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Protective Effect of Clofibrate Against *S. pneumoniae* Infection in Rats[1] (39414)

MICHAEL C. POWANDA AND PETER G. CANONICO

United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701

Pneumococcal sepsis is associated with decreased plasma albumin concentration and increased acute phase globulin synthesis (1-3) even in protein deficient or starved animals, implying that altered plasma protein metabolism is a fundamental host response to infection. Hepatic peroxisome content is decreased during pneumococcal sepsis; and it has been suggested that the increased acute phase serum protein synthesis occurs at the expense of peroxisomal protein synthesis (4). Increased cholesterolgenesis (5) and triglyceride synthesis (6) have also been reported to occur in pneumococcal infection. The inverse relationship between hepatic peroxisomal content and lipid metabolism during infection may be more than coincidence in that peroxisomes are thought to control intracellular cholesterol level by regulating its degradation (7) and drugs which lower serum cholesterol and triglyceride concentration induce hepatic peroxisomal proliferation (8). Clofibrate (ethyl-*p*-chlorophenoxyisobutyrate), an antihyperlipidemic agent produces diametrically opposite changes in metabolism compared to pneumococcal sepsis, i.e., increased plasma albumin concentration and decreased acute phase globulin levels (9), increased catalase synthesis (10), and decreased plasma cholesterol and triglyceride concentrations (11). It was therefore of interest to ascertain whether clofibrate would mute or enhance the severity of pneumococcal sepsis in rats.

Materials and methods. Fisher Dunning rats (Microbiological Associates, Walkersville, Md.) weighing 150-200 g were housed in a room maintained at 25-28° and were fed *ad libitum* on commerical rat chow (Purina). Clofibrate, provided by Dr. George Brice (Ayerst Laboratories, N.Y.) was dissolved in 95% ethanol and added to ground commercial chow on a wt/wt basis. The drug-treated chow was allowed to air-dry to remove the ethanol before being fed to the rats. Virulent *Streptococcus pneumoniae* type 1, strain 5, served as the infectious agent. Infection was initiated by the sc injection of the requisite number of organisms in 0.1 ml of saline. Bacteremia was quantitated by plating 0.1 ml of serial 10-fold dilutions of whole blood onto blood agar plates and counting the resulting colonies after 24 hr of incubation at 37°. Plasma chlorophenoxyisobutyrate (CPIB) content was determined by the spectrophotometric method of Barret and Thorpe (11). Clofibric acid for standardization was obtained from Dr. Dvornik (Ayerst Laboratories, Montreal, Canada).

For carbon clearance studies rats were lightly anesthetized with halothane and then injected via the dorsal penile vein with a suspension of colloidal carbon (Pelikan ink, Gunther Wagner) at a dose of 8 mg/100 g body wt. At various intervals 25-μl blood samples were obtained from the tail vein, mixed with 2.0 ml of 0.1% Na_2CO_3, and the optical density measured at 620 nm. The clearance rate (K) was calculated according to Gorelkin and Jahrling from the formula $K = \log C_1 - \log C_2/t_2 - t_1$. C_1 and C_2 are colloidal carbon concentrations in mg/100 ml of blood, $C = \text{O.D.}_{620} \times 48.5$, and t_1 and t_2 are times of sampling in minutes (12).

Results. Rats were fed a normal diet or one containing either 0.25 or 1.25% clofibrate (wt/wt) for 1 week before challenge with 3×10^2 *S. pneumoniae* injected sc. All untreated rats were dead in 7 days, while 5 of 20 fed the 0.25% diet and 17 of 20 fed the 1.25% diet survived (Fig. 1). Clofibrate

