# EFFECTS OF *IN VIVO* TREATMENT WITH A NEW FLUORINATED MACROLIDE (P-0501A) AND OTHER ERYTHROMYCINS ON DRUG CLEARANCE AND HEPATIC FUNCTIONS IN PERFUSED RAT LIVER

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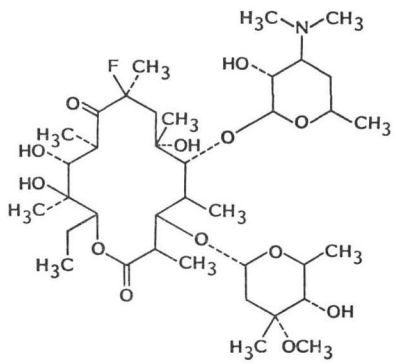
(Received for publication December 2, 1985)

The hepatic clearance and the effects of a new fluorinated macrolide (P-0501A) on the functions of the isolated, perfused rat liver were compared with two known erythromycins – the base and the estolate – after 7 days of treatment (1.36 mmol/kg po daily). The *in vitro* metabolism of the antibiotics was induced to different extent but only the base and P-0501A were cleared from the perfusate and the liver faster than in untreated animals. In untreated rats the therapeutically active form of P-0501A was excreted in the bile more than the base and the estolate; after pretreatment, biliary excretion of all erythromycins was nearly double. The content of inactive, complexed cytochrome P-450 was increased only by the base and estolate, with various effects on microsomal activities (some induced, *e.g.* aminopyrine demethylation, other reduced, *e.g.* pentobarbital clearance). The clearance and biliary excretion of sulphobromophthalein was not affected by treatment with P-0501A or the base, but was significantly reduced by estolate.

Erythromycin base and estolate are orally active antibiotics which concentrate in the liver where they are partly inactivated by demethylation and partly excreted in an active form in the bile[1]. Both drugs have major limitations: The base is inactivated by gastric juice and the estolate, though more gastro-resistant, is hepatotoxic in humans[2] and in experimental models[3~6]. Moreover repeated administration of erythromycins to humans[7] and rats[8~10] induces biosynthesis of microsomal enzymes and promotes formation of metabolites which bind and inactivate cytochrome P-450; hepatic mixed function oxidase activities are variously affected, some being induced but most inhibited[2,10]. It was found that a new fluorinated macrolide (8*S*)-8-fluoroerythromycin A (P-0501A, see Fig. 1)†[11], was more active when given orally[12,13] and less toxic in liver cell culture[6] than erythromycin base; it does not form inactive cytochrome P-450-metabolite complexes and does

Fig. 1. Flurithromycin (P-0501A, (8*S*)-8-fluoroerythromycin A).



† INN: Flurithromycin.

not affect the metabolism of other compounds after prolonged treatment[10]. In order to compare the effects of the new derivative, the base and the estolate on hepatic clearance and liver functions we used perfused livers isolated from animals after 7 days of treatment (1.36 mmol/kg daily).

## Materials and Methods

### Chemicals

The three macrolides, P-0501A (flurithromycin, see Fig. 1), erythromycin base and estolate were kindly supplied by Pierrel S.p.A., Milan, Italy. All other chemicals were analytical grade.

### Animals

Male Crl: CD (SD) BR rats (Charles River, Calco, Italy) weighing 160~180 g were fed a standard diet *ad lib.* (Altromin MT, Rieper, Vandoies, Italy) and housed under controlled conditions (22±0.5°C, 55% relative humidity, 12/12 light/dark cycle).

### Treatments

Rats were given suspensions of the erythromycins in corn oil orally (by gastric tube), 1.36 mmol per kg divided into two daily doses, for 7 consecutive days. Control animals received 8 ml of corn oil/kg/day. Animals were used as liver donors 24 hours after the last dose.

### Liver Perfusion

Livers were isolated by the usual surgical technique[14] under Na-phenobarbital (50 mg/kg) and chloralose (60 mg/kg) anesthesia; the biliary duct and portal vein were cannulated. The perfusion medium contained one-third homologous difibrinated blood, two-thirds Krebs-Ringer bicarbonate buffer pH 7.4 with 4% of bovine serum albumin and 0.1% of glucose.

