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Tetrachloroauric Acid Depresses the Activation Processes of Phagocytic Cells

Chung Soo Lee

Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul 156−756, Korea

Gold compounds depress phagocytic cell responses, including chemotaxis, and respiratory burst. However, the effects of gold compounds on the function of phagocytic cells are variable according to the preparation of medicine. In this study, effect of tetrachloroauric acid on activated neutrophil responses, including respiratory burst, lysosomal enzyme release and change of intracellular Ca^{2+} level and on the synthesis of interleukin-8 and granulocyte-macrophage colony stimulating factor by macrophages was studied. This study further examines how gold compounds affect the activation processes.

The respiratory burst stimulated by complement C5a, degraded IgG and PMA in neutrophils was inhibited by tetrachloroauric acid. In contrast to C5a and degraded IgG, PMA-stimulated superoxide production was weakly inhibited by tetrachloroauric acid. Staurosporine, genistein, EGTA and verapamil inhibited superoxide and H_2O_2 production caused by C5a and degraded IgG. PMA-stimulated superoxide production was inhibited by staurosporine but was not affected by genistein. Tetrachloroauric acid, genistein, EGTA and verapamil inhibited the release of acid phosphatase and myeloperoxidase, while the effect of staurosporine was not detected. The synthesis of interleukin-8 and granulocyte-macrophage colony stimulating factor by interleukin-1 β in macrophages was inhibited by tetrachloroauric acid. Preincubation with tetrachloroauric acid, genistein, EGTA and verapamil, the elevation of $[Ca^{2+}]_i$ evoked by C5a was inhibited. Store-regulated Ca^{2+} entry in thapsigargin-pretreated neutrophils was decreased by the addition of tetrachloroauric acid and genistein. The effect of staurosporine on intracellular Ca^{2+} mobilization was not observed. In conclusion, tetrachloroauric acid may suppress neutrophil responses through its inhibitory action on elevation of intracellular Ca^{2+} level and protein kinase C. It might exhibit an inhibitory effect on the action of protein tyrosine kinase. Tetrachloroauric acid depresses cytokine production by macrophages.

Key Words: Tetrachloroauric acid, Human neutrophils, Murine macrophages, Functional depression

INTRODUCTION

Neutrophils and macrophages play an important role in host defense against bacterial infections and inflammatory reactions. Activation of neutrophils results in increased phagocytosis, bacterial killing, release of lysosomal enzymes and increased respiratory burst (Fantone & Ward, 1982). In addition, activated neutrophils are thought to regulate other inflammatory and immune cells by rapidly expressing interleukin-1 β and several other cytokins (Lloyd & Oppenheim, 1992). Neutrophils and macrophages are considered to be involved in tissue destruction in inflammatory disease, such as rheumatoid arthritis and myocardial reperfusion injury (Fantone & Ward, 1982; Weiss, 1989).

An early stage of neutrophil activation involves phosphoinositide hydrolysis by phospholipase C (Korchak et al, 1988). Protein kinase C is well known to be involved in activation process of neutrophil responses. The inhibitors of protein tyrosine kinase, such as genistein and ST638, inhibit superoxide and

Corresponding to: Chung Soo Lee, Department of Pharmacology, College of Medicine, Chung-Ang University, Seoul 156-756, Korea

prostaglandin production by chemoattractants in a primed phagocytic cells (Berkow et al, 1989; Tanimura et al, 1992). Protein tyrosine phosphorylation is also involved in activation process of neutrophils.

