S.D. *$P < 0.05$. P value was calculated by the data points after various treatment with the respective control value.

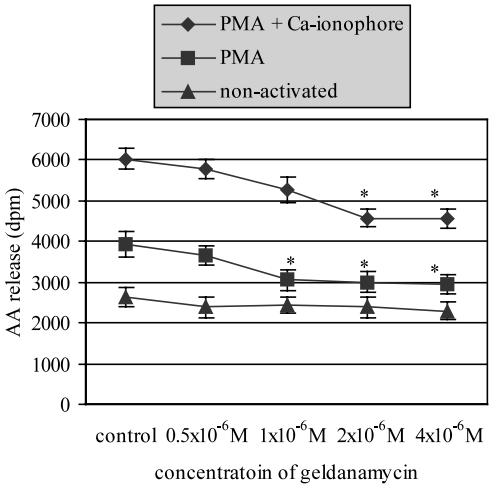

Fig. 4. Dose dependent inhibitory effect of GA on the AA release by PBMC. Cells were incubated with various doses of GA for 1 h before the activation by PMA and PMA+calcium ionophore. Data represent the mean of 4 independent experiments and the bar shows the S.D. $P < 0.5$. P value was calculated by the data points after various treatment with the respective control value.

3.5. Effects of combined applications of heat shock and geldanamycin on the release of AA in PBMC.

When geldanamycine and HS were applied concurrently, the AA release was further decreased compared to the effects of single treatments in PBMC stimulated by PMA+calcium ionophore. As shown in Table 1, both HS and GA (2×10^{-6} M) applied alone resulted in inhibitions almost to a similar extent (24, 22%). The combination of HS with GA, however, increased the percent of inhibitions (34%). (These data are shown in Table 1).

4. Discussion

AA functions as a second messenger [19] and as a precursor of eicosanoids, which are potent mediators of inflammation and signal transduction[20]. Among the PLA_2s group IV $cPLA_2$ is believed to play a pivotal role in providing free AA for eicosanoid biosynthesis [21]. However, group VI $iPLA_2$ and DAG lipase also can contribute to the production of AA to a lesser extent [22]. The activation of $cPLA_2$ is taking place by an ERK dependent phosphorylation process requiring the participation of MAPK and protein kinase C [23]. GCSs and the 70 and 90 kDa HSPs are natural inhibitors of the activation of $cPLA_2$ through the regulating effect of these HSPs on the steroid receptor activity [24].

In our experimental model, PMA, a DAG analogue and an activator of PKC resulted in a well detectable release of AA from human PBMC where the main sources of AA were the monocytes [25]. The addition of calcium ionophore to PMA enhanced the AA release further [18]. It was, however, a new observation that the HS treatment of human PBMC resulted in a significant inhibition of AA release. It had to be stressed that this effect of HS could be observed at least 4 h after as the treatment was finished. Since, we measured the significant inhibitions 20 h after the treatments, the involvement of newly synthesized HSPs (Hsp 90 and Hsp 70) could be suggested. Both Hsp 90 and Hsp 70 bound to Raf and MEK could block the activation of ERK, the key enzyme of the phosphorylation of $cPLA_2$. They could be involved in the HS induced blocking of AA release in PBMC [26,27]. This pathway can play an essential role in the natural cellular adaptation to the environmental stresses or unfavourable conditions.

GA can be linked to these processes in two points. a) It can disrupt the complex of Hsp 90 and glucocorticoid receptor [13]. b) GA can inhibit the Raf-1-MEK-mitogen-activated-protein kinase pathway [28] involved in the phosphorylation of $cPLA_2$ [23].

GA dose dependently decreased the AA release of PBMC stimulated both by PMA or PMA+calcium ionophore after a preincubation for 60 min already, in contrast to the 20 h required for the appearance of the HS induced inhibition. This finding is in agreement with the time course of other GA-effects [15,16] where the disruption of the Raf–MEK signaling pathway took place. A smaller amount (10^{-6} M) of GA was needed to cause the same significant inhibition in the PMA stimulated cells,—producing smaller amouts of AA—than in the PMA+calcium ionophore stimulated ones (2×10^{-6} M of GA) producing more AA. The combined application of HS and GA resulted in an enhanced inhibition of AA release compared to effects of single treatments. This augmentation could possibly derive from the parallel involvement of two pathways starting separately but converging to the blocking of the same enzyme finally, to the inhibition of $cPLA_2$ producing AA. In the time (20 h) consuming inhibition caused by HS, newly synthesized HSPs could block the ERK dependent phosphorylation of this enzyme [23]. On the other hand, GA was able to produce the same effect, possibly on the same enzyme, after 60 min already [28].

In the present study, we firstly provided data on the HS induced inhibition of AA release from human PBMC. In addition, we showed that the blocking effect of GA on the AA release from PBMC could be a new element in the wide antiinflammatory pharmacological effects of this drug.

Table 1
Effects of HS and GA treatments on the AA release of PBMC stimulated by PMA+calcium ionophore

	Percent of inhibition	
Treatment	Treatment alone	HS+GA
HS (43 °C, 60 min)	24	34
GA (2×10^{-6} M)	22	

Cells were incubated with 2×10^{-6} M of GA and/or incubated for 1 h at 43 °C as given in Section 2. Data represent the average of inhibition of AA release compared with the control cells (5 independent experiments).

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