The drugs, dissolved in dimethyl sulfoxide, were used at the concentration of $68\times10^{-6}$ M (equivalent to 50 $\mu$g erythromycin base/ml perfusion medium). The initial volume of medium was proportional to the liver weight, 9 ml/g tissue and flow rate was 1 ml/g liver/minute. Sulfobromophthalein (BSP) was added at a concentration of 1 mg/g liver 10 minutes after perfusion was started. In some experiments pentobarbital was added to the perfusion medium at the concentration of 25 $\mu$g/ml in order to measure the influence of the *in vivo* treatment with erythromycin on the metabolism of this xenobiotic.

### Analysis of the Perfusate

The following parameters were studied during 3-hours' perfusion: The concentration of the erythromycins[15], BSP[16] and pentobarbital[17], the activity of glutamic-oxalacetic transaminase (GOT) and urea levels (Boehringer assay kits, Mannheim, West Germany). The perfusion medium and BSP did not interfere with erythromycin determination.

### Bile Analysis

The bile flow and the excretion of erythromycins[15] and BSP[16] were determined. At the end of the perfusion the liver was weighed and one lobe was used to measure ATP[18] and erythromycins[15]. The remaining tissue was homogenized, microsomes were prepared as previously described[19] and uncomplexed cytochrome P-450[20] was determined. The total amount of cytochrome P-450 was measured by the same procedure[20] after first adding 50 $\mu$M potassium ferricyanide to another batch of microsomes. *N*-Demethylation of erythromycin[21] and aminopyrine[19] were assayed using microsomes treated with 50 $\mu$M ferricyanide to break the inactive cytochrome P-450-metabolite complex.

### Kinetic and Statistical Calculations

The disappearance of erythromycins, BSP and pentobarbital from the perfusion medium followed first order kinetics:

$$C_t = C_0 \cdot e^{Kt}$$

where $C_0$ is the initial concentration of the compounds, K is the first order elimination rate constant and $C_t$ is the concentration at time t. Mean hepatic clearance was calculated from the formula:

Table 1. Comparison of erythromycin clearance by perfused liver and amount of erythromycins (E) in liver tissue and in bile after 3-hours' perfusion of untreated and pretreated rats.

| Drug | Clearance (ml/minute) | | E in liver (total $\mu$g in liver) | | E in bile (total $\mu$g in bile) | |
|---|---|---|---|---|---|---|
| | Untreated | Pretreated[a] (%)[b] | Untreated | Pretreated (%) | Untreated | Pretreated (%) |
| E-Base | 2.02±0.25 | 6.65±1.04 (329) | 1,022±130 | 110±20 (11) | 373±53 | 741±101 (199) |
| E-Estolate | 1.49±0.26 | 1.40±0.27[d] (94) | 905±186 | 935±120[e] (103) | 143±28[e] | 262±71[e] (183) |
| P-0501A | 1.65±0.19 | 3.29±0.61[e] (200) | 944±78 | 469±29[d] (50) | 564±35[d] | 848±86 (150) |

[a] Each group of rats was treated with one of the erythromycins (1.36 mmol/kg po daily) for 7 days and 24 hours after the last dose the livers were perfused with the same antibiotic (50 $\mu$g/ml medium). The results are means±SE of 3~4 rats.

[b] Percentage of the untreated rats.

[c] $P<0.05$ vs. E-Base, [d] $P<0.01$ vs. E-Base, [e] $P<0.01$ vs. E-Base and P-0501A.

$$CL_H=(A_0/C_0)K$$

where $A_0$ is the initial amount of the compounds in the total volume of medium[22]. The statistical significance of the results was established by one-way analysis of variance and DUNCAN's[23] and DUNNETT's tests[24].

## Results

### Clearance and Metabolism of Erythromycins

#### Perfusion of Isolated Liver

The fate of erythromycin base, estolate and P-0501A added to perfused rat liver was followed in perfusion medium, liver tissue and bile after 7-days' treatment with the same drugs (1.36 mmol/kg daily). The clearance of erythromycin base and P-0501A by liver of treated rats was respectively three and two times that in untreated animals (Table 1). The amount of active drug in the liver after 3-hours' perfusion with 50 $\mu$g/ml of erythromycin base and P-0501A was considerably lower (11% the base and 50% P-0501A), than in untreated animals (Table 1). Biliary excretion of P-0501A in untreated rats was significantly higher than that of the base and estolate; after *in vivo* treatment excretion of all three erythromycins in bile was about double that in untreated animals (Table 1). Pretreatment with erythromycin estolate did not affect its hepatic clearance or the amount of active drug in the liver after perfusion (Table 1).