Gold compounds have minimal anti-inflammatory effects and cause a gradual reduction of the signs and symptoms of inflammation associated with rheumatoid arthritis (Kavanaugh & Lipsky, 1992). They depress neutrophil responses to exhibit inhibition of superoxide production and lysosomal enzyme release. Gold compounds interfere with the functions of mononuclear phagocytes, including HLA-DR expression and collagenase production. The effects of gold compounds on the function of phagocytic cells are variable according to the preparation of medication (Day, 1994). Aurothiomalate (IM preparation) have little or no effect on cytokine production, phagocytosis and respiratory burst of neutrophils, whereas auranofin (oral medication) significantly inhibits those functions. Unlike injectable compounds, auranofin does not affect macrophage functions. Auranofin appears to act multiple sites in the early activation processes of neutrophils (Rudkowski et al, 1991). However, it has no effect on the function of neutrophils which are already activated. Although an action site of auranofin is suggested as protein kinase C, auranofin has variable effects on the activity (Parente et al, 1987; Kavanaugh & Lipsky, 1992). In addition, influence of auranofin on intracellular Ca^{2+} mobilization in phagocytic cells is uncertain (Rudkowski et al, 1992; Ishitani et al, 1995). Gold compounds also appear to react with sulfhydryl group of cells and exert their actions (Day, 1994). As above views, the action mechanism of gold compounds and their biological effects are not clearly elucidated.

In this study, effect of tetrachloroauric acid, which is not a sulfur containing complex compared to gold medicines, on the activated neutrophil responses, including respiratory burst, lysosomal enzyme release and change of intracellular Ca^{2+} level and on the synthesis of interleukin-8 and GMCSF by macrophages was studied. Its action on the activation processes was also examined.

METHODS

Materials

Tetrachloroauric acid, C5a, IgG (Human), 12-myristate 13-acetate (PMA), human recombinant interleukin-1 β (IL-1 β), staurosporine, genistein, verapamil, ferricytochrome c, scopoletin, o-dianisidine hydrochloride, diagnostic kit for acid phosphatase, cytochalasin B, fura-2/AM, thapsigargin and Ficoll-Hypaque solution were purchased from Sigma Chemical Co. The ELISA assay kits for interleukin-8 (IL-8) and granulocyte-macrophage colony stimulating factor (GMCSF) were bought from Amersham Life Science. All other reagents were of analytic grade.

Preparation of human neutrophils

Neutrophils were prepared from fresh whole human blood, anticoagulated with 10% acid-citrate-dextrose, by dextran sedimentation, hypotonic lysis of erythrocytes and Ficoll-Hypague density centrifugation (Markert et al, 1984). The neutrophils were suspended in Dulbecco's phosphate-buffered saline (PBS), pH 7.4 at a concentration of 1×10^7 cells/ml. Final suspensions of neutrophils were comprised of about 97% neutrophils as judged by Wright-Giemsa stain, and viability was more than 98% as judged by trypan blue dye exclusion.

Cytochalasin B treatment: After neutrophils were pretreated with cytochalasin B (5 μg/ml for 10^7 cells) for 5 min, the assay for the respiratory burst and degranulation was done.

Macrophage elicitation and cultivation

Macrophages were elicited by injection of 1 ml of 3% thioglycollate medium into the peritoneal cavity of ICR female mouse weighing about 20 g. Cells were harvested from peritoneum 4 days after the injection and were cultured by the method of Johnston et al. (1978).

Assay of superoxide production

The superoxide dependent reduction of ferricytochrome c was measured by the method of Markert et al. (1984). The reaction mixtures in plastic microfuge tubes contained 2×10^6 neutrophils, 75 μM ferricytochrome c, tetrachloroauric acid, stimulating agent, 20 mM HEPES-tris and Hanks' balanced salt solution (HBSS), pH 7.4 in a total volume of 1.0 ml. The reactions were performed in a 37°C shaking water bath for stated times. The amount of reduced cytochrome c was calculated by using an extinction

coefficient of 2.1×10^4 $M^{-1}cm^{-1}$ at 550 nm (Cohen & Chovaniec, 1978).

Assay of hydrogen peroxide

H_2O_2 produced from activated neutrophils was measured by a change of scopoletin fluorescence. Reaction mixtures contained 2×10^6 neutrophils, 2.5 μM scopoletin, 5 μg/ml horse radish peroxidase, tetrachloroauric acid, 20 mM HEPES-tris and HBSS buffer, pH 7.4 in a total volume of 2.0 ml and were treated with 20 nM C5a. The change of scopoletin fluorescence by H_2O_2 produced was read at the wavelengths of excitation, 343 nm and emission, 460 nm (Root et al, 1975).

