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EFFECTS OF THIAZINAMIUM CHLORIDE, PROMETHAZINE AND CHLORPROMAZINE ON THROMBOXANE B_2 SYNTHESIS, PHAGOCYTOSIS AND RESPIRATORY BURST BY RAT ALVEOLAR MACROPHAGES

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Abstract—The effects of three phenothiazines, promethazine, thiazinamium chloride and chlorpromazine, on macrophage function were investigated in rat alveolar macrophages. The study focused on thromboxane B_2 (TxB_2) synthesis, zymosan phagocytosis, and hexosemonophosphate (HMP) shunt activity in these phagocytes. TxB_2 synthesis by resting macrophages was inhibited by thiazinamium chloride and promethazine in a dose-dependent manner. However, chlorpromazine was inhibitory only at 10^{-3} M. Promethazine treatment of zymosan-activated macrophages led to a concomitant reduction in both phagocytosis and TxB_2 synthesis. Thiazinamium chloride inhibited TxB_2 synthesis but had no effect on the ingestion of zymosan particles. In contrast, chlorpromazine inhibited phagocytosis but not TxB_2 synthesis except at 10^{-3} M. The effects of these agents on the formation of TxB_2 synthesis from exogenous arachidonic acid were also investigated. Under these conditions where indomethacin, a known cyclooxygenase inhibitor, was inhibitory, promethazine but not thiazinamium chloride inhibited TxB_2 synthesis from exogenous arachidonic acid. Treatment of macrophages with promethazine and chlorpromazine but not thiazinamium chloride results in a reduction in the oxidative burst during phagocytosis. The results suggest that the phenothiazines used in this study differ from one another in their actions on macrophage function. Furthermore, the ability of thiazinamium chloride to selectively inhibit arachidonic acid metabolism may contribute to its bronchodilator/antiallergic activity.

Various attempts using both cell-free preparations and intact cells have been made to elucidate the mechanisms of action of phenothiazines on arachidonic acid metabolism. In spite of this, it is unclear whether these agents have a negative or positive influence on the synthesis of prostaglandins. For example, chlorpromazine has been shown either to inhibit [1–4] or to stimulate [5] prostaglandin (PG) synthesis by cell-free systems such as bovine seminal vesicles and guinea pig lung homogenate preparations. Similarly, equivocal data have been reported using intact cells. In RBL-1 cells and several other established cell lines, Rigas *et al.* [6] reported that phenothiazines such as chlorpromazine stimulate PGE_2 and $PGF_{2\alpha}$ synthesis at doses ranging from 10 to 40 μM, whereas in platelets it was shown that chlorpromazine at 200 μM inhibits arachidonic acid release and reduces the levels of arachidonic acid metabolites, notably thromboxane A_2 (TxA_2) [7, 8].

Prostaglandins are known to have a role in the regulation of airway reactivity in the lung [9, 10] and of cellular immune and allergic responses [11, 12]. Alterations in the synthesis of these metabolites could, therefore, potentially affect these regulatory mechanisms in the lung. Furthermore, alveolar macrophages play a critical role in host defense mechanisms and are known to synthesize and release PGE_2 and TxA_2 [13]. Several studies suggest that phenothiazines may act at the level of phagocytic cells. They inhibit phagocytosis [14], the respiratory burst [15, 16], phospholipase C activity [17], superoxide generation [18, 19], lysosomal alterations [20], and chemotaxis [21].

Recently, thiazinamium chloride, a quaternary analog of phenothiazine, has been shown to differ markedly in its *in vivo* pulmonary pharmacology from promethazine itself [22, 24]. Thiazinamium chloride possesses greater anticholinergic and antiallergic activity than promethazine without affecting motor activity. Further, unlike promethazine, which is highly lipid soluble, thiazinamium chloride, because of its quaternary nature, does not diffuse easily across cell membranes. Consequently, thiazinamium chloride is under clinical investigation as an aerosol form since it is poorly absorbed orally.

We now wish to report our investigations examining the effects of thiazinamium chloride and several reference drugs, including other phenothiazines, on TxB_2 synthesis, the hexosemonophosphate (HMP) shunt activity, and phagocytosis in rat alveolar macrophages.

METHODS

Materials. Female Wistar rats (125–150 g) were purchased from the Harlan Co., Indianapolis, Ind.

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Lung lavage fluid was prepared according to the following specifications: 0.85% NaCl, 0.1% dextrose, 0.1% Na_2EDTA, 20 mM HEPES, 100 units/ml penicillin, and 100 μg/ml streptomycin. Media 199 (M199), newborn calf serum (NCS), Hanks' Balanced Salt Solution (HBSS), and Dulbecco's phosphate buffered saline containing calcium and magnesium were purchased from Grand Island Biological Co., New York, NY. Prior to use, NCS was heat inactivated and M199 was buffered with HEPES and supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. [1-^{14}C]arachidonic acid (AA) (sp. act. 52.7 mCi/mmole), [^{3}H]TxB_2 (sp. act. 125 Ci/mmole), [1-^{14}C]glucose and [6-^{14}C]glucose (sp. act. 55–60 mCi/mmole) were obtained from New England Nuclear Corp., Boston, MA. Thin-layer chromatography (TLC) plates for analysis used for prostaglandin separations were purchased from VWR Scientific, San Francisco, CA.

