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Computer-aided prediction of macrolide antibiotic concentrations in human circulating polymorphonuclear leucocytes

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The relative in-vivo intracellular concentration of various macrolides in phagocytes cannot be directly extrapolated from in-vitro experiments that use a fixed and constant extracellular concentration for all compounds, since this fails to consider different rates of intracellular penetration, dosage regimens and pharmacokinetic data. In the proposed model, which takes into account the free plasma concentrations and accumulation characteristics of three antibiotics, roxithromycin, azithromycin and erythromycin, we show that roxithromycin and azithromycin may reach similar concentrations in human polymorphonuclear leucocytes when conditions mimic clinical administration of these drugs, while erythromycin concentrations are lower. This approach may be useful to predict the behaviour of other drugs or other cells, and to assist in the design of rational treatment schemes.

Introduction

Macrolide antibiotics concentrate to a large but variable extent in phagocytic cells, both *in vitro* and *in vivo*. *In vitro*, intracellular concentrations in human polymorphonuclear leucocytes (PMNs) are proportional to extracellular concentrations within the 0–20 mg/L range; this encompasses the plasma concentration range observed in humans during treatment with standard dosing regimens.

Erythromycin accumulates only to a modest extent in cells. Its plasma concentrations are low and erratic, due in part to its acid lability and to a short half-life (approximately 2 h).[1] Roxithromycin, an oxime derivative of erythromycin,[2] is more stable than erythromycin in acidic conditions, and so achieves higher and more reproducible plasma concentrations. Its elimination half-life is about six times that of erythromycin, and it achieves significant tissue concentrations.[3] Azithromycin, an azalide antibiotic,[4,5] is more stable than erythromycin in the presence of acids, but it has very low plasma concentrations because it is concentrated to a large extent in tissues.[1,6] Azithromycin has a very long terminal half-life, in keeping with its unusually large volume of distribution.

A question often raised is to what extent such different pharmacokinetic behaviours may influence in-vivo cell accumulation when the drugs are used under clinical conditions. In this paper we propose a model in which macrolide intracellular concentrations in circulating PMNs *in vivo* can be predicted from the specific accumulation characteristics of each antibiotic and by the free (non-protein bound) plasma concentrations. Using this model, we simulated the concentrations of erythromycin, azithromycin and roxithromycin within circulating PMNs after usual dosage regimens.

Materials and methods

Cell culture data

The data regarding intracellular accumulation of the macrolides were obtained from the in-vitro studies of Gladue *et al*.[7] and Carlier *et al.*,[8] who used cultured cells. Transfer constants K_{in} and K_{out} between the extracellular and intracellular compartments were calculated using the results of studies of accumulation or release of azithromycin,[7] roxithromycin[8] and erythromycin[8] from PMNs at extracellular concentrations of 10 mg/L. A linear, single-compartment model was used:

$$dC_{ic}/dt = (K_{in} \times C_{ec}) - (K_{out} \times C_{ic}) \quad [1]$$

where C_{ec} and C_{ic} are the extracellular and intracellular concentrations, respectively.

The calculated values of K_{in} and K_{out} are shown in Table I. The half-life of antibiotic release from the cells was calculated as $t_{1/2} = \log_2/K_{out}$. The accumulation ratio at steady-state is given in this model by the ratio K_{in}/K_{out}.

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Pharmacokinetic data

Free plasma concentration profiles were constructed from pharmacokinetic studies in healthy volunteers and protein-binding data for roxithromycin,[3,9] azithromycin[1,6] and erythromycin.[10,11] The free plasma fractions of the three antibiotics are shown in Figure 1.

Dosage simulation

The following dosage regimens were simulated: (i) roxithromycin 150 mg po bd for 10 days; (ii) azithromycin 500 mg po (loading dose) followed by 250 mg po qds for 4 days; (iii) erythromycin (stearate) 500 mg po tds for 10 days.

Concentrations in PMNs were calculated by stepwise numerical integration over time of Equation 1, using the free plasma concentration profiles as the extracellular concentration. All calculations were performed using the RS/1 statistical software package (Bolt Beranek and Newman Inc., Cambridge, MA, USA). The main pharmacokinetic parameters for concentrations within PMNs and the total and free plasma concentrations are shown in Table II.

