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Acetaminophen inhibits the human polymorphonuclear leukocyte function in vitro

Ehsan Ali Shalabi

Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia

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Abstract: The aim of the study is to investigate the effect of Acetaminophen (Am) on the oxidative respiratory burst of isolated human polymorphonuclear leukocytes (PMNs). Acetaminophen inhibited the luminolchemiluminescence (CL) peak response of PMNs stimulated with phorbol myristate acetate (PMA) or opsonized zymosan in a concentration dependent manner. The inhibitory effect of Am on PMA-stimulated PMNs-CL response was partially reversible. The level of CL inhibition with Am plus the hydroxyl radical scavengers allopurinol, dimethyl sulfoxide (DMSO) or superoxide dismutase (SOD) is greater than that with Am alone. Generation of superoxide (O_2^-) by stimulated PMNs, as assayed by superoxide dismutase inhibitable reduction of Ferricytochrome *c*, was markedly inhibited by Am. Furthermore, the phagocytic activity of PMNs as tested for by the ingestion of opsonized dead yeast was significantly reduced in Am-treated cells. These results indicate clearly that Am causes significant inhibition of the human PMNs function in vitro.

Key words: Human polymorphonuclear leukocyte; Acetaminophen; Chemiluminescence; Phagocytosis; Superoxide

Introduction

Acetaminophen (Am), *N*-acetyl-*p*-Aminophenol, is a widely used analgesic and antipyretic drug and has been reported to have few side effects when taken in low doses. High doses, however, lead to hepatic injury in humans (Davidson and Eastham, 1966) and in experimental animals (Laskin et al., 1984; Laskin and Pilaro, 1986). The toxicity of Am is thought to be related to its conversion to a highly reactive electrophilic benzoquinone imine intermediate by oxidative enzymes (Jungermann and Sasse, 1978). These toxic intermediates can bind to cellular components causing liver cell damage (Jollow et al., 1973; Potter et al., 1974).

Polymorphonuclear leukocytes (PMNs) are the first cells to appear at the site of infection and play a crucial role against bacterial, viral and parasitic infections. Stimulation of PMNs induces several phenomena known collectively as the respiratory oxidative burst (Klebanoff and Clark, 1978; Babior and Cohen, 1981). Characteristics of this increase in oxidative metabolism include a rapid uptake of molecular oxygen and the production of oxygen-free radicals (ORFs). These ORFs include superoxide (O_2^-), hydrogen

Correspondence to: E.A. Shalabi, Department of Physiology, College of Medicine, King Saud University, P.O. Box 2925 (Riyadh 11461) Saudi Arabia

Abbreviations: Am, acetaminophen; PMNs, polymorphonuclear leukocytes; CL, chemiluminescence; PBS, phosphate buffered saline; PMA, phorbol myristate acetate; DMSO, dimethyl sulfoxide; SOD, superoxide dismutase; O_2^-, superoxide; OFRs, oxygen-free radicals; OH, hydroxyl radical; BSS, balanced salt solution; MPO, myeloperoxidase

peroxide (H_2O_2) and hydroxyl radicals (OH) (Badwey and Karnovsky, 1980; Babior and Cohen, 1981). These reactive metabolites are shown to have very powerful bactericidal activity (Klebanoff, 1988). Light emission or chemiluminiscence of PMNs, a phenomenon related to the respiratory burst activation, was originally described by Allen et al. (1972).

Van Zyl et al. (1989) found that Am binds to purified myeloperoxidase (MPO) via its acetamido side chain and competes effectively with Cl^- as MPO substrate. They stated that high concentrations of Am not only inhibit HOCl formation, but also scavenge available HOCl and thus paralyze the MPO-H_2O_2-Cl^- antimicrobial system of PMNs. Abramson et al. (1990) reported that Am has no effect on the viscosity of isolated human PMNs plasma membrane preparations or on the binding of *n*-formyl-methionyl-leucyl-phenylalanine (FMLP) to it nor did it cause any change in the ability of PMNs to aggregate in response to this chemoattractant (FMLP). They also stated that Am exerted some inhibition on superoxide anion generation as well as degranulation of PMNs.