The following compounds were obtained from the sources within parentheses: zymosan (Sigma Chemical Co., St. Louis, MO), hyamine hydroxide (J. T. Baker, Phillipsburg, NJ), trichloroacetic acid (TCA) (Aldrich Chemicals, Milwaukee, WI), Hydrofluor (National Diagnostics, Somerville, NJ), Diff-quick (Harleco Co. Gibbstown, NJ), and prostaglandins (The Upjohn Co., Kalamazoo, MI). Thiazinamium chloride and promethazine were synthesized at Wyeth Laboratories, Philadelphia, PA. Chlorpromazine was a gift from Smith Kline & French, Philadelphia, PA. Ipratropium was donated by C. H. Boehringer, Ridgefield, CT. Indomethacin was a gift from Merck, Rahway, NJ.

Macrophage cultures. Rats were anesthetized with an intraperitoneal injections of sodium pentobarbital (43 mg/kg body wt) and exsanguinated by cardiac puncture; their lungs were isolated and lavaged with a total of 50 ml of lavage solution. Lungs that exhibited infection or gross pathological changes were not used. The lavage fluid containing macrophages was centrifuged at 300 *g* for 10 min, and the cell pellet was resuspended in serum free M199. Macrophage monolayers were established by incubating 1.5×10^6 cells in petri dishes (35×10 mm) for 2 hr at 37° in an atmosphere of 95% room air and 5% carbon dioxide. After washing with HBSS to remove non-adherent cells, the cultures were incubated in serum-free M199 with or without zymosan (100 μg/ml) in the absence or presence of drugs. Before experimentation, cultures contained 95.0 ± 0.6% alveolar macrophages, as determined by non-specific esterase staining, adherence, and the capacity for phagocytosis. Viability of macrophages assayed at the end of each experiment by trypan blue exclusion was always >90% unless stated otherwise.

TxB_2 radioimmunoassay. Macrophage cultures were incubated with or without drugs for 2 hr since preliminary work showed that TxB_2 production at this time was suboptimal (data not shown). Experiments performed at this time thus allowed an assessment of both inhibitory and enhancing effects of the drugs. Following incubation, the media were then removed for analysis of TxB_2 content by radioimmunoassay. TxB_2 was measured in the culture media by radioimmunoassay according to the method of Flynn [25] with slight modifications. Briefly, [^{3}H]TxB_2 (10,000 dpm) was equilibrated with TxB_2 antiserum (50 μl) and known or unknown samples at ambient temperature in 12 × 75 mm polystyrene culture tubes for 2 hr. Following incubation, the antibody bound [^{3}H]TxB_2 was adsorbed to 100 μl of 10% dextran–charcoal solution, vortexed, and pelleted at 1200 *g* for 10 min. An aliquot of the supernatant fluid (250 μl) was then transferred into a scintillation vial and counted for radioactivity in 10 ml of Hydrofluor. The cross-reactions of the antiserum with other prostaglandins were: 0.0005% (6-keto-$PGF_{1\alpha}$), 0.0006% ($PGF_{1\alpha}$), 0.0014% ($PGF_{2\alpha}$), 0.0006% (PGE_2), and 0.031% (PGD_2). The concentrations of unknown samples were derived from a standard curve for TxB_2.

Determination of hexose-monophosphate shunt activity. The oxidation of [1-^{14}C]glucose was determined with modifications, as described by De Chatelet and Parce [26]. Briefly, alveolar macrophages were isolated as described and suspended in PBS at a concentration of 5×10^6 macrophages/ml. All assays were performed in a final volume of 3 ml in 25 ml Erlenmeyer flasks fitted with rubber stoppers to which center wells containing pieces of filter paper saturated with 250 μl of 1 M hyamine hydroxide were attached. The reaction mixture consisted of 0.2 μCi D-[1-^{14}C]- or D-[6-^{14}C]glucose, 1.3×10^{-6} M glucose, 100 μg/ml of zymosan, and appropriate test compounds. The reaction was started by the addition of 5×10^6 macrophages to the reaction mixture and incubated at 37° for 1 hr. Following incubation, the reaction was stopped by adding 1 ml of 10% TCA to the mixture. After 20 min, the center wells were removed and placed in scintillation vials and counted for radioactivity in 10 ml Hydrofluor.

