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Effects of Promethazine Hydrochloride on the Metabolism of Rabbit Alveolar Macrophages[1] (39553)

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Promethazine hydrochloride has been used clinically with impressive results to ameliorate the effects of erythroblastosis fetalis (1). Initial studies to define a mechanism of action demonstrated that administration of promethazine to experimental animals resulted in inhibition of both the primary and secondary immune response as well as delayed hypersensitivity (2). However, depression of circulating anti-Rh antibody titers has been noted in only some of the patients treated with the drug (J. P. Gusdon, Jr., unpublished data). The possibility that the drug was acting at the level of phagocytic cells was suggested by studies demonstrating that promethazine *in vitro* markedly inhibits oxidative events associated with phagocytosis by human polymorphonuclear leukocytes (3). It has been shown in animals that both the primary and secondary responses to red blood cell antigens are macrophage dependent (4, 5). We have recently demonstrated that fetal macrophages *in vitro* bind Rh-positive red blood cells which have been coated with anti-Rh antibody; this binding is inhibited *in vitro* by the addition of promethazine · HCl (6). The present report extends these studies by demonstrating that the drug inhibits macrophage metabolism *in vitro* in a fashion previously demonstrated for polymorphonuclear leukocytes (3). Further, administration of the drug *in vivo* to rabbits partially blocked the cellular activation induced by heat-killed Bacillus-Calmette-Guerin.

Materials and methods. In vitro studies. Alveolar macrophages were collected from female New Zealand White Rabbits by the lung lavage technique of Myrvik *et al.* (7), 3-4 weeks after the animals were injected iv with a sonic suspension of heat-killed Bacillus-Calmette-Guerin (BCG) in Bayol F (100 μg in 0.10 ml). The heat-killed BCG was kindly supplied by Dr. Quentin Myrvik. Lungs were lavaged with 80 ml of isotonic saline followed by two washes with the same medium. Contaminating red blood cells were lysed for 20 sec in deionized water, and the suspension was brought to isotonicity with 3.5% saline. Differential counts were performed by phase microscopy in a white cell-counting chamber and the isolated cells were suspended in Dulbecco's phosphate-buffered saline (PBS) to a concentration of 5×10^6 macrophages per ml. Cell purity was routinely greater than 80%, with the bulk of the contamination consisting of nonphagocytic lymphocytes.

The oxidation of [1-^{14}C]glucose was determined as previously described for neutrophils; all solutions were prepared in PBS (3). The reaction was routinely run in the presence of 1 m*M* cyanide to inhibit mitochondrial oxidation, which is significant in this cell type. Phagocytosis was initiated in appropriate flasks by the addition of 1.0 ml of heat-killed *Escherichia coli* B. The bacterial suspension was prepared in PBS and was standardized to give an absorbance of 1.00 at 525 nm on a Beckman DU spectrophotometer.

The kinetics of the phagocytic process were determined by measuring the uptake of radiolabeled *E. coli* B as previously described (8). The buffer employed was PBS containing a final concentration of 10% nor-

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mal rabbit serum to provide a source of opsonins.

In vivo studies. In order to determine whether the effects observed *in vitro* might be physiologically significant, a series of *in vivo* studies was initiated. Animals were divided into Four groups. Control: These animals received no treatment. BCG: These animals were injected iv with 100 μg of heat-killed BCG in Bayol F 3.5 weeks prior to sacrifice. Promethazine: Animals were injected daily with promethazine·HCl, 30 mg/kg body weight (im). BCG–Promethazine: Animals were first vaccinated with BCG and then injected with promethazine·HCl on a daily basis as above.