The aim of this study was to examine the effect of Am on PMNs ability to generate OFRs upon stimulation with a soluble stimulus, phorbol myristate acetate (PMA) using the luminol-dependent chemiluminescence (CL) technique. Furthermore, the influence of Am on superoxide production by PMA-stimulated PMNs was assayed by using superoxide dismutase inhibitable reduction of Ferricytochrome *c*. In addition, the effect of Am on isolated human PMNs phagocytosis of opsonized dead yeast cells was tested.

Materials and Methods

Reagents

N-acetyl-*p*-aminophenol (Acetaminophen or Am, Sigma Chemical Co.) was dissolved in saline 0.9% (w/v). Nycodenz monocyte solution (Nyegaard & Co., As, Torshov, Norway), is a ready-made sterile solution for the isolation of PMNs. Luminol was dissolved in dimethyl sulfoxide (DMSO) in concentration of 10^{-2} M. This stock was further diluted in PBS to 10^{-4} M prior to use. A stock solution of phorbol myristate acetate (PMA, Sigma Chemical Co.) of 2 mg/ml in DMSO was prepared and kept in the freezer until used. This stock solution was further diluted by adding 50 μl PMA stock solution to 10 ml PBS before use. Zymosan (Sigma Chemical Co.) was opsonized by suspending 50 mg in 3 ml human serum and 1 ml PBS, incubated for 30 min at 37 °C and then centrifuged at 300 × *g* for 10 min. The supernatant was then removed and the pellet was washed twice with 4 ml buffer. After a second centrifugation, the pellet was resuspended in PBS at a concentration of 12.5 mg/ml and stored in the freezer until use. The concentration of opsonized zymosan which has been used is 2 mg/ml. Superoxide dismutase (Sigma Chemical Co.) was dissolved in PBS just before use. Allopurinol (Alhekmah, Jordan) was dissolved in PBS. Ferricytochrome *c* (Sigma Chemical Co.) was dissolved in Earle's balanced salt solution without phenol red (GIBCO, Grand Island, NY), xanthine oxidase from butter milk (Sigma Chemical Co.), 100 U/1.5 ml suspended in 2 M ammonium sulphate containing 0.02% sodium salicylate. Xanthine from Merck, Darmstadt.

PMNs isolation

Blood was collected by vene puncture from apparently healthy, middle-aged male donors in a sterile container containing heparine (10 IU/ml, Fisher Scientific Co., NJ). The heparinized blood was mixed with Dextran T 500 (6% w/v) and allowed to stand for 30 min at room temperature. The leukocyte-rich plasma layer was removed after the erythrocytes were settled. About 5–6 ml of the leukocyte-rich plasma was layered over 3 ml Nycodenz solution and centrifuged at 400 × *g* in a Heraues centrifuge (model GmBH, Osterode) for 10 min at 22 °C. To the bottom PMNs-rich portion, 10 ml of PBS was added and centrifuged at 350 × *g* for 10 min. Erythrocytes were hemolysed by hypotonic PBS (1:1

with H_2O). The cells were then centrifuged as above and resuspended in PBS to 5×10^6 cells/ml and examined microscopically for viability.

Effect of Am on PMNs-CL response

The PMNs oxidative burst was measured by CL. The principle of oxidation of luminol, 5-amino-2, 3-dehydro-1, 4-phthalazinedione, by reactive oxygen species produced by stimulated PMNs was employed to increase the amount of measurable light (Allen and Loose, 1976). The CL assay was carried out in an LKB (WALLAC) 1251 luminometer as described by Al Tuwaijri et al. (1990) and Kato et al. (1981). The light output in mV was continuously recorded on an LKB (WALLAC) chart recorder and maximum peak response was printed.

The effect of different concentrations of Am (1×10^{-7} M to 1×10^{-2} M) and different incubation times (0–90 min) on PMA-stimulated PMNs-CL was tested. The influence of superoxide scavenger, superoxide dismutase, and hydroxyl radical scavengers, allopurinol and DMSO on PMNs-CL response were examined after stimulation of PMNs with PMA at a final concentration of 1 μg/ml in the presence or absence of Am.