Phagocytosis assay. The extent of phagocytosis was determined by the following method. Macrophage monolayers were prepared as described previously. Zymosan particles were added to media of cultures at a final concentration of 100 μg/ml, and phagocytosis was allowed to proceed for 1 hr at 37°. After incubation, the monolayers were washed extensively to remove free zymosan particles, air-dried, fixed with methanol, and stained with Diff-quick. The ingestion of particles by 100 or more cells per culture were evaluated by direct microscopic counting and the phagocytic index was defined by the equation:

$$\% \text{ Phagocytosing cells} = \frac{\text{Cells that have ingested 2 or more particles}}{\text{Total number of cells counted}} \times 100$$

Metabolism of exogenous [^{14}C]arachidonic acid by rat alveolar macrophages. Macrophages were prepared as described and incubated with 1 μCi of [^{14}C]arachidonic acid for 3 hr at 37° in the absence or presence of the drugs. After the incubation, the culture media were rapidly removed and extracted by solvent partitioning with diethyl ether at pH 3.5 to 4.0. The ether extracts were evaporated, residues redissolved, and aliquots applied to thin-layer chromatographic plates (0.2 mm thickness, Merck). The chromatograms were developed in the following solvent systems: ethyl acetate–formic acid (80:1)

(R_f: $PGE_2 = 0.41$; $TxB_2 = 0.57$; 6-keto-$PGF_{1\alpha}$ = 0.22; $PGF_{2\alpha}$ = 0.23; arachidonic acid = 0.90) and ethyl acetate–iso-octane–acetic acid–water (110:50:20:100) (R_f: $PGE_2 = 0.45$; $TxB_2 = 0.63$; 6-keto-$PGF_{1\alpha} = 0.35$; $PGF_{2\alpha} = 0.23$; arachidonic acid = 1.0). After development, the marker compounds were identified by brief exposure of the chromatogram to iodine vapour. The distribution of radioactivity along the thin-layer chromatogram was determined by cutting each channel into appropriately sized strips, eluting with methanol (1 ml) in liquid scintillation vials for 5 min, and then adding scintillation fluid (Hydrofluor).

Statistics. All data were analyzed using Student's *t*-test.

RESULTS

Effect on resting macrophages. Rat alveolar macrophages, when placed in culture, synthesized and released low but measurable amounts of TxB_2 over a period of 2 hr. Using 1-day culture conditions, PGE_2 was also synthesized but to a lesser extent and was not investigated further in this study. This is in contrast to the mouse pulmonary macrophages where both PGE_2 and TxB_2 are synthesized approximately to the same level [13]. Rat alveolar macrophages treated with doses of phenothiazines that were non-cytotoxic did not differ morphologically from control macrophages. When examined under an inverted tissue culture microscope, drug-treated macrophages remained adherent and retained the characteristic morphology of alveolar macrophages: large (16–20 μm diameter), round, extended, and containing many vacuoles and granules. Figure 1 illustrates the effects of thiazinamium chloride, promethazine, chlorpromazine, indomethacin and ipratropium on this basal synthesis. The data indicate that, when alveolar macrophages were treated with thiazinamium chloride or its parent compound, promethazine, the synthesis of TxB_2 was reduced in a dose-related fashion. Thiazinamium chloride at the highest dose (10^{-5} M) inhibited TxB_2 synthesis by more than 70%, and promethazine had the capacity to abolish TxB_2 synthesis almost completely within the dose range of 10^{-4}–10^{-3} M. The IC_{50} values for thiazinamium chloride and promethazine were 2×10^{-7} M and 2×10^{-5} M respectively. Chlorpromazine, on the other hand, failed to affect TxB_2 levels except at 10^{-3} M where a loss of cell viability was evident. It should also be noted that, at this dose, promethazine caused a similar loss of cell viability whereas thiazinamium chloride was non-cytotoxic at all doses. As expected, indomethacin, a known cyclooxygenase inhibitor, reduced TxB_2 synthesis markedly; at 10^{-5} M, indomethacin reduced TxB_2 synthesis greater than 80%. Ipratropium, a potent anticholinergic agent, had virtually no effect.