Results

Total and free plasma concentration profiles are shown in Figures 2 and 3, respectively. Simulated concentrations of roxithromycin, azithromycin and erythromycin in PMNs are shown in Figure 4. At extracellular concentrations matching in-vivo free plasma concentrations, predicted in-vivo concentrations of roxithromycin and azithromycin in PMNs are similar due to the compensatory effect of their pharmacokinetic properties (despite the 15-fold greater in-vitro accumulation ratio of azithromycin). Azithromycin has a large free fraction but low total plasma concentrations, with a very large volume of distribution, probably due to a high degree of tissue binding. Roxithromycin has a smaller free plasma fraction but higher total plasma concentrations, with less extensive tissue binding.

Table I. In-vitro transfer constants and accumulation ratios (K_{in}/K_{out}) in human PMNs

	K_{in} (min^{-1})	K_{out} (min^{-1})	$t_{1/2}$ (out) (min)	K_{in}/K_{out}
Roxithromycin	1.402	0.0943	7.4	14.9
Azithromycin	0.873	0.00384	180	227
Erythromycin	0.233	0.0228	30.4	10.2

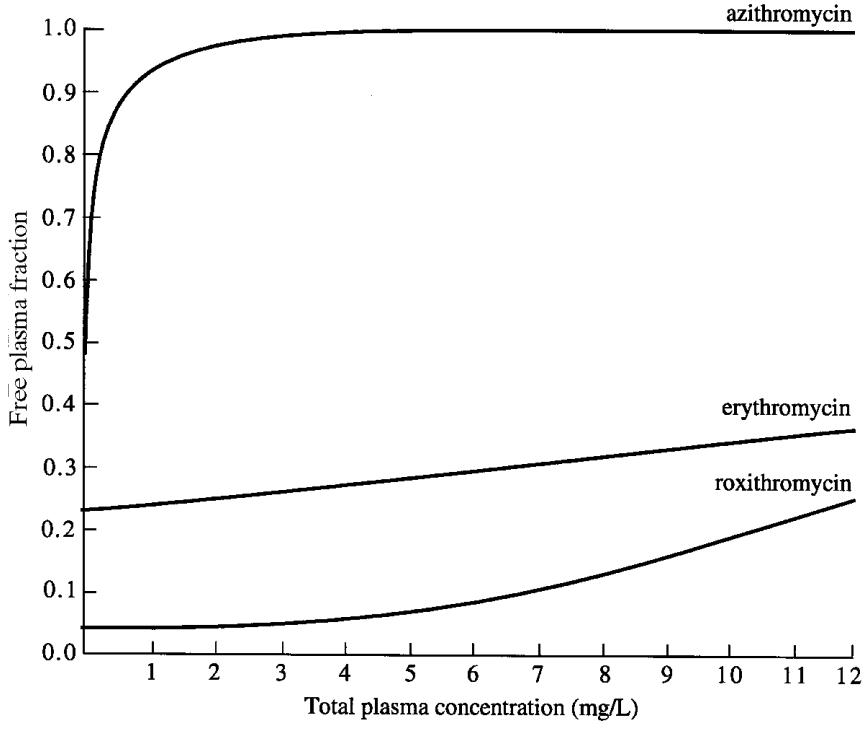

Figure 1. Free plasma fractions of azithromycin, erythromycin and roxithromycin.

