In vitro inhibition of lipolysis
by pyrimethamine in blood from ducks
parasitized with Plasmodium lophurae

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SUMMARY

The effect of pyrimethamine on lipolysis was studied in systems of blood from normal ducks and ducks infected with Plasmodium lophurae. After incubation with sodium (1-14C) acetate, total lipids were extracted from erythrocytes (RBC) and plasma and separated into classes by thin-layer chromatography, and levels of radioactivity were measured.

Pyrimethamine induced a reduction in the uptake of 1-14C acetate into the total lipid fraction of RBC and the excretion of lipids by blood cells into plasma. The percent incorporation of 1-14C acetate into the phospholipid fraction of RBC appeared to be proportional to the concentration of pyrimethamine, particularly in parasitized RBC. The activity in the triglycerides increased more in normal RBC than in infected cells. In both instances, percent incorporation in the free fatty acid and sterol fractions decreased with an increase in pyrimethamine concentration. It also appeared that pyrimethamine induced an increase in the excretion of labeled phospholipid, triglyceride, sterol, and sterol ester into the plasma, whereas there was a decrease in the level of labeled free fatty acid.

INTRODUCTION

It is a characteristic of the antimalarial drugs used more commonly that they partially or completely inhibit lipolysis, leading to subsequent production of free fatty acids (FFA) in rat epididymal adipose tissue (8,10).

The finding that stearic acid was a requirement for in vitro cultivation of Plasmodium (11) led to the present study. It was
suggested that the antimalarial drugs owed their effectiveness, in part, to a reduction of FFA available to the parasite (8).

The lipids of fowl blood have been shown to incorporate labeled acetate (1,13). Furthermore, an absolute increase in the weight of total lipid of erythrocytes (RBC) infected with *Plasmodium* has been confirmed (6,9). Therefore, the lipids of blood offered a system to extend the aforementioned studies on antimalarial drugs and their effects on lipolytic processes.

This paper presents data on the effects of another antimalarial drug, pyrimethamine, on the incorporation of sodium (1-14C) acetate by the lipids of blood from normal ducks and ducks infected with *P. lophurae*. The mechanism of action of pyrimethamine has been precisely defined as selectively inhibiting the enzyme, dihydrofolate reductase of *Plasmodium* (2,4,5). Its effects on lipids have not been reported.

**MATERIALS AND METHODS**

Blood from normal Pekin ducklings and ducklings infected with *P. lophurae* was collected by cardiac puncture in heparin. The degree of parasitemia was determined from blood smears stained by the Giemsa method. Packed-cell volumes averaged 35% for normal ducklings and ranged from 11 to 19% for the acutely-infected ducklings.

Fifty ml of blood were added to a 250-ml Erlenmeyer flask containing Versene (50 µg), penicillin (50 mg), and streptomycin (62 mg). Sodium (1-14C) acetate (specific activity 40 mc/mM) was diluted in 0.85% NaCl solution and added at the rate of 1 µc/ml of blood. Pyrimethamine was added to the incubation mixture to give final concentrations of 2.0 and 6.0 mM. The flasks were plugged with cotton and incubated for 5 hours in an agitating water-bath at 39 C. Aseptic techniques were employed throughout the study.

After the incubation period, plasma and erythrocytes (RBC) were separated by centrifugation and the buffy coat discarded. The RBC were centrifugally-washed 3 times in 0.85% NaCl solution. Total lipids from both plasma and RBC were extracted overnight with chloroform:methanol (2:1, v/v) on a magnetic stirrer. The lipids were recovered as described by Folch et al. (3).