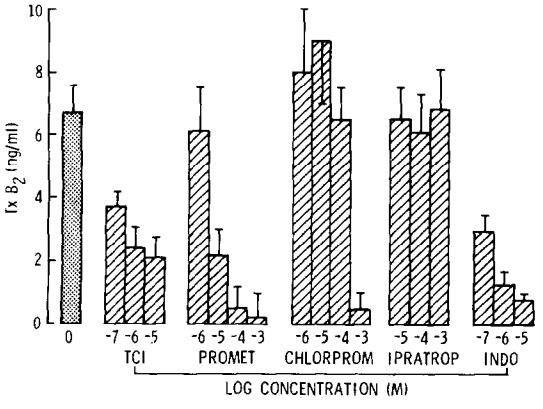
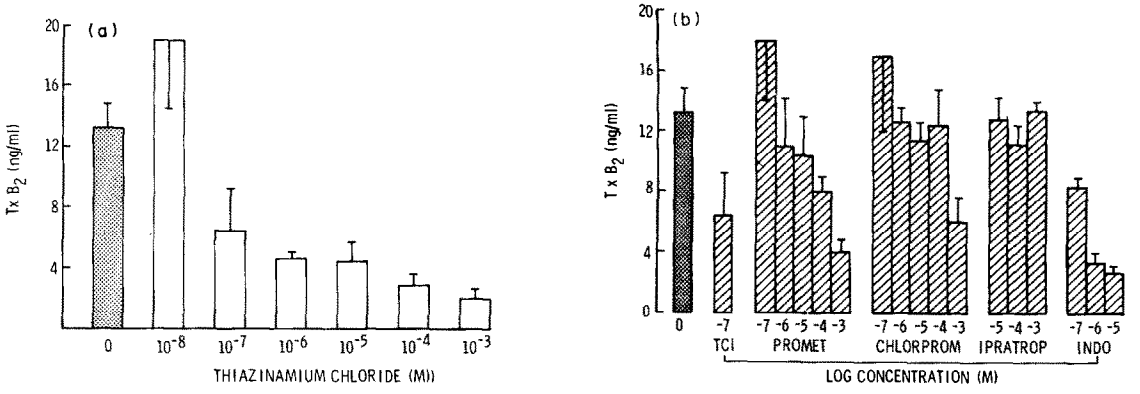


Fig. 1. Effects of various drugs on TxB_2 synthesis by resting macrophages. Macrophage monolayers (1.5×10^6 cells/plate) were incubated at 37° for 2 hr in the absence or presence of drugs at various concentrations. Following incubation, the culture media were removed and analyzed for TxB_2. All values are expressed as the mean TxB_2 equivalents (ng/ml ± S.E.M.) of at least four determinations. Abbreviations; TCl, thiazinamium chloride; Promet, promethazine; Chlorprom, chlorpromazine; Ipratrop, ipratropium; and Indo, indomethacin.

Effect on zymosan-induced TxB_2 synthesis. Because resting macrophages produced relatively low quantities of TxB_2, it was difficult to assess accurately the pharmacological actions of the drugs under study. We, therefore, examined the effects of the various drugs on zymosan-treated macrophages where TxB_2 synthesis was increased. Macrophages (1.5×10^6 cells/plate) phagocytosing zymosan particles synthesized 2 to 2.5 times more TxB_2 than resting macrophages (13.3 ± 1.4 vs 6.5 ± 0.8 ng). Under these conditions, the addition of thiazinamium chloride at various concentrations caused a well-defined dose-related inhibition ($IC_{50} = 6.64 \times 10^{-8}$ M (Fig. 2A). No loss of cell viability was observed even at 10^{-3} M. Figure 2B summarizes the results obtained with the various drugs in comparison with thiazinamium chloride. Promethazine inhibited the formation of TxB_2 by approximately 40% at 10^{-4} M and, although there was a slight decrease at doses of 10^{-5} and 10^{-6} M, the inhibition was not significant. Chlorpromazine had virtually no effect on zymosan-induced TxB_2 synthesis except at 10^{-3} M where there was a significant loss of cell viability. At 10^{-7} M, chlorpromazine and thiazinamium chloride had a slight but non-significant stimulatory effect. Ipratropium, under these conditions, also failed to have any effect. Predictably, indomethacin caused a marked decrease in TxB_2 synthesis by these activated cells.

Effect on phagocytosis. One possible explanation for the inhibitory action of the phenothiazines on zymosan-activated TxB_2 synthesis was a reduction in the ingestion of zymosan particles. The phagocytosis data obtained in the presence of the various drugs are presented in Table 1. It is evident that the inhibitory actions of thiazinamium chloride and indomethacin on TxB_2 synthesis were not due to the decreased ability of the cells to phagocytose zymosan. In contrast, promethazine inhibited the ingestion of zymosan particles within the same dose range that inhibited the formation of TxB_2 by alveolar macrophages. For example, where TxB_2 synthesis was reduced by 39% at 10^{-4} M, a similar reduction in phagocytosis was observed. Interestingly, although chlorpromazine was inactive against TxB_2 synthesis at doses where no loss of cell viability



(A) (B)

Fig. 2 (A). Effect of thiazinamium chloride (TCl) on TxB_2 synthesis by zymosan-activated alveolar macrophages. Macrophage monolayers (1.5×10^6 cells/plate) were incubated with various concentrations of TCl in serum-free M199 for 10 min prior to the addition of zymosan (100 μg/ml). Following a further 2-hr incubation, the culture media were removed and analysed for TxB_2. Values are expressed as mean TxB_2 equivalents ± S.E.M. of at least four separate macrophage cultures. (B) Effects of various drugs on TxB_2 synthesis by zymosan-activated alveolar macrophages. Macrophage monolayers were treated with the various drugs at the indicated concentrations for 10 min before the addition of the stimulus, zymosan (100 μg/ml). Following zymosan addition, the macrophage cultures were further incubated for 2 hr at 37°. After incubation, the culture media were removed and analyzed for TxB_2. Values are expressed as TxB_2 equivalents ± S.E.M. of at least four separate cultures. Abbreviations: TCl, thiazinamium chloride; Promet, promethazine; Chlorprom, chlorpromazine; Ipratrop, ipratropium; and Indo, indomethacin.