Assay of acid phosphatase activity

Released amount of acid phosphatase from activated neutrophils was measured using Sigma diagnostic kit. The reaction mixtures contained 2×10^6 neutrophils, C5a, tetrachloroauric acid, 20 mM HEPES-tris and HBSS buffer, pH 7.4 in a total volume of 0.5 ml. Reaction was performed for 15 min at 37°C, and supernatants were obtained. The absorbance was read at 405 nm. Activity of acid phosphatase was estimated from the standard curve using p-nitrophenol standard solution and is expressed as the mUnit/2×10^6 cells.

Assay of myeloperoxidase release

Measurement of myeloperoxidase release was done by the method of Spangrude et al. (1985). A 5×10^6 cells/ml neutrophils in HBSS buffer with or without tetrachloroauric acid were stimulated by adding C5a for 15 min. A 250 μl of 0.2 M phosphate buffer, pH 6.2 and 250 μl of an equal mixture of 3.9 mM o-dianisidine HCl and 15 mM H_2O_2 were added to reaction mixtures. After 10 min of reincubation, the reaction was stopped by the addition of 250 μl of 1% sodium azide. Myeloperoxidase activity was determined by the change in absorbance at 450 nm (ΔA_{450}) using the equation, dianisidine oxidation (n mol) = $50 \times \Delta A_{450}$ (Burt et al, 1994).

Assay of synthesis of interleukin-8 and GMCSF

Murine macrophages (2.5×10^5cells/250 μl of mixtures) were treated with 10 ng/ml interleukin-1 β for 18 h at 37°C. The amounts of IL-8 and GMCSF produced were measured by using ELISA assay kits.

Assay of cytosolic free calcium

Fura-2 loading and Ca^{2+} measurement were performed by the method of Luscinskas et al. (1990). Neutrophils (5×10^7 cells/ml) were loaded with 2 mM fura-2/AM (1 μM/10^7 cells) at 37°C for 10 min in the reaction mixtures containing Hanks' balanced salt solution (HBSS) buffer without calcium and magnesium (HBSS-CMF), and 20 mM HEPES-tris, pH 7.4. After loading, the suspension was centrifuged at 200 g for 10 min, and neutrophils were resuspended in 0.1 % bovine serum albumin containing HBSS-CMF. This procedure was performed twice. Neutrophils were finally suspended in bovine serum albumin-free HBSS-CMF at 5×10^7 cells/ml. Fluorescence measurements were done with a Turner Spectrofluorometer (Model 430). Preloaded neutrophils (4×10^6) were suspended in the same reaction mixture in a final volume of 1.0 ml. After preincubation at 37℃ for 5 min with compounds, the response was initiated by the addition of C5a. A fluorescence change was read at an excitation wavelength of 340 nm and an emission wavelength of 505 nm.

Assay of capacitative Ca^{2+} entry

In thapsigargin (TG)-treated neutrophils, Ca^{2+} entry was measured (Sargeant et al, 1993). The reaction mixtures contained fura-2 loading neutrophils (4×10^6 cells/ml), 200 μM EGTA, 20 mM HEPES-tris and HBSS buffer without calcium, pH 7.4. After 5 min of preincubation with inhibitors, neutrophils were treated with 50 nM TG for 90 sec, and then 1 mM Ca^{2+} was added to induce Ca^{2+} influx.

Data analysis

The results in experiments were analyzed for the level of significance using the Student's *t*-test.

RESULTS

Inhibition of the respiratory burst in neutrophils by tetrachloroauric acid

The respiratory burst in neutrophils was stimulated

Table 1. Inhibition of neutrophil superoxide production by tetrachloroauric acid

Compounds	Superoxide n mol/2×10^6 cells		
	C5a	Degraded IgG	PMA
No addition	9.05±0.74	10.19±0.93	28.38±2.82
Tetrachloroauric acid 5 μM	3.08±0.25**	–	24.57±0.79
Tetrachloroauric acid 10 μM	0.61±0.39**	1.12±0.23**	20.27±0.33*
Staurosporine 100 nM	5.32±0.35**	5.63±0.45**	2.83±0.77**
Genistein 10 μM	3.53±0.28**	4.73±1.06**	30.89±1.13
EGTA 5 mM	0.98±0.40**	–	–
Verapamil 100 μM	5.95±0.32**	–	–