Effect of Am on PMNs viability

In order to examine if Am, SOD or allopurinol have a direct cytotoxic effects upon PMNs, the effect of Am (1×10^{-2} M) alone or in combination with SOD or allopurinol on PMNs viability were tested at 30 min interval for 3 h following incubation at 37 °C. The percentage of viable PMNs was estimated by Trypan blue exclusion which was carried out by a microscopic count of cells not stained by 0.2% Trypan blue and was expressed as percent of unstained cells to total cell numbers.

Effect of Am on superoxide generation by PMNs

The reduction of Ferricytochrome *c* by O_2^- (Babior et al., 1973) is the basic principle of this assay. The amount of reduced Ferricytochrome *c* is determined by measuring its absorbance at 550 nm in the Micro ELISA reader Dynatech MR 580 as described by Pick and Mizel (1981). Briefly, 100 μl PMNs suspended in BSS without phenol red and 100 μl Ferricytochrome *c* (160 μM) were placed in the microtiter wells. To test the effect of Am on O_2^- production, 100 μl of PMA (10 μg/ml) and 50 μl Am (1×10^{-3} M) or (1×10^{-4} M) were added. In the control, Am was replaced by 50 μl saline. 100 μl SOD (0.2 mg/ml) and 50 μl saline were used instead of PMA and AM respectively for the blank. The plate is covered and placed in a 37 °C humidified incubator, gassed with 95% air and 5% CO_2 for 15 min followed by measuring the absorbance value for each well. Superoxide value was calculated as follows:

$$O_2^- \text{ (nmol)} = \frac{(\text{test OD} - \text{assay blank OD}) \times 100}{6.3}$$

Effect of acetaminophen on superoxide production by the xanthine–xanthine oxidase system

In order to rule out any non-specific effect of Am on PMNs, a cell-free superoxide generating system was employed. It consisted of the xanthine–xanthine oxidase system. Xanthine oxidase (0.5 U) and 50 μl Am (1×10^{-3} M) or (1×10^{-4} M) were added to 250 μl of reaction mixture containing 10 mM sodium phosphate buffer pH 7.4, 0.1 mM EDTA, 0.1 mM xanthine, 0.1 mM Ferricytochrome *c*. The plate was left at room temperature (25 °C) for 15 min followed by measuring the absorbance value. Superoxide was calculated as above.

Effect of Am on phagocytic capacity

Phagocytic capacity was measured by using opsonized dead yeast cells. Dead yeast cells were prepared by washing 5 g of pure Baker's yeast cells in PBS twice and resuspended in 20 ml in PBS. Yeast cells were killed by boiling for 30 min to ensure that the cells were killed. The yeast viability was examined by culturing them in Sabouraud's agar. Yeast cells were adjusted to a concentration of 2×10^8 cells per ml in PBS. This

TABLE I

Effect of various concentrations of Am on human PMNs chemiluminescence stimulated by PMA or opsonized zymosan

Am concentration (M)	Chemiluminescence (CL) maximum peak response (mV)		
	No stimulant	PMA	Opsonized zymosan
0 (Control)	55.23 ± 10.2	340.00 ± 17.6 (-)	417.30 ± 42.6 (-)
1×10^{-7}	34.52 ± 11.3 (37)*	271.16 ± 14.8 (20)*	364.55 ± 43.7 (13)*
1×10^{-6}	26.09 ± 1.8 (53)**	214.48 ± 12.7 (37)*	293.11 ± 35.8 (30)*
1×10^{-5}	14.10 ± 2.0 (74)**	199.77 ± 12.0 (41)*	210.89 ± 36.1 (50)**
1×10^{-4}	8.80 ± 0.32 (84)**	126.29 ± 10.0 (63)**	97.64 ± 12.3 (77)**
1×10^{-3}	1.95 ± 0.13 (96)**	40.29 ± 2.2 (88)**	29.65 ± 5.6 (93)**
1×10^{-2}	0.64 ± 0.14 (99)**	2.30 ± 1.0 (99)**	8.72 ± 1.9 (98)**

PMNs count = 5×10^5 cells/ml, PMA concentration = 1 μg/ml, opsonized zymosan = 2 mg/ml, luminol concentration = 10^{-4} M. Each value was expressed as the mean of five experiments ± SE. The numbers in parentheses represent the percentage of CL inhibition. Incubation temperature was 37 °C for 30 min. % CL inhibition = (Control – experiments)/control × 100.