Table 1. Phagocytosis of zymosan particles by rat alveolar macrophages treated with various drugs*

Drug (M)	Phagocytic index	% Inhibition of phagocytosis
None	90 ± 2	
Thiazinamium Cl		
10^{-5}	92 ± 2	0
10^{-4}	83 ± 4	0
10^{-3}	82 ± 10	0
Promethazine		
10^{-5}	86 ± 2	5
10^{-4}	57 ± 5†	37
10^{-3}	13 ± 7†	86
Chlorpromazine		
10^{-5}	66 ± 2‡	27
10^{-4}	15 ± 8†	84
10^{-3}	5 ± 2†	95
Indomethacin		
10^{-5}	94 ± 1	0
Ipratropium		
10^{-3}	92 ± 1	0

* Macrophage monolayers were established in petri dishes and treated with drugs 5 min before the addition of zymosan particles (100 μg/ml). Phagocytosis was allowed to proceed for 2 hr at 37°. The phagocytic index was calculated as described in Methods. Results are expressed as the mean phagocytic index ± S.E.M. of at least four separate experiments. Statistical significance was determined using Student's *t*-test, by comparing to the zymosan control.
†$P < 0.001$.
‡$P < 0.05$.

occurred, zymosan phagocytosis was inhibited in a dose-related fashion. Indeed, at 10^{-4} M, the phagocytic process was inhibited by greater than 80%.

Effect on the metabolism of exogenous arachidonic acid. When alveolar macrophages were incubated with [^{14}C]arachidonic acid for 3 hr, significant

Table 2. Effects of phenothiazines and indomethacin on exogenous arachidonic acid conversion to thromboxane B_2*

Treatment	N	Concn (M)	[^{14}C]TxB_2 (dpm)	Inhibition (%)
None	5		4194 ± 459	
Thiazinamium Cl	6	10^{-3}	4135 ± 296	0
Promethazine	6	10^{-4}	2486 ± 403†	41
Chlorpromazine	6	10^{-4}	4148 ± 703	0
Indomethacin	6	10^{-5}	854 ± 148†	80

*Macrophage monolayers were established at a cell density of 1.5×10^6 cells/plate. [^{14}C]Arachidonic acid (0.5 μCi) was added to each culture in the presence or absence of drugs and incubated for 3 hr at 37°. Following incubation, the culture media were removed, extracted, and analyzed for radioactive TxB_2 as described in Methods. Results are expressed as the mean dpm ± S.E.M. N = number of experiments. Percent inhibition was calculated relative to the control TxB_2 level. Statistical significance was determined using Student's *t*-test.
†$P < 0.01$.
‡$P < 0.001$.

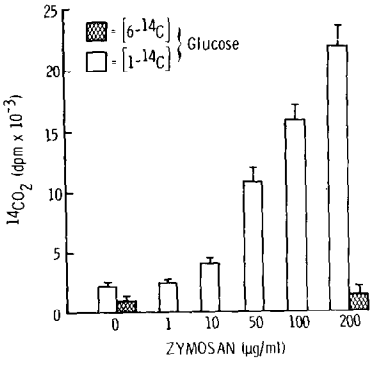


Fig. 3. Ability of alveolar macrophages to oxidize [1-^{14}C]glucose to $^{14}CO_2$ via the HMP shunt. Macrophages (5×10^6 cells/flask) were incubated at 37° for 1 hr with [1-^{14}C]glucose or [6-^{14}]glucose in the presence or absence of zymosan at various concentrations as described in Methods. After termination of the reaction, the radiolabeled CO_2 that had evolved from the reaction was absorbed into hyamine hydroxide and counted for radioactivity. Values are expressed as the mean dpm ± S.E.M. of at least four separate reactions.

amounts of radiolabeled TxB_2 could be found in the medium. Table 2 shows the effects of the various drugs under these conditions. Addition of indomethacin to the incubation medium markedly reduced the conversion of [^{14}C]arachidonic acid to TxB_2. In contrast, thiazinamium chloride at the highest dose tested (10^{-3} M) had no effect, whereas promethazine inhibited the formation of [^{14}C]TxB_2 by approximately 40% at 10^{-4} M. Chlorpromazine at the highest dose (10^{-4} M), which did not affect cell viability, did not significantly reduce the synthesis of TxB_2 from exogenous arachidonic acid.