Neutrophils were treated with 20 nM C5a for 5 min, degraded IgG for 15 min and PMA for 10 min. Values are means±SD, n=5. *$p<0.05$, **$p<0.01$ by Student's *t*-test.

by the addition of immune complement C5a, degraded immunoglobulin G (IgG) and PMA. Effect of gold compound on the respiratory burst in stimulated neutrophils was examined. Tetrachloroauric acid inhibited superoxide production in neutrophils stimulated by 20 nM C5a. At the concentration of 10 μM, tetrachloroauric acid inhibited superoxide production by 93.3% (Table 1). Preincubation with 100 nM staurosporine, an inhibitor of protein kinase C, 10 μM genistein, an inhibitor of protein tyrosine kinase, 5 mM EGTA, a Ca^{2+} chelator or 100 μM verapamil, a Ca^{2+} channel blocker, inhibited superoxide production caused by C5a. Degraded IgG (0.5mg/ml)-stimulated superoxide production was depressed by 10 μM tetrachloroauric acid, 100 nM staurosporine and 10 μM genistein. Tetrachloroauric acid showed a similar potency of effect on superoxide production by both stimulators. Alteration of protein kinase C-mediated superoxide production by tetrachloroauric acid was studied. As shown in data, 5 μM and 10 μM tetrachloroauric acid exhibited a depressant action on the superoxide production stimulated by PMA, a protein kinase C activator, by 13.4% and 28.6%. Staurosporine (100 nM) inhibited superoxide production by PMA, while 10 μM genistein did not affect it.

Hydrogen peroxide (H_2O_2) production was measured by oxidation of scopoletin. The oxidation of scopoletin by neutrophils was stimulated by C5a. Change of scopoletin fluorescence occurred immediately after addition of C5a (20 nM). Influence of tetrachloroauric acid on H_2O_2 production by neutrophils was observed. Table 2 shows that C5a-induced H_2O_2 production was inhibited by 5 μM and 10 μM tetrachloroauric acid by 36.6% and 68.8%. Staurosporine (100 nM), 10 μM genistein, 100 μM verapamil and 5 mM EGTA inhibited H_2O_2 production by C5a-stimulated neutrophils.

Table 2. Effect of tetrachloroauric acid on H_2O_2 production by C5a

Compounds	Fluorescence units
No addition	20.5±0.3
Tetrachloroauric acid 5 μM	13.0±0.4**
Tetrachloroauric acid 10 μM	6.4±0.3**
Staurosporine 100 nM	6.8±0.3**
Genistein 10 μM	2.2±0.2**
EGTA 5 mM	9.0±0.5**
Verapamil 100 μM	15.1±0.4**

Neutrophils were stimulated with 20 nM C5a for 5 min. Values are expressed as fluorescence units and are means ±SD, n=3. **$p<0.01$ by Student's *t*-test.

Effect of tetrachloroauric acid on lysosomal enzyme release from activated neutrophils and synthesis of cytokins by macrophages

The secretion of lysosomal enzymes from activated neutrophils was assayed by measuring the release of acid phosphatase and myeloperoxidase. Table 3 shows that 5 μM and 10 μM tetrachloroauric acid significantly inhibited the release of acid phosphatase and myeloperoxidase from neutrophils activated by 20 nM C5a, and 24.0% to 84.0% of inhibition was observed. Involvement of protein kinases in activation

Table 3. Inhibition of lysosomal enzyme release by tetrachloroauric acid

Compounds	Acid phosphatase (⊿absorbance)	Myeloperoxidase (n mol)
No addition	0.153±0.005	9.35±0.78
Tetrachloroauric acid 5 μM	–	7.11±0.85*
Tetrachloroauric acid 10 μM	0.027±0.005**	1.50±1.18**
Staurosporine 100 nM	0.149±0.007	10.44±3.30
Genistein 10 μM	0.135±0.005*	1.09±0.31**
EGTA 5 mM	0.085±0.003**	–
Verapamil 100 μM	0.110±0.006**	–

Neutrophils (2×10^6 cells/0.5 ml for acid phosphatase, 5×10^6 cells/ml for myeloperoxidase) were treated with 20 nM C5a in the presence of compounds for 10 min. Values are means±SD, n=4. *p<0.05, **p<0.01 by Student's *t*-test.