* $p < 0.01$.

** $p < 0.001$.

stock suspension was kept frozen until used. Opsonization of yeast cells was performed by incubating 100 μl of human serum from healthy individuals and 900 μl of dead yeast cells suspension at 37 °C for 30 min. The isolated human PMNs were suspended in a culture medium which was made up of 4 ml Eagle's minimum essential medium with Earle's salts supplemented with 0.5 ml heat inactivated fetal calf serum (GIBCO) and 0.05 ml penicillin (1000 IU/ml). For phagocytosis assays, 500 μl PMNs (5×10^6 cells/ml), 500 μl Am (1×10^{-3} M) and 50 μl yeast suspension (2×10^8 cells/ml) were added to 4 ml culture medium. This mixture was incubated at 37 °C for 30 min. It was placed in ice for 15 min to stop further phagocytosis and centrifuged at 350 × *g* for 10 min. The pellet was spread on a slide stained by Trypan blue and examined under

TABLE II

Effect of various concentrations of Am on the CL of isolated human PMNs stimulated with PMA at different time intervals of incubation

Am concentration (M)	Time of incubation			
	0	30	60	90
Control	422.20 ± 20.7	410.30 ± 17.8	390.22 ± 12.0	350.84 ± 10.2
1×10^{-4}	85.75 ± 5.7 (79.69)**	82.39 ± 4.7 (79.92)**	75.04 ± 4.5 (80.77)**	64.49 ± 3.2 (81.56)**
1×10^{-3}	39.68 ± 1.1 (90.33)**	39.68 ± 2.2 (90.33)**	37.27 ± 2.3 (90.45)**	29.40 ± 0.9 (91.62)**
1×10^{-2}	13.18 ± 2.1 (96.73)**	11.36 ± 1.4 (97.23)**	10.38 ± 0.17 (97.34)**	12.84 ± 0.9 (96.34)**

Each number represents the maximum peak response (mV) for five experiments ± SE, the numbers in parentheses represent the percentage of CL inhibition. PMNs count = 5×10^5 cells/ml. PMA concentration = 1 μg/ml. Luminol concentration = 10^{-4} M. Incubation temperature = 37 °C.

* $p < 0.01$.

** $p < 0.001$.

a light microscope. The phagocytic capacity was determined by counting the number of ingested yeast particles in 200 PMNs. The number of engulfed particles per one PMN was calculated.

Statistical analysis

The data were represented as mean ± standard error (SE) and the differences between the groups were analyzed for significance according to Student's *t*-test. When p is less than 0.05, it is considered significant.

Results

Effect of Am on luminol-dependent chemiluminescence of PMNs

Am markedly inhibited the PMNs-CL response. In the range of concentration used (1×10^{-2} M to 1×10^{-7} M), Am showed a concentration-dependent inhibitory response. The percentage of Cl inhibition reached 99% at high concentration (1×10^{2} M). In order to exclude that this inhibitory effect was not due to interaction between Am and PMA, another PMNs stimulant (opsonized zymosan) was used with different concentrations of Am. A marked concentration-dependent inhibition of PMNs-CL response was also observed (Table I). On the other hand, incubation of various concentrations of Am with PMNs at different incubation periods (0, 30, 60 and 90 min) showed that the inhibitory effect of Am is not time-dependent (Table II).

Reversibility of Am effect

In an attempt to determine at what level Am inhibited CL response, PMNs were incubated with Am (1×10^{-4} M) at 37 °C for 30 min. The PMNs were then washed several times with PBS to remove Am prior to the measurement of CL responses. The CL response of Am-treated PMNs washed with PBS was significantly lower than the control washed cells (Table III). This test was repeated several times to confirm the finding. Similar results were obtained. Therefore, the results indicate that the inhibitory effect of CL with AM was partially reversible during the range of the time used.