#### Microsomal Metabolism

After pretreatment and 3-hours' perfusion demethylation of the base was highly induced (four times); P-0501A and estolate metabolism was also significantly induced but to a lower extent (Table 2). The demethylation of another substrate, aminopyrine, was induced by treatment with the base and the estolate but not by P-0501A (Table 2). Correspondingly the total content of cytochrome P-450 was not affected by pretreatment with P-0501A but was significantly raised by the base and estolate; part of it (30% and 55% respectively) was bound in complexes which could be broken *in vitro* with potassium ferricyanide (Table 2). The formation of inactive complexes affected the metabolism of some substrates, *e.g.* pentobarbital, whose hepatic clearance was significantly reduced in perfused livers isolated from rats pretreated with the base and estolate (Table 3), in agreement with *in vivo* results[10].

#### Effects of Erythromycins on Hepatic Functions

In untreated rats clearance and bile excretion of BSP were affected to different extents by the three erythromycins at the concentration of 50 $\mu$g/ml (Table 4). Both parameters were slightly reduced by

Table 2. Hepatic mono-oxygenase activities after 3-hours' perfusion of pretreated rats.

| Group | Cytochrome P-450 (nmol/mg protein) potassium ferricyanide | | Erythromycin demethylase nmol HCHO/10 minutes/mg protein (%)[a] | | Aminopyrine demethylase nmol product/ 30 minutes/mg protein (%) |
|---|---|---|---|---|---|
| | Without (Uncomplexed) | With (Total) | | | |
| Perfused untreated control | 0.52±0.01 | 0.43±0.01 | Substrate | | 2.9±0.5 (100) |
| | | | E-Base | 6.8±0.2 (100) | |
| | | | E-Estolate | 7.2±0.1 (100) | |
| | | | P-0501A | 11.9±0.7[d] (100) | |
| *In vivo* treatment (1.36 mmol/kg daily for 7 days) | | | | | |
| E-Base | 0.80±0.13[b] | 1.15±0.15[c] | | 28.6±3.2[c] (419) | 7.4±0.6[c] (252) |
| E-Estolate | 0.65±0.04 | 1.43±0.23[c] | | 23.0±4.7[c] (319) | 8.6±0.8[c] (296) |
| P-0501A | 0.48±0.05 | 0.39±0.03 | | 19.2±1.6[c] (160) | 3.8±0.8 (130) |

Results are means±SE of 3~4 rats.

[a] The percentage was calculated *versus* the corresponding macrolide in perfused untreated control.

[b] $P<0.05$ vs. control, [c] $P<0.01$ vs. control, [d] $P<0.01$ vs. E-Base and E-Estolate.

Table 3. Hepatic clearance of pentobarbital during liver perfusion of pretreated rats.

| Group | No. of perfusions | Clearance (ml/minute) (mean±SE) (%) | Rate constant/minute ($K_1\times10^{-3}$±SE) (%) |
|---|---|---|---|
| Perfused untreated control | 5 | 1.70±0.10 (100) | 15.6±0.8 (100) |
| *In vivo* treatment (1.36 mmol/kg daily for 7 days) | | | |
| E-Base | 3 | 1.09±0.26[a] (59) | 8.3±1.4[b] (53) |
| E-Estolate | 3 | 1.00±0.06[a] (59) | 8.9±1.2[b] (57) |
| P-0501A | 3 | 1.97±0.22 (116) | 16.5±0.8 (106) |

The rats were treated with one of the erythromycins (E) (1.36 mmol/kg po daily) for 7 days and 24 hours after the last dose the livers were perfused with pentobarbital (25 μg/ml medium).

[a] $P<0.05$ vs. control, [b] $P<0.01$ vs. control.

the base and P-0501A (about 25%) and much more by the estolate (about 50%). Pretreatment with the base and P-0501A did not affect clearance and biliary excretion of BSP. When 50 μg/ml of these drugs were added to the perfusion medium both parameters were reduced to the same extent (about 30%, Table 4). Clearance and biliary excretion of BSP were significantly reduced by *in vivo* treatment with the estolate. The presence of this antibiotic in the perfusion medium (50 μg/ml) reduced these parameters by about 70% (Table 4). Other parameters of liver function, bile production, urea production, GOT leakage into the perfusion medium and ATP content in the liver were not affected by pretreatment with the antibiotics (data not shown).