[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

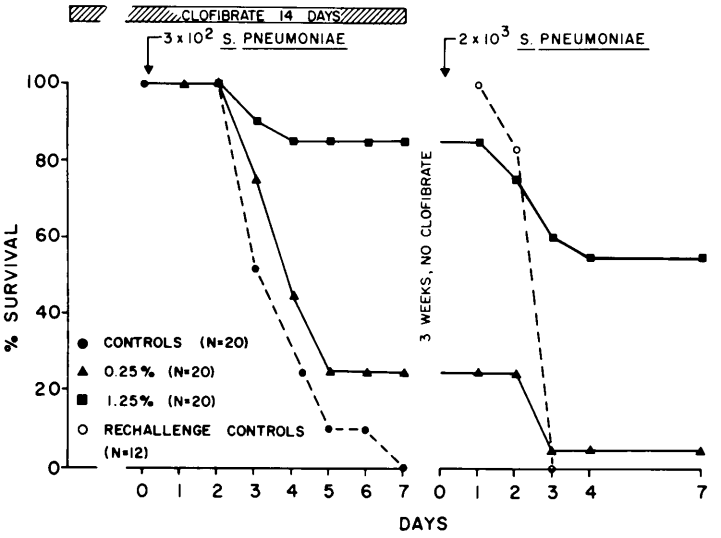

FIG. 1. Effect of dietary clofibrate on survival in rats inoculated with *S. pneumoniae*.

was removed from the diet 7 days postinfection and 3 weeks later the survivors plus a new set of controls were challenged with 2 × 10^3 organisms of the same strain of *S. pneumoniae*. All control rats died within 3 days, only 1 of 5 of those rats which originally had been on the 0.25% diet survived while 11 of 17 of those that had been on the 1.25% diet survived.

Since clofibrate protected some rats against death due to pneumococcal sepsis, another set of control and drug-treated (1.25%) rats were inoculated sc with 10^3 *S. pneumoniae* and animals were sacrificed at 24-hr intervals to determine bacteremia. Pretreatment with clofibrate caused a significant decrease in bacteremia at 48 hr as well as a decrease in the number of animals with bacteremia. Although both the number of bacteria/ml blood and the number of rats with positive cultures were equally low in the drug-treated group at 72 hr, no statistically significant difference was measurable owing both to the paucity of survivors in the untreated group and the large standard deviation in the drug-treated groups (Table I).

Since clofibrate not only protected rats against *S. pneumoniae* infection but also decreased the incidence and extent of bacteremia, plasma from rats fed various levels of dietary clofibrate were tested to determine if the drug had a bactericidal or bacteriostatic activity. Nine-hundred *S. pneumoniae* were incubated with plasma; samples were withdrawn for plating at 30 min and 3 hr. There was no change in bacterial count after 30-min incubation, indicating that the active form of clofibrate in the plasma (CPIB) does not have bactericidal activity. The comparable growth evident at 3 hr in all instances indicated that CPIB does not have a bacteriostatic effect (Table II).

TABLE I. BACTEREMIA[a] AFTER SUBCUTANEOUS INJECTION OF 10^3 *S. Pneumoniae*.

	Control		1.25% Clofibrate	
Time (hr)	Log titer/ml	No. positive/total	Log titer/ml	No. positive/total
24	0	0/5	0	0/5
48	5.2	5/5	3.8[b]	3/5
72	5.7	3/3	3.9	3/5
96	No survivors		0	0/3

[a] Bacteremia determined in duplicate.
[b] $P < 0.01$ vs. control.

Since clofibrate at the levels used in this study induces hepatomegaly, it is possible that clofibrate might induce a proliferation of the reticuloendothelial system as well and thus enhance the phagocytosis of the *S. pneumoniae*. As can be seen in Fig. 2 the rate of clearance of colloidal carbon from the blood of rats fed clofibrate for 10 days was the same as that of control rats. The *K* value for control rats was 0.035 ± 0.008 (mean ± SD), a value similar to that previously reported for normal rats (13).

Discussion. Clofibrate added to the diet

TABLE II. PLASMA CPIB CONTENT AND ANTIBACTERIAL ACTIVITY.

Clofibrate in diet (%)	Plasma CPIB μg/ml[a] Mean ± SE	Range	Organisms/ml + SE[b] 30 min	3 hr
0	–	–	1095 ±140	3148 ±472
0.05	33 ±5	(28–44)	1095 ±260	2856 ±723
0.25	109 ±8	(86–174)	1035 ±95	2592 ±476
1.25	246 ±31	(114–372)	1165 ±250	2856 ±728

[a] $N = 10$.