The concentration of promethazine in these studies is about 6× the maximal dose used in patient studies (1); it was selected because it was the dose employed in previous *in vivo* animal studies (2). We have not, however, determined serum levels of the drug in rabbits, nor have serum levels in man following ingestion of the drug been published, so direct comparison of the *in vivo* and *in vitro* studies is not possible. The numbers of animals in each group and physical data on the groups of animals are presented in Table I. Vaccination of the animals with BCG caused a marked increase in weight of the lungs and a 10-fold increase in the quantity of cells obtained from the lung in accord with previous reports (9, 10). Simultaneous treatment with promethazine partially blocked this response, but the differences are not impressive. This dose of promethazine (either alone or in vaccinated animals) appeared to cause some weight loss over the 3.5-week period of the experiment. Cells from all control animals were pooled, as were the cells from the promethazine-treated and the BCG–promethazine animals because of the low yield of cells per animal; the cells from the three BCG-treated animals were assayed separately.

The oxidation of [1-^{14}C]glucose was determined on these cells as previously described. Serum was replaced in the incubation mixture by an equivalent amount of glucose solution (80 mg/dl). This was done to eliminate effects of different sera on the cells. Latex spherules (0.81 μm) were employed as the challenge particle because they do not require opsonization for phagocytosis.

Results. Table II illustrates the effect of the *in vitro* addition of promethazine·HCl on the oxidation of [1-^{14}C]glucose by BCG-induced rabbit alveolar macrophages. Resting cells exhibit an appreciable capacity to oxidize glucose via the hexose monophosphate shunt; this is stimulated two- to threefold by the initiation of phagocytosis, in substantial agreement with previously published reports (9, 10). The addition of promethazine to a final concentration of 0.033 mg/ml causes a profound inhibition of glucose oxidation under either resting or phagocytizing conditions. This inhibition is dose dependent; increasing the concentration of

TABLE I. ANIMAL DATA FOR *In vivo* PROMETHAZINE·HCl EXPERIMENT.[a]

Treatment	Weight of start (kg)	Weight at end (kg)	Lung weight (g)	Packed cell volume
Control (8)	2.06 ± 0.06	2.25 ± 0.09	8.98 ± 0.51	0.18 ± 0.03
Promethazine (8)	2.07 ± 0.11	1.65 ± 0.08	7.58 ± 0.40	0.10 ± 0.02
BCG–Promethazine (6)	2.11 ± 0.08	1.85 ± 0.09	12.90 ± 0.64	1.20 ± 0.10
BCG (3)	2.02 ± 0.19	2.22 ± 0.14	16.30 ± 1.44	1.83 ± 0.44

[a] Values represent the mean ± SE for the number of animals given in parentheses.

TABLE II. EFFECT OF PROMETHAZINE·HCl ON OXIDATION OF [1-^{14}C]GLUCOSE BY BCG-INDUCED RABBIT ALVEOLAR MACROPHAGES.[a]

Description	Counts per minute in $^{14}CO_2$	
	Resting cells	Phagocytizing cells
Control	3204 ± 78	7983 ± 285
+ 0.033 mg/ml of promethazine·HCl	949 ± 21	1284 ± 60
+ 0.16 mg/ml of promethazine·HCl	408 ± 4	393 ± 10

[a] Values represent the mean ± SE for triplicate determinations.

promethazine to 0.16 mg/ml results in virtually complete inhibition of glucose oxidation. In experiments not shown, promethazine caused a similar inhibition of oxygen consumption by intact cells under both resting and phagocytizing conditions. These results cannot be attributed to an effect on cell viability since greater than 80% of the cells remained viable at the end of the incubation, as monitored by trypan blue exclusion.

This effect can be partially explained by an effect on ingestion as illustrated in Table III. Promethazine at a concentration of 0.16 mg/ml inhibits the uptake of radiolabeled bacteria at all time points examined. The inhibition of uptake, however, is not nearly as pronounced as the inhibition of cellular metabolism; also the effect on resting cells must be independent of any effects on particle uptake. Thus, the drug must exert a specific effect on macrophage oxidative metabolism which is independent of any effect on ingestion.

In an attempt to determine whether the effect of promethazine might be physiologically significant, we turned to *in vivo* experiments, as described in Materials and Methods. Results of these experiments are listed in Table IV. Vaccination of an animal with BCG results in marked activation of the alveolar macrophage, as indicated by the dramatic increase in [1-^{14}C]glucose oxidation. This activation is most apparent in resting cells, but is also observed in cells phagocytizing latex. The daily administration of promethazine to the BCG-treated animals causes a significant ($P < 0.001$) inhibition of the cellular activation. This inhibition is observed under both resting and phagocytizing conditions.