Effect on the hexosemonophosphate (HMP) shunt. We also examined the effect of these drugs on the HMP shunt, a further index of macrophage function which has been reported to be affected by phenothiazines [14, 15]. The degree of glucose oxidation via the HMP shunt depends on the ability of macrophages to oxidize preferentially [1-^{14}C]glucose over [6-^{14}C]glucose to $^{14}CO_2$. In contrast, [6-^{14}C]glucose is solely oxidized through the Krebs cycle, whereas [1-^{14}C]glucose can be metabolized by either the HMP shunt or the Krebs cycle. It can be seen from Fig. 3 that actively phagocytosing macrophages exhibited an increased capacity to oxidize [1-^{14}C]glucose in response to increasing doses of zymosan. In contrast, there was no increase in Krebs cycle activity throughout the dose range.

Table 3 depicts the effects of the various drugs on the oxidation of [1-^{14}C]glucose by rat alveolar macrophages. The addition of promethazine or chlorpromazine caused a dose-dependent inhibition of the HMP activity. At 10^{-4} M, both promethazine and chlorpromazine reduced the level of HMP activity to levels even lower than the basal oxidation of glucose by resting macrophages. Thiazinamium chloride and indomethacin, even at a dose where TxB_2 synthesis was reduced by greater than 70%, under these conditions were inactive. Ipratropium equally failed to affect the HMP shunt.

DISCUSSION

In the present study we have examined the effects of phenothiazines on a number of macrophage functions. The data establish that: (a) both thiazinamium chloride and promethazine, but not chlorpromazine, inhibited TxB_2 synthesis in resting macrophages in a dose-related fashion; (b) in actively phagocytosing macrophages, thiazinamium chloride also inhibited TxB_2 synthesis without affecting phagocytosis, whereas promethazine inhibited both processes; (c) metabolism of exogenous [^{14}C]arachidonic acid in resting macrophages was unaffected by thiazinamium chloride and chlorpromazine but inhibited by promethazine; and (d) zymosan-activated oxidative

Table 3. Effects of various drugs on the hexosemonophosphate shunt in rat alveolar macrophages*

Agent	Concn (M)	HMP shunt activity (dpm in $^{14}CO_2$ formed from [1-^{14}C]glucose)
None		1.012 ± 196
Zymosan (Z) (100 μg/ml)		12,861 ± 535
Z + TCl	10^{-3}	11,212 ±578
Z + promethazine	10^{-3}	< 100†
Z + promethazine	10^{-4}	500 ± 196†
Z + promethazine	10^{-5}	9,119 ± 1,186‡
Z + chlorpromazine	10^{-3}	< 100†
Z + chlorpromazine	10^{-4}	< 100†
Z + chlorpromazine	10^{-5}	11,955 ± 540
Z + indomethacin	10^{-5}	14,792 ± 842
Z + ipratropium	10^{-3}	13,820 ± 1,490

*Macrophages were incubated with or without drugs as described in Methods. HMP shunt activity was calculated as the difference between dpm from [1-^{14}C]glucose and dpm from [6-^{14}C]glucose. Values are expressed as the mean dpm ± S.E.M. of at least four separate determinations.

†$P < 0.001$.

‡$P < 0.01$.

burst as measured by the HMP shunt was similarly unaffected by thiazinamium chloride but inhibited by the other phenothiazines.

Our observation that phenothiazines within the dose range 10^{-3} to 10^{-7} M caused a reduction in TxB_2 synthesis is consistent with the inhibitory effect seen in platelets [7, 8] but does not resemble the stimulatory activity described in RBL-1 cells [6]. Certainly, the inhibitory activity of promethazine in actively phagocytosing macrophages can be mainly attributed to a diminution in phagocytosis. The degree of inhibition of both processes in the presence of promethazine was similar which suggests that decreased TxB_2 synthesis may be a consequence of impaired zymosan uptake by macrophages. However, promethazine also inhibited the basal synthesis of TxB_2 synthesis in the absence of zymosan which tends to suggest that inhibition of particle ingestion may not be the sole mechanism of action of the drug. Indeed, in cell-free macrophage homogenates, Wightman *et al.* [17] have shown that promethazine is inhibitory against phospholipase C (an enzyme involved in free arachidonic acid release), suggesting a direct action on the arachidonic acid metabolic pathway by the drug.

In direct contrast to promethazine, chlorpromazine selectively inhibited zymosan phagocytosis by alveolar macrophages in a dose-dependent manner. This inhibitory action resembles the effect of chlorpromazine on phagocytosis of *Staphylococcus epidermis* [27] and zymosan [14] by polymorphonuclear neutrophils. While zymosan phagocytosis was reduced at lower doses, chlorpromazine reduced TxB_2 synthesis only at 10^{-3} M in the actively phagocytosing macrophages where there was a clear loss of cell viability; even in resting macrophages, the drug was only mildly inhibitory. The reason for its lack of inhibitory activity on TxB_2 synthesis is unclear. Studies with chlorpromazine examining prostanoid synthesis have been contradictory. The drug has been reported to be stimulatory in RBL-1 cells but inhibitory in platelets. In cell-free systems such as bovine and ram seminal vesicles, both stimulatory and inhibitory activities have been reported [1–4]. Such diverse results could perhaps be due to the free radical scavenging nature of chlorpromazine. Depending on existing conditions such as presence of cofactors and substrate conditions, the drug may display opposite effects, as suggested by Gryglewski [28].