TABLE III

Effect of washing Am with PBS from the incubation medium on the chemiluminescence of PMNs stimulated with PMA

Group	Am	CL peak response (mV)	Inhibition (%)
Not washed (control)	–	520.11 ± 18.00	–
Not washed	+	130.03 ± 3.90**	79.98
Washed (control)	–	386.60 ± 10.40	–
Washed	+	243.82 ± 14.50*	36.93

PMNs count = 5×10^5 cells/ml, PMA concentration = 1 μg/ml, luminol concentration = 10^{-4} M, Am = 10^{-4} M. Incubation time was 30 min. Incubation temperature = 37 °C. Each value was expressed as the mean of five experiments ± SE.

* $p < 0.01$.

** $p < 0.001$.

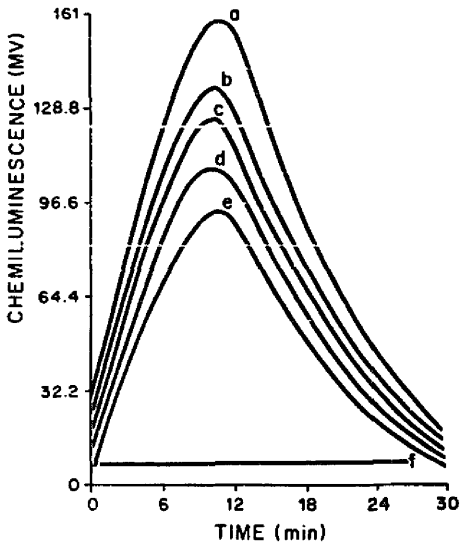

Fig. 1. Time trace of CL emitted from human PMNs (5×10^5 cells) stimulated with PMA (1 μg/ml); effect of hydroxyl radical scavengers on Am-induced PMNs inhibition; (a) PBS, (b) Am (1×10^{-6} M), (c) allopurinol (25 mM), (d) DMSO (10 mM), (e) Am + allopurinol. Results represent the means of five experiments with different PMNs donors.

Effect of oxygen-free radical scavengers SOD (0.2 mg/ml), allopurinol (25 mM) and DMSO (10 mM) on CL

When SOD was added to Am-pretreated PMNs, the level of inhibition with Am plus SOD was greater than that with Am alone (Fig. 1). The same was true with DMSO plus Am; however, very marked CL inhibition was noticed when allopurinol was added to Am pretreated PMNs (Fig. 2).

The amount of DMSO used as a solvent in this study did not affect the magnitude of CL response of PMNs to PMA or zymosan (data not shown).

Effect of Am, SOD and allopurinol on cell viability

The viability of PMNs was not significantly changed following incubation of PMNs with high concentration of Am (1×10^{-2} M) either alone or in combination with the SOD or Allopurinol (Table IV).

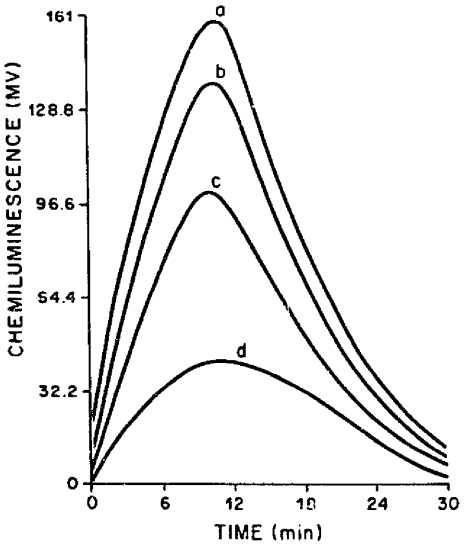

Fig. 2. Time trace of CL emitted from human PMNs (5×10^5 cells) stimulated with PMA (1 μg/ml); effect of the superoxide scavenger SOD on Am-induced PMNs inhibition; (a) PBS, (b) Am (1×10^{-6} M), (c) SOD (0.2 mg/ml), (d) Am + SOD. Results represent the means of five experiments with different PMNs donors.