TABLE III. EFFECT OF PROMETHAZINE·HCl ON PHAGOCYTOSIS OF *E. coli*-^{14}C BY BCG-INDUCED RABBIT ALVEOLAR MACROPHAGES.[a]

	Cell-associated bacteria (cpm)	
Time (min)	Control	Promethazine
0	936 ± 99	846 ± 7
5	5,987 ± 585	3,140 ± 241
10	14,152 ± 610	8,344 ± 263
15	18,023 ± 471	12,279 ± 676
20	21,644 ± 128	12,257 ± 276

[a] Values represent the mean ± SE for triplicate determinations. Promethazine was added, where indicated, to a final concentration of 0.16 mg/ml. Cells were challenged with 86,400 cpm of radiolabeled *E. coli* B.

Discussion. In a doctoral thesis in France, promethazine was first reported to be effective in ameliorating the effects of erythroblastosis in babies (Bierme Alie Enjalbert, Doctoral Thesis, Centre Regional de Transfusion Sanguine et d'Hematologie, Toulouse, France, 1967). Published clinical experiments conducted at this institution have generally supported this observation (1). To date, the effectiveness of promethazine hydrochloride in ameliorating the disease has been studied over the past 7 yr in more than 40 patients. It appears as though the fetal mortality has been reduced by at least 60% in this disease process (J. P. Gusdon, Jr., unpublished observations). More extensive clinical trials are currently underway with six other collaborating institutions.

The mechanism whereby the drug accomplishes this effect is uncertain, but promethazine has been demonstrated to cross the placenta (11, 12). It is likely that the effectiveness of this drug in ameliorating the effects of erythroblastosis is a function of its ability to impair the fetal reticuloendothelial cells (macrophages and/or lymphocytes) which are responsible for red cell lysis. In a previous study, we demonstrated that the *in vitro* addition of promethazine inhibited the ability of fetal macrophages to bind Rh-positive red blood cells (6); this has recently been demonstrated in the neonatal infant as well (13). The present study extends these observations by describing specific effects of promethazine hydrochloride on macrophage metabolism both *in vitro* and *in vivo*, thus, lending support to the concept that the drug acts at the level of the macrophage.

It seems possible that the primary effect of the drug might be at the level of the cell membrane, resulting both in decreased binding to opsonized red blood cells and to altered cellular metabolism.

Summary. The addition of promethazine hydrochloride to a suspension of rabbit alveolar macrophages *in vitro* results in an inhibition of cellular glucose oxidation under both resting and phagocytizing conditions as well as in an inhibition of phagocytosis of radiolabeled bacteria. The *in vivo*

TABLE IV. OXIDATION OF [1-^{14}C]GLUCOSE BY ALVEOLAR MACROPHAGES OBTAINED FROM RABBITS UNDER VARIOUS EXPERIMENTAL CONDITIONS.[a]

Treatment	Counts per minute in $^{14}CO_2$	
	Resting cells	Phagocytizing cells
Control (8)	4,563 ± 63	10,708 ± 87
Promethazine (8)	3,894 ± 40	6,714 ± 127
BCG–Promethazine (6)	10,250 ± 213	16,432 ± 322
BCG	22,399 ± 150	38,018 ± 805
BCG	31,513 ± 299	40,558 ± 231
BCG	21,834 ± 15	29,711 ± 1390

[a] Values represent the mean ± SE of triplicate determinations. Cells were pooled from the number of animals indicated in parentheses; data for the three BCG-induced animals are given separately.

injection of the compound into rabbits partially suppresses the BCG-induced activation of the alveolar macrophages. These results suggest that the mechanism of action of promethazine hydrochloride in ameliorating the effects of erythroblastosis might lie, at least in part, in its ability to suppress the fetal macrophages which are responsible for red cell lysis.

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