Table 4. Depressant action of tetrachloroauric acid on synthesis of cytokines

Compounds	pg/2.5×10^5 cells	
	IL-8	GMCSF
No addition	40.3±3.0	86.8±6.0
Tetrachloroauric acid 5 μM	36.5±5.4	60.3±2.9*
Tetrachloroauric acid 10 μM	30.0±4.3	55.5±1.7**

Macrophages (2×10^5 cells/250 μl) were incubated with 10 ng/ml interleukin-1β for 18h at 37°C. Values are means±SD, n=3. *p<0.05, **p<0.01 by Student's *t*-test.

process of lysosomal enzyme release was studied. Ten μM genistein, 5 mM EGTA and 100 μM verapamil inhibited release of acid phosphatase and myeloperoxidase, whereas the effect of 100 nM staurosporine was not detected. IL-1β (10 ng/ml) stimulated synthesis of IL-8 and GMCSF in murine peritoneal macrophages, and 40.3 pg/2.5×10^5 cells and 86.8 pg/2.5×10^5 cells were produced, respectively. The productions of IL-8 and GMCSF were depressed by tetrachloroauric acid, and at 10 μM, they were inhibited by 25.7% and 36.1%, respectively (Table 4).

Effect of tetrachloroauric acid on intracellular Ca^{2+} mobilization

In 1.23 mM Ca^{2+}-containing media, 20 nM C5a evoked an elevation of intracellular Ca^{2+} level ($[Ca^{2+}]_i$) in neutrophils. The $[Ca^{2+}]_i$ rose to a maximum level

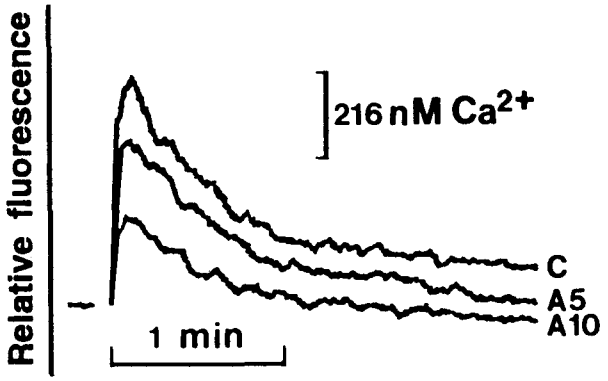

Fig. 1. Effect of tetrachloroauric acid on C5a-evoked elevation of $[Ca^{2+}]_i$. Fura-2 loaded neutrophils (4×10^6 cells/ml) were preincubated with tetrachloroauric acid, and then 20 nM C5a added. C, no addition; A5, 5 μM tetrachloroauric acid; A10, 10 μM tetrachloroauric acid. The traces are representative of three experiments.

within 15 sec post addition, and then elevated $[Ca^{2+}]_i$ was gradually declined to the resting level over the subsequent several minutes. The effect of tetrachloroauric acid on increased $[Ca^{2+}]_i$ in C5a-stimulated neutrophil was studied. As shown in Fig. 1, preincubation with tetrachloroauric acid (5 μM and 10 μM) decreased elevation of $[Ca^{2+}]_i$. Regulatory action of protein kinases on intracellular Ca^{2+} mobilization was examined. Table 5 shows that 10 μM genistein, 5 mM EGTA and 100 μM verapamil addition depressed elevation of $[Ca^{2+}]_i$ caused by C5a, while 100 nM staurosporine did not have any effect on it.

The elevation of $[Ca^{2+}]_i$ is attained by both release of Ca^{2+} from intracellular stores and subsequent Ca^{2+}

Table 5. Effect of various compounds on the elevated of $[Ca^{2+}]_i$

Compounds	Intracellular Ca^{2+} n mol at peak
No addition	580.1±12.6
Staurosporine 100 nM	583.7±35.0
Genistein 10 μM	467.1±29.4*
EGTA 5 mM	202.9±40.0**
Verapamil 100 μM	364.0±35.4*

Neutrophils were stimulated with 20 nM C5a in the presence of compounds. Values are means±SD, n=3-4. *$p<0.05$, **$p<0.01$ by Student's *t*-test.