## Discussion

Erythromycin derivatives are widely used for the treatment of various human infections, but some of them may induce hepatotoxicity (estolate)[2~6] or interfere with drug metabolism (base, estolate[7~10]). More stable, less toxic derivatives have been sought and a new fluorinated erythromycin, P-0501A (see Fig. 1), has been found to be more bioavailable when given orally[12,13], less toxic in liver cell culture than the base[6] and without influence on the metabolism of other xenobiotics[10]. As shown in previous communications[12,13] the new derivative was well absorbed and homogeneously distributed in all considered rat organs after oral administration. Similar distribution characteristics were also found in dogs[25].

Table 4. Effects of erythromycins (E) on clearance and biliary excretion of BSP during liver perfusion of untreated and pretreated rats.

| Group | Initial erythromycin concentration ($\mu$g/ml) | Clearance (ml/minute) | | Biliary excretion of BSP (mg) | |
|---|---|---|---|---|---|
| | | Untreated (%) | Pretreated (%) | Untreated (%) | Pretreated (%) |
| Perfused control | — | 1.26±0.06 (100) | 0.97±0.06 (100) | 6.9±0.1 (100) | 7.0±0.3 (100) |
| E-Base | — | | 0.80±0.22[c] (82) | | 6.0±1.0[d] (86) |
| | 50 | 0.89±0.08[b,c](71) | 0.63±0.13[a,c](65) | 5.2±0.2[b,c](75) | 4.8±0.7[b,d](69) |
| E-Estolate | — | | 0.39±0.06[b] (40) | | 3.6±0.3[b] (51) |
| | 50 | 0.57±0.05[b] (45) | 0.29±0.09[b] (30) | 3.9±0.3[b] (56) | 2.4±0.8[b] (34) |
| P-0501A | — | | 1.28±0.12[d] (132) | | 7.1±0.4[d] (101) |
| | 50 | 0.94±0.06[b,d](75) | 0.65±0.11[a,c](67) | 5.9±0.3[b,d](86) | 5.2±0.3[a,d](74) |

Each group of rats was treated either with oil (controls) or with one of the erythromycins (1.36 mmol/kg po daily) for 7 days and 24 hours after the last dose the livers were perfused with DMSO or the same antibiotics. BSP (1 mg/g liver) was added 10 minutes after starting perfusion. Results are means± SE or 3~4 rats.

[a] $P<0.05$ vs. perfused control, [b] $P<0.01$ vs. perfused control, [c] $P<0.05$ vs. E-Estolate, [d] $P<0.01$ vs. E-Estolate.

Actually studies on metabolite formation of P-0501A are in progress.

The results of this study show that P-0501A and the base do not interfere with some functions of the perfused liver isolated from rats pretreated with the same drugs for 7 days. BSP removal from the perfusate and its excretion in bile were not affected by the base or P-0501A treatment but they were greatly reduced by the estolate. It has been reported that the physico-chemical characteristics of the estolate, which lowers surface tension more than the base and is more lipophilic than both the base and the new derivative, might affect the membrane interphase, possibly result in modified uptake of other compounds (*e.g.* BSP)[5]. The uptake of estolate itself from the liver is also impaired compared with the base and P-0501A, which are cleared sooner from the perfusate and are left in only small amounts in the liver after 3-hours' perfusion.

Therefore it seems that after *in vitro* treatment the uptake of these erythromycins from the liver is the result of a compromise between induced metabolism and the condition of the cell membrane. The estolate is also excreted much less in its therapeutically active form in bile compared with the base and P-0501A. After pretreatment excretion of all erythromycins was nearly double than in untreated liver, but the same order was kept: P-0501A > base ≫ estolate–suggesting marked enterohepatic recycling of the new fluorinated derivative. In agreement with previous *in vivo* results[10] the base and the estolate, but not P-0501A, by inducing the formation of inactive cytochrome P-450 complexes, variously affect mono-oxygenase activities, some being increased (aminopyrine demethylation) and others reduced (pentobarbital clearance).

In conclusion, in perfused liver isolated after 7-days' treatment of rats our observations show that:

1) The new fluorinated derivative does not affect the liver functions, and its clearance from the perfusate and the liver being increased, like the base.

2) Although the metabolism of P-0501A is induced, mono-oxygenase activities are not affected, unlike both the base and estolate.

3) The therapeutically active form of P-0501A is excreted more in bile than the estolate and the base.

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