The third phenothiazine examined in our study is the quaternary promethazine analog, thiazinamium chloride. Owing to its quaternary structure, the drug is not expected to cross cell membranes easily. Such a proposal is supported by the work of Elferink [29], who showed that in open and resealed erythrocyte ghost membranes the quaternary analog of chlorpromazine is restricted to the outside face of the membrane. In spite of this, thiazinamium chloride was more potent than promethazine in inhibiting TxB_2 synthesis in both resting and actively phagocytosing macrophages. Yet, it virtually left the phagocytic process intact. Even at a dose of 10^{-3} M where there was almost complete cessation of TxB_2 synthesis, the ingestion of zymosan particles was unaffected. In addition, preliminary data using 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as a soluble stimulus showed that thiazinamium chloride still inhibited TxB_2 synthesis (unpublished observations).

Thiazinamium chloride and promethazine both possess anticholinergic activity in animals [22]. In view of the fact that anticholinergic agents have been reported to inhibit TxB_2 synthesis in the guinea pig lung [30], we also examined a specific anticholinergic agent, ipratropium, under our conditions. The data indicate that such inhibitors do not block TxB_2 synthesis by macrophages. The reason for the discrepancy is unclear but may suggest that anticholinergics exert their inhibitory actions on TxB_2 synthesis via a different cell type in the lung.

Earlier studies with platelets indicated that phenothiazines may interfere with TxB_2 synthesis at the phospholipase step prior to the conversion of arachidonic acid to TxB_2 by cyclooxygenase. We, therefore, have investigated the metabolism of exogenous radiolabeled arachidonic acid in macrophages. Under these conditions, indomethacin, a known cyclooxygenase inhibitor, was inhibitory at doses which inhibit the endogenous synthesis of TxB_2. In contrast, thiazinamium chloride was ineffective in blocking exogenous TxB_2 synthesis even at 10^{-3} M while promethazine was still inhibitory at 10^{-4} M.

These results, therefore, strongly suggest that thiazinamium chloride inhibits TxB_2 synthesis by reducing the availability of the endogenous arachidonic acid substrate. It is, however, not known at present whether it is inhibiting phospholipase A_2 or C since both enzymes have been described in macrophages [17, 31] and both are capable of generating free arachidonic acid. We have not ruled out the unlikely possibility that thiazinamium chloride, while inhibiting TxB_2 synthesis, may enhance the synthesis of other arachidonic acid metabolites and alter the spectrum of products synthesized by the alveolar macrophages. It appears that promethazine may also be acting directly on phospholipase although it is also possible that, at higher doses, it affects cyclooxygenase activity as well.

Phagocytes such as the alveolar macrophages possess the distinctive feature of undergoing oxidative metabolism via the HMP shunt during phagocytosis to generate oxygen-free radicals for host defense purposes [32]. Since the plasma membrane is a significant target for phenothiazines, one would expect that the HMP shunt would be affected. Indeed, both promethazine and chlorpromazine reduced HMP shunt activity. This is consistent with the observations of DeChatelet *et al.* [15, 16], where a similar reduction is seen in rabbit alveolar macrophages. In contrast, thiazinamium chloride has no effect on the HMP shunt suggesting that host defense mechanisms may be left intact during treatment with this drug.

The mechanism whereby promethazine and chlorpromazine impair the HMP shunt is unkuown. Conceivably, because of the high affinity of these phenothiazines for the plasma membrane and the fact that the (NADPH–NADH) oxidase system is also situated at this location, a situation might be created in which the enzyme is either inhibited or made unavailable for interaction in the HMP shunt. Additionally, a direct consequence of this inhibition would be a reduction of oxygen-free radicals in macrophages

since the reducing equivalents for the formation of these products are generated through the HMP shunt.

In conclusion, the present studies show that the phenothiazines differ both qualitatively and quantitatively in their biological actions on alveolar macrophage. However, it is possible that phenothiazines affect macrophage functions non-specifically. This is unlikely since the three macrophage functions examined in this study, i.e. TxB_2 synthesis, phagocytosis and HMP shunt, were not affected to the same degree at a particular concentration. There was no direct correlation between the concentration of phenothiazine necessary to demonstrate inhibition of TxB_2 synthesis with that needed to affect changes in phagocytosis and HMP shunt activity. Certainly, with regard to thiazinamium chloride inhibitory actions on arachidonic acid metabolism, our working hypothesis that it inhibits early in the pathway would predict an additional inhibitory effect on leukotriene synthesis as well. Our observations raise the interesting possibility that the bronchodilator effect of phenothiazines such as thiazinamium chloride may involve modulation of the arachidonic acid cascade, in addition to the antihistaminic, anticholinergic and mast cell stabilizing (antiallergic) properties already reported.