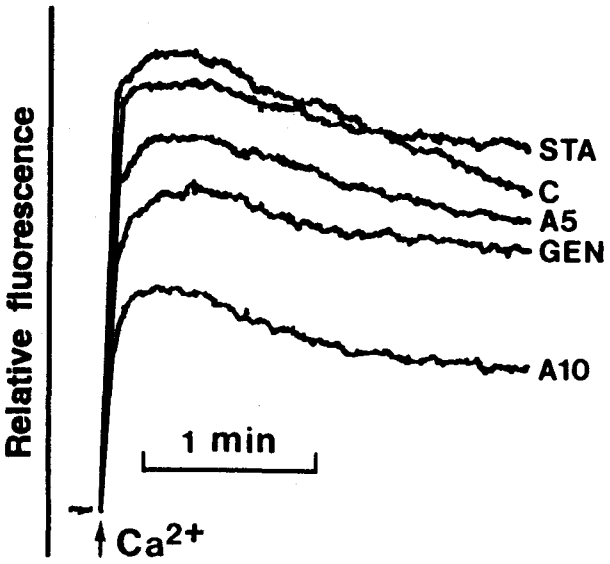

Fig. 2. Inhibitory effect of tetrachloroauric acid on thapsigargin-induced Ca^{2+} entry. In Ca^{2+} free media, neutrophils were preincubated with 5 μM and 10 μM tetrachloroauric acid (A5 and A10), 100 nM Staurosporine (STA) and 10 μM genistein (GEN), and then 50 nM thapsigargin (TG) was added. C, no addition of inhibitors. The traces are representative of three experiments.

influx across the plasma membrane (Westwick & Poll, 1986; Cobbold & Rink, 1987). Thapsigargin (TG), an inhibitor of the endomembranous Ca^{2+}-ATPase, depletes the intracellular Ca^{2+} stores without increasing cellular $InsP_3$ (Sargeant et al, 1993). The depletion of intracellular Ca^{2+} pools appears to induce Ca^{2+} entry. In Ca^{2+} free media, fura-2-loaded neutrophils were treated with TG for 90 sec to deplete intracellular Ca^{2+} stores and then were exposed to high concentration of Ca^{2+}. TG itself did not cause any recognizable change in fluorescence for the stated time. Fig. 2 shows that the addition of 1 mM Ca^{2+} to TG-treated neutrophils evoked a marked elevation of $[Ca^{2+}]_i$. The TG-induced Ca^{2+} influx was inhibited by 5 μM and 10 μM of tetrachloroauric acid and 10 μM genistein. In this reaction, the effect of 100 nM staurosporine was not detected.

DISCUSSION

Chemoattractants and immune complexes are thought to exert their action by binding to G protein-linked, cell surface receptors at the plasma membrane (Sicilliano et al, 1990). Resultant elevated cytosolic free Ca^{2+} and activated protein kinase C are considered to play an important role in activation of neutrophil responses. In addition, receptor binding of chemoattractants is reported to induce activation of protein tyrosine kinase. Complement C5a is a potent chemotaxin for neutrophils and macrophages (Goldstein, 1992). It stimulates these cells to produce superoxide anion, to release lysosomal enzymes and alters their surface properties leading to enhanced adhesion and aggregation. The activation processes, which transduce response changes after C5a receptors binding, appear to involve pertussis toxin-sensitive G proteins (Becker et al, 1985). C5a caused an immediate response of neutrophils, respiratory burst and lysosomal enzyme release after a short lag period.