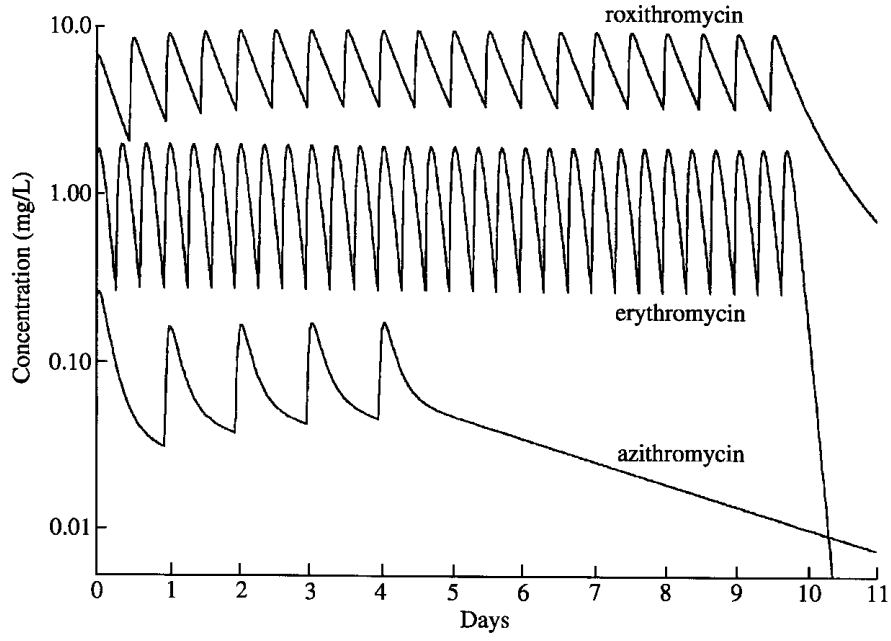

Figure 2. Simulated total plasma concentrations of azithromycin (500 mg + 250 mg od for 4 days), erythromycin (500 mg tds for 10 days) and roxithromycin (150 mg bd for 10 days).

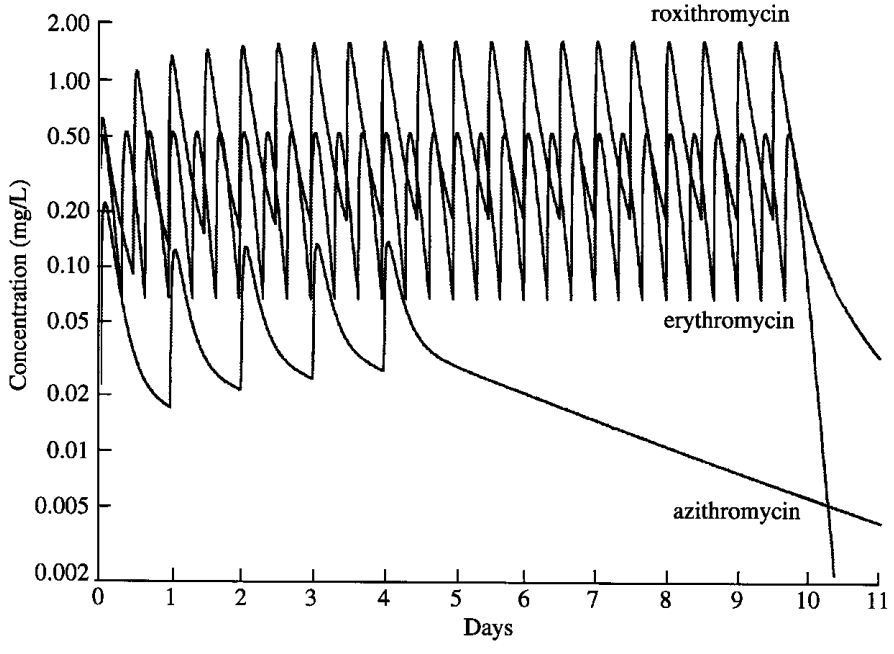

Figure 3. Stimulated free plasma concentrations of azithromycin (500 mg + 250 mg od for 4 days), erythromycin (500 mg tds for 10 days) and roxithromycin (150 mg bd for 10 days).

In terms of free fraction and total plasma concentrations, erythromycin is in an intermediate position; its predicted in-vivo concentration in PMNs is approximately four times lower than those of roxithromycin and azithromycin.

The time course of the ratio between PMN concentrations and free plasma concentrations of the three antibiotics is illustrated in Figure 5. This figure, derived from the data in Figures 3 and 4, demonstrates that the final values at pseudo-equilibrium (end of the profiles) were close to those described *in vitro*, despite the different kinetics of extracellular concentrations *in vitro* (constant) and *in vivo* (variable over time).

Discussion

The intracellular concentrations of macrolides depend both on the extracellular concentrations and on the intrinsic capacity of each molecule to accumulate in