Total lipids in chloroform were dried over Na2SO4 and reduced in volume by evaporation under nitrogen. The lipid classes were separated by thin-layer chromatography (TLC) on Silica Gel G in the solvent system of Mangold (7), consisting of petroleum ether, diethyl ether, and formic acid (84:15:1, v/v/v).
Table 1. Effect of pyrimethamine on the incorporation of sodium (1-14C) acetate into lipid classes of RBC from normal ducks and ducks infected with *Plasmodium lophurae*.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Normal RBC</th>
<th>Parasitized RBC^B^</th>
<th>Parasitized RBC^C^</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyrimethamine (mM)</td>
<td>Pyrimethamine (mM)</td>
<td>Pyrimethamine (mM)</td>
</tr>
<tr>
<td></td>
<td>Control 2.0 6.0</td>
<td>Control 2.0 6.0</td>
<td>Control 2.0 6.0</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>20.7 22.7 22.9</td>
<td>22.0 35.5 35.9</td>
<td>29.8 34.8 35.8</td>
</tr>
<tr>
<td>Sterol</td>
<td>35.7 36.7 24.8</td>
<td>34.8 28.7 29.7</td>
<td>40.8 38.2 37.2</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>24.1 20.0 14.3</td>
<td>36.8 26.4 25.2</td>
<td>20.5 14.3 11.4</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>15.5 18.5 34.3</td>
<td>5.5 8.3 8.1</td>
<td>7.9 12.4 15.0</td>
</tr>
<tr>
<td>Sterol ester</td>
<td>3.7 2.1 3.4</td>
<td>1.0 1.1 1.0</td>
<td>0.8 1.2 1.6</td>
</tr>
<tr>
<td>Total counts per minute</td>
<td>63,560 39,397 33,051</td>
<td>89,230 61,121 57,960</td>
<td>110,960 56,340 50,710</td>
</tr>
</tbody>
</table>

^A^Means of at least 4 trials each.

^B^30-35% parasitemia.

^C^90-95% parasitemia.

TLC plates were developed in iodine vapor. A mixture of standards assisted identification of the classes. Zones containing lipid corresponding to a standard lipid class were scraped from the plate and placed in glass columns which has been plugged with fat-free cotton. Methanol:chloroform (2:1, v/v) was employed to elute phospholipid and diethyl ether for all other classes of lipid.

The eluates were collected in scintillation vials and dried in a vacuum oven at 30 C for 3 hr. To each vial were added 15 ml of methylcellosolve, 2,5-bis-5'-tert-buty1-benzoxazoyl (2')-thiophene (BBOT) scintillation mixture (12). Twenty-minute counts were taken on each sample with a Beckman liquid scintillation spectrometer, model LS-250.

### RESULTS

**Effect of *P. lophurae* on in vitro lipolysis in blood.** Table 1 gives data on the effect of parasitemia on incorporation of 1-14C-acetate into extractable lipids of RBC. Total counts per minute (cpm) increased with the degree of parasitemia. Significant changes occurred in the relative percent of total radioactivity in the lipid classes of RBC parasitized with *P. lophurae*. When 30–35% of the RBC were parasitized, the mean relative percent of radioactivity incorporated into FFA increased approximately 50% above that of normal RBC and the level of labeled TG decreased approximately 66% below that of normal cells. The level of radioactivity in the PL class increased approximately 10% above that of normal RBC. When 90–95% of the RBC were parasitized, the amount of radioactivity in the FFA class was about 17% below that of normal cells. The level of radioactivity of PL increased approximately 50%
above that found in normal RBC. In parasitized cells, radioactivity in ST increased approximately 14% and SE decreased approximately 78% from normal values.

After incubation of blood parasitized with *P. lophurae*, the FFA class of plasma lipids contained a higher percent of the total radioactivity than that of normal RBC (Table 2). When 90–95% of RBC were parasitized, the percent of radioactivity of FFA was comparable to that observed when 30–35% of the RBC were parasitized with *P. lophurae*. The percentage of radioactivity of the PL and TG classes in plasma from blood from infected ducks decreased below that of normal plasma.

**Effect of pyrimethamine on lipolysis of normal duck blood.** Incubation of normal duck blood with 2.0 mM and 6.0 mM pyrimethamine resulted in a decrease of total radioactivity in lipid extracted from RBC (Table 1). Pyrimethamine at a 2.0 mM concentration reduced total cpm approximately 30% below normal, and 6.0 mM pyrimethamine reduced the total cpm approximately 50% below that of normal RBC. The drug had a significant effect on the distribution of radioactivity among the lipid classes. Labeled FFA of blood incubated with 6.0 mM pyrimethamine decreased about 25% below that of the control, and the mean relative percent of radioactivity in TG increased approximately 125% above that of the control. A slight increase above the control level was detected in the level of radioactivity incorporated into the PL class. Pyrimethamine at 2.0 mM had little effect on the percent radioactivity incorporated into the ST class; however, 6.0 mM pyrimethamine de-