Gold compounds are used to reduce or prevent joint damage and to preserve joint function in rheumatoid arthritis patients. The effects of gold compounds on functions of phagocytic cells, which play an important role in inflammatory processes, are variable (Day, 1994). Auranofin in vitro at pharmacological concentrations has been shown to inhibit functional response of neutrophils, including phagocytosis, while aurothiomalate has little or no effect on neutrophil response. Auranofin depresses early responses of neutrophils and macrophages exposure to stimulators but has little or no effect on late responses (Rudkowski et al, 1991b; Yamashita et al, 1997). Anti-inflammatory effect of gold compounds may relate to their capacity to alter protein kinase C activity. It has been reported that auranofin has variable effects on the activity of protein kinase C, which include inhibition (Kavanaugh & Lipsky, 1992), stimulation (Parente et al, 1987) or no effect (Herlin

et al, 1989). Thus, biological effect and action mechanism of gold compounds on neutrophil responses has not been elucidated.

Superoxide production by the respiratory burst is catalyzed by a membrane-associated enzyme that utilizes NADPH as the electron donor. C5a and degraded IgG-induced superoxide and H_2O_2 production was inhibited by staurosporine, genistein, EGTA and verapamil. This finding indicates that protein kinase C, protein tyrosine kinase and Ca^{2+} may be involved in the activation process of superoxide production in neutrophils. The stated concentrations of tetrachloroauric acid significantly inhibited superoxide and H_2O_2 production by C5a or degraded IgG. In contrast to aurothiomalate and auranofin, tetrachloroauric acid is not a sulfur containing complex. It is suggested that inhibitory effect of tetrachloroauric acid is ascribed to gold itself.

PMA is able to substitute 1,2-diacylglycerol on activation of protein kinase C. PMA activates protein kinase C directly, bypassing activation of the membrane linked-G proteins (Tauber, 1987). The stimulatory action of PMA on superoxide production, which is responsible for staurosporine, was inhibited by tetrachloroauric acid (5 μM and 10 μM). In constrast to C5a or degraded IgG, PMA-stimulated superoxide production was inhibited weakly by tetrachloroauric acid by 13.5% to 28.6%. It has been shown that PMA stimulates neutrophil responses without elevation of intracellular Ca^{2+} level. Thus, tetrachloroauric acid appears to have an weak inhibitory action on protein kinase C.

Lysosomal enzyme release from neutrophils stimulated by C5a is Ca^{2+} dependent and may be regulated by protein tyrosine kinase. On the contrary, the effect of protein kinase C inhibitor, staurosporine, on releases of acid phosphatase and myeloperoxidase was not observed. The result partly supports that the activation process of degranulation appears to be different from the respiratory burst. Tetrachloroauric acid may depress the release process of lysosomal enzymes. Inhibition of synthesis of IL-8 and GMCSF in IL-1β-activated macrophages by tetrachloroauric acid also contributes to the anti-inflammatory action of gold compounds.

Changes in intracellular Ca^{2+} level are thought to play an important role in the activation process of neutrophil responses (Smolen et al, 1981). The receptor binding of chemoattractants to the plasma membrane elicits a biphasic increase in $[Ca^{2+}]_i$ (Westwick & Poll, 1986). A rapid and transient initial phase is attained by release from the intracellular Ca^{2+} stores, and a sustained phase, which is maintained by influx across the plasma membrane, is followed. Inhibition of C5a-evoked elevation of $[Ca^{2+}]_i$ by EGTA and verapamil supports the view on intracellular Ca^{2+} mobilization. Protein tyrosine kinase may be involved in the elevation of $[Ca^{2+}]_i$ caused by C5a, while regulatory action of protein kinase C is not suggested. The mechanism underlying receptor-mediated Ca^{2+} entry in neutrophils is uncertain. Depletion of intracellular Ca^{2+} stores is thought to promote Ca^{2+} influx across the plasma membrane (Vostal et al, 1991; Sargeant et al, 1993). In TG-treated neutrophils, extracellular addition of high concentration of Ca^{2+} caused a marked elevation of $[Ca^{2+}]_i$. The store-regulated Ca^{2+} entry appears to be regulated by protein tyrosine kinase but not controlled by protein kinase C. Auranofin is reported to inhibit Ca^{2+} uptake into neutrophils. The depressant effect of tetrachloroauric acid on the designated Ca^{2+} entry postulates that it might depress the action of protein tyrosine kinase, and this causes attenuation of intracellular Ca^{2+} mobilization. These results indicated that gold itself may exert a depressant action on the activation processes of phagocytic cells.

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