TABLE IV

Influence of Am and oxygen scavengers on the viability of polymorphonuclear leukocytes following incubation at different time intervals

Incubation time (min)	Percentage viability			
	Control	Am	Am + Allopurinol	Am + SOD
0	96	95	96	98
30	96	97	90	94
60	95	94	89	92
90	91	90	87	89
120	90	83	85	89
150	87	85	80	83
180	86	78	79	80

PMNs count = 5×10^6 cells/ml, allopurinol concentration = 25 mM, SOD concentration = 0.2 mg/ml. Am concentration is 1×10^2 M. Viability was examined by Trypan blue exclusion.

The influence of Am on O_2^- production by activated PMNs and the xanthine–xanthine oxidase system

Upon stimulation of PMNs with PMA, the quantity of O_2^- released was 5.49 ± 0.58 nmol per 10^6 PMNs. Addition of Am (final concentrations 1.5×10^{-4} M, 1.5×10^{-5} M) to PMNs, significantly reduced the O_2^- production by 19.49% and 21.31%, respectively (Table V).

TABLE V

Effects of various concentrations of Am on superoxide (O_2^-) production by human polymorphonuclear leukocytes stimulated with PMA

Am final concentration (M)	Superoxide nmol/1×10^6 PMNs	Inhibition (%)
Control	5.49 ± 0.26	–
1.4×10^{-5}	4.42 ± 0.27*	19.49
1.4×10^{-4}	4.32 ± 0.27*	21.31

PMNs count = 1×10^7 cells/ml, PMA final concentration = 3 μg/ml. Ferricytochrome *c* concentration = 160 mM, incubation time = 15 min, incubation temperature = 37 °C. Each cell sample was assayed in triplicate. Each value was expressed as the mean of five experiments ± SE.
O_2^- nmol = (Test OD – Blank OD)/6.3 × 100.
* $p < 0.01$.

TABLE VI

Effects of various concentrations of Am on superoxide (O_2^-) production by the xanthine–xanthine oxidase system

Am final concentration (M)	Superoxide production in 15 min (nmol)	Inhibition (%)
Control	6.40 ± 0.28	–
1.4×10^{-5}	4.52 ± 0.15*	29.7
1.4×10^{-4}	3.64 ± 0.11**	43.0

The xanthine oxidase concentration in the reaction mixture was 10 mM; sodium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.1 mM xanthine, 0.1 mM Ferricytochrome *c*; 0.5 U xanthine oxidase was added to each well. SOD concentration, 0.2 mg/ml. Room temperature, 25 °C. Each well was assayed in triplicate. Each value was expressed as the mean of five experiments ± SE.

* $p<0.01$.

** $p<0.001$.

TABLE VII

The effects of Am on phagocytic capacity of isolated human PMNs at different times of incubation

Incubation time	Number of ingested opsonized yeast particles/cell		Inhibition (%)
	Without Am	With Am	
10	3.25 ± 0.30	1.20 ± 0.14**	63.08
20	5.33 ± 0.59	1.98 ± 0.19**	62.85
30	8.93 ± 1.06	2.56 ± 0.22**	60.13

PMNs count, 5×10^6 cells/ml, opsonized yeast count, 2×10^8 cells/ml, final concentration of Am, 1×10^{-5} M, incubation temperature, 37 °C. Each value was expressed as the mean of five experiments ± SE.

* $p<0.01$.

** $p<0.001$.

Similarly, Am (final concentrations 1.5×10^{-4} M, 1.5×10^{-5} M) significantly decreased O_2^- released by the cell-free superoxide generating system (xanthine–xanthine oxidase). The degree of inhibition was about 30% and 43%, respectively (Table VI).

Effect of Am on the phagocytic capacity

Phagocytic capacity of PMNs was significantly inhibited by Am (final concentration 1×10^{-5} M) regardless of the incubation time used in this study (Table VII).