### Table 2. Effect of pyrimethamine on levels of radioactivity found in the lipid classes of plasma of normal ducks and ducks infected with *Plasmodium lophurae* in which RBC were incubated with sodium (1-14C) acetate.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Normal plasma</th>
<th>Plasma from infected ducks&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Plasma from infected ducks&lt;sup&gt;B&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyrimethamine (mM)</td>
<td>Pyrimethamine (mM)</td>
<td>Pyrimethamine (mM)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>5.9</td>
<td>10.7</td>
<td>12.5</td>
</tr>
<tr>
<td>Sterol</td>
<td>3.8</td>
<td>7.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Free fatty acid</td>
<td>85.8</td>
<td>79.2</td>
<td>77.3</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>2.8</td>
<td>5.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Sterol ester</td>
<td>1.0</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Total cpm per minute</td>
<td>22,180</td>
<td>8,708</td>
<td>6,258</td>
</tr>
</tbody>
</table>

<sup>A</sup>Companion samples of plasma from blood incubated as shown in Table 1.

<sup>B</sup>30–35% parasitemia.

<sup>C</sup>90–95% parasitemia.
creased the level of radioactivity in the ST class approximately 35% below that of the control.

**Effect of pyrimethamine on lipolysis of blood parasitized with P. lophurae.** Table 2 indicates that plasma lipids from blood incubated with pyrimethamine possessed reduced levels of radioactivity. The distribution of $^{14}$C among plasma lipid classes generally followed that of the lipids extracted from RBC.

Table 1 indicated that a decrease in the incorporation of $^{14}$C-labeled acetate occurs into RBC lipids when blood parasitized with *P. lophurae* is incubated with pyrimethamine. The distribution of radioactivity among the lipid classes is similar to that of normal RBC incubated with pyrimethamine with one important exception. Whereas the mean relative percent of radioactivity found in the PL class changes little when normal RBC are incubated with pyrimethamine, a significant increase in radioactivity occurs in the PL of RBC parasitized with *P. lophurae*. Plasma lipids in blood from parasitized ducks incubated with $^{14}$C-labeled acetate and pyrimethamine showed a decrease in total cpm (Table 2). The mean relative percent of radioactivity associated with the FFA decreased significantly below that of normal plasma, and the level of radioactivity incorporated into PL and TG increased above the value for normal plasma.

**DISCUSSION**

A marked increase in lipids of RBC parasitized with malarial parasites has been found (6,9). Since parasitized RBC exhibit an increased biosynthetic activity, one might expect antimalarial drugs to alter lipid metabolism of these RBC. Quinine and several of its derivatives inhibit *in vitro* lipolytic processes (8,10).

Our data indicate that pyrimethamine inhibits *in vitro* lipase activity in both normal duck RBC and duck RBC parasitized with *P. lophurae* as evidenced by uptake of $^{14}$C-labeled acetate. The increase in radioactivity found in the TG class and the concomitant decrease in FFA indicate that pyrimethamine inhibits the hydrolysis of fatty acids in TG. During the early course of the parasitemia, FFA and PL increase and TG decrease. When 90–95% of the RBC are parasitized, the levels of FFA and TG decrease, while that of PL increases. In normal RBC incubated with pyrimethamine, TG increases and FFA decreases. Pyrimethamine reduced total cpm in extractable lipid in both normal blood and blood parasitized with *P. lophurae*. This decrease in incorporation of labeled acetate was also reflected in the amount of total labeled lipid found to be ex-
creted by the blood cells into plasma during incubation. Apparently, the drug has an adverse effect on incorporation of acetate into lipid.

The accumulation of labeled phospholipid indicates that pyrimethamine may also inhibit phospholipases. It has been reported that another antimalarial drug, primaquine, stimulates the incorporation of long-chain fatty acids into phospholipids (14).

All of the more commonly employed antimalarial drugs inhibit lipolysis as measured by various means employing rat epididymal adipose tissue as the test system (8). Apparently pyrimethamine is similar to other antimalarial drugs in that it has antilipolytic activity.

REFERENCES


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