[b] $N = 5$. Nine-tenths milliliter of plasma was mixed with 0.1 ml of saline containing 900 *S. pneumoniae* and incubated at 37°. Duplicate 0.2-ml samples were taken at 30 min and 3 hr; bacteria were quantitated by plating 0.1 ml of serial 10-fold dilutions onto blood agar plates and counting the resulting colonies after 24 hr of incubation at 37°.

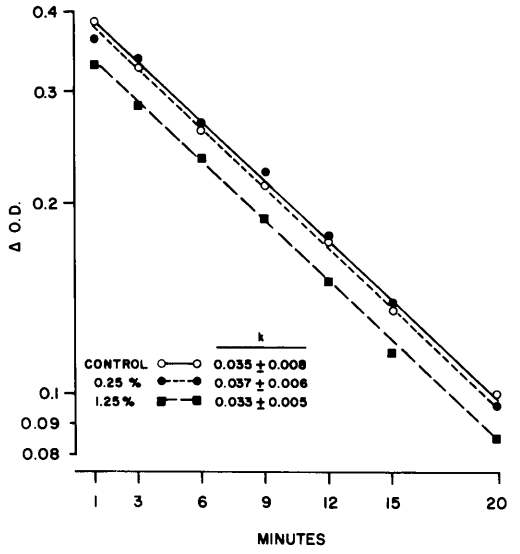

FIG. 2. Carbon clearance in normal rats and in those fed various amounts of clofibrate in the diet.

for a week prior to infection with *S. pneumoniae* protects some rats from what would otherwise be a uniformly lethal infection. Rechallenge of the survivors with the same strain of *S. pneumoniae* in the absence of the drug indicates that many of the rats previously had been exposed to sufficient antigenic mass to induce immunity. Despite the hepatomegaly induced by clofibrate, it is unlikely that the drug diminishes bacteremia and death by enhancing the phagocytosis of the bacteria since carbon clearance, an indicator of phagocytic activity, is unaltered in drug-treated rats. Nor is there any consistent, significant effect of the drug on total leukocyte or differential count which might account for the protection afforded. It is apparent that although plasma containing the active form of the drug is neither bactericidal nor bacteriostatic *in vitro*, clofibrate still decreases the incidence and extent of bacteremia *in vivo*. Although clofibrate does not exhibit bactericidal or bacteriostatic action *in vitro*, it is nonetheless still possible that the drug may deleteriously affect the metabolism of bacteria which are growing within an animal or restrict the availability of substrate, thereby lessening microorganism replication. Grossberg *et al.* (14) have proposed that clofibrate decreases viral replication in some instances by limiting the supply of lipids necessary for virus membrane formation. Weinberg (15) has suggested that the decrease in plasma iron which occurs in numerous infectious illnesses may, at least in some instances, be beneficial to the host in restricting microbial growth. Clofibrate does, in fact, occasion a decrease in plasma iron concentration and in that of zinc, iron, and copper (unpublished data), as well as decreasing plasma cholesterol and triglyceride concentrations (11) and altering serum protein patterns (9).

The precise mechanism of action of clofibrate's protection against pneumococcal sepsis in the rat, as well as the implications of this protection as regarding the essentially of certain of the host's responses to infection (1–6), remains to be determined.

Summary. Clofibrate, nominally an antihyperlipemic agent, added to the diet for a week prior to the inoculation of rats with *S. pneumoniae* protected some of the rats from what would normally be a uniformly lethal infection. Plasma from clofibrate-treated rats was neither bactericidal nor bacteriostatic, yet drug-treated rats displayed significantly diminished incidence and extent of bacteremia. Clofibrate does not alter carbon clearance, thus it is unlikely that clofibrate affords protection through enhanced reticuloendothelial removal of microorganisms. The mechanism and implications of clofibrate protection remain to be determined.

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