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REFERENCES

1. R. E. Lee, *Prostaglandins* **5**, 63 (1974).
2. A. Schaefer, M. Kolmos and A. Seregi, *Biochem. Pharmac.* **27**, 213 (1978).
3. H. Kunze, E. Bohn and G. Bahrke, *J. Pharm. Pharmac.* **27**, 880 (1975).
4. P. Krupp and M. Wesp, *Experientia* **15**, 330 (1975).
5. J. Robak, H. Kasperczyk and A. Chytkowski, *Biochem. Pharmac.* **28**, 2844 (1979).
6. V. A. Rigas, H. Van Vunakis and L. Levine, *Prost. Med.* **7**, 183 (1981).
7. J. Y. Vanderhoek and M. B. Feinstein, *Molec. Pharmac.* **16**, 171 (1979).
8. J. Maclouf, H. de la Baume. S. Levy-Toledano and J. P. Caen, *Biochim. biophys. Acta* **711**, 377 (1982).
9. A. L. Hyman, E. W. Spannhake and P. J. Kadowitz, *Am. Rev. resp. Dis.* **117**, 111 (1978).
10. J. Svensson, K. Strandberg, T. Tuvemo and M. Hamberg, *Prostaglandins* **14**, 425 (1977).
11. J. S. Goodwin and D. R. Webb, *Clin. Immun. Immunopath.* **15**, 106 (1980).
12. M. E. Goldyne and J. D. Stobo, *CRC Crit. Rev. Immun.* **2**, 189 (1981).
13. C. A. Rouzer, W. A. Scott, A. L. Hamill and Z. A. Cohn, *J. exp. Med.* **155**, 720 (1982).
14. J. G. Elferink, *Biochem. Pharmac.* **28**, 965 (1979).
15. L. R. DeChatelet, P. S. Shirley, P. Wang, L. C. McPhail and J. P. Gusdon, JR., *Proc. Soc. exp. Biol. Med.* **153**, 392 (1976).
16. L. R. DeChatelet, D. Qualliotine-Mann, R. Caldwell, C. E. McCall and J. P. Gusdon, *Infect. Immunity* **7**, 403 (1973).
17. P. D. Wightman, M. E. Dahlgren, J. C. Hall, P. Davies and R. J. Bonney, *Biochem. J.* **197**, 523 (1981).
18. H. J. Cohen, M. E. Chovaniec and S. E. Ellis, *Blood* **56**, 23 (1980).
19. D. L. Ochs and P. W. Reed, *Biochem. biophys. Res. Commun.* **102**, 958 (1981).
20. D. Drenckhahan, L. Kleine and R. Lullmann-Rauch, *Lab. Invest.* **35**, 116 (1976).
21. B. Bjorksten and P. G. Quie, *Infect. Immunity* **14**, 948 (1976).
22. A. J. Lewis, A. Dervinis and M. E. Rosenthale, *Eur. J. Pharmac.* **80**, 171 (1982).
23. R. P. Carlson, R. J. Capetola, J. M. DiLuigo, A. J. Lewis and D. H. Yu, *Fedn. Proc.* **40**, 1025 (1981).
24. A. J. Lewis, A. Dervinis, R. P. Carlson, M. E. Rosenthale and W. C. Daniel, *J. Allergy clin. Immun.* **69**, 155 (1982).
25. J. T. Flynn, *Adv. Shock Res.* **10**, 149 (1983).
26. L. R. DeChatelet and J. W. Parce, in Methods for Studying Mononuclear Phagocytes (Eds. D. O. Adams, P. J. Edelson and H. Koren), p. 477. Academic Press, New York (1981).
27. T. Huutu, *Ann. Med. exp. Biol. Fenn.* **50**, 24 (1972).
28. R. J. Gruglewski, *Agents Actions* (Suppl. 7), 247 (1980).
29. J. G. Elferink, *Biochem. Pharmac.* **24**, 2411 (1977).
30. G. Folco, C. Omini, G. Rossoni, T. Vigano and F. Berti, *Eur. J. Pharmac.* **78**, 159 (1982).
31. W. Hsueh, U. Desai, F. Gonzalez-Crussi, R. Lamb and A. Chu, *Nature, Lond.* **290**, 710 (1981).
32. F. Rossi, P. Patriaca and D. Romeo, in *The Reticuloendothelial System, A Comprehensive Treatise* (Eds. A. J. Sbarra and R. R. Strauss). p. 153. Plenum Press, New York (1980).