Discussion

The results of the present study show that Am produces a marked and concentration-dependent inhibition of PMA-CL response of isolated human PMNs. This effect was partially reversible when phagocytes pretreated with Am were washed with PBS. Since two structurally different stimuli, viz., PMA and particulate opsonized zymosan were used to stimulate PMNs and in both cases a concentration-dependent inhibition of CL response were observed, it is therefore unlikely that the effect of Am could be due to a nonspecific interaction between Am and PMA. We have also found that Am reduces superoxide generation by PMA-stimulated PMNs when it is measured by the Ferricytochrome *c* reduction method. Consistent with this findir · are the results previously reported by Abramson et al. (1990) which show that Am inhibits superoxide production by PMNs. Recent studies have shown that generation of oxygen reactive metabolites induced by PMA from phagocyte cells is due to direct activation of the phospholipid dependent kinase, protein kinase C (Niedel et al., 1983; Yeh et al., 1987) and that protein kinase C plays an important role in the activation of a membrane NADPH oxidase which is responsible for superoxide generation in PMNs (Fujita et al., 1984). It is possible therefore that Am may have acted as a competitive inhibitor either to protein kinase C or to NADPH oxidase enzymes to produce its effects.

Two mechanisms are believed to be responsible for luminol-dependent CL of PMNs; one involving myeloperoxidase system and the other superoxide anion production (Rosen and Klebanoff, 1976; Klebanoff and Clark, 1978). Since Am exhibited a strong inhibitory effect on PMA-induced CL, it is, therefore, likely that Am can

inhibit both superoxide production and the myeloperoxidase system in PMNs. Indeed it has been reported by Van Zyl et al. (1989) that Am binds via its acetamido side chain to purified myeloperoxidase. The latter authors have also stated that Am competes effectively with Cl^- as a myeloperoxidase substrate and thus suppresses HOCl formation. Moreover, they have also found that Am can act as HOCl scavenger.

The addition of Am to a cell-free superoxide generating system (xanthine–xanthine oxidase) was found to inhibit the generation of superoxide from such a system. This result suggests that Am is either acting as a superoxide scavenger or as an inhibitor of xanthine oxidase.

Inhibition of PMA-stimulated Cl of PMNs, when Am was used in combination with the superoxide scavenger, superoxide dismutase (SOD), was greater than that when Am was used alone. Allopurinol, the hydroxyl radical scavenger (Moorhouse et al., 1987) and the potent inhibitor of xanthine oxidase enzyme (Williams and Bray, 1981), when added to Am pretreated PMNs, produced a marked inhibitory effect on the CL response. The other hydroxyl radical scavenger DMSO (Parks et al., 1983) when used in combination with Am also produced an inhibitory effect on the CL response but to a lesser degree than allopurinol. High concentration of Am, either alone or in combination with the oxygen-free radical scavengers, SOD and allopurinol, had no significant effect on the viability of PMNs as it was measured by the Trypan blue exclusion method.

To evaluate the effect of Am on the phagocytic capacity of PMNs, the drug was incubated with PMNs at 37 °C. It produced a significant decrease in the phagocytic capacity of PMNs to ingest opsonized yeast particles. The results of several studies (Ragsdale and Arend, 1980; Griffin and Mullinax, 1981) demonstrated that for a receptor to be able to mediate phagocytosis it must have the ability to move within the plasma membrane. The viscosity of the lipid bilayer affects the capacity of receptors to move within it (Yuli et al., 1982). It was reported by Abramson et al. (1990) that Am has no effect on plasma membrane viscosity. Therefore, the inhibitory effect of Am is not due to an action to alter membrane viscosity of PMNs. So the reduction of phagocytic capacity by Am may be due to either decreasing the affinity of the specific receptors to attach to the opsonized yeast cells or by interfering with the phagocytic signal.

It is known that the therapeutic plasma level of Am is 10–20 μg/ml, which is equivalent to 7×10^{-5} M to 1×10^{-4} M (Penet and Cheiner, 1985). This plasma level is within the range of the Am concentrations (1×10^{-7} M to 1×10^{-2} M) used in the present study, which produced a significant inhibitory effect on the function of PMNs in vitro. Although there is no direct evidence, it is possible that Am may have the same effect on PMNs function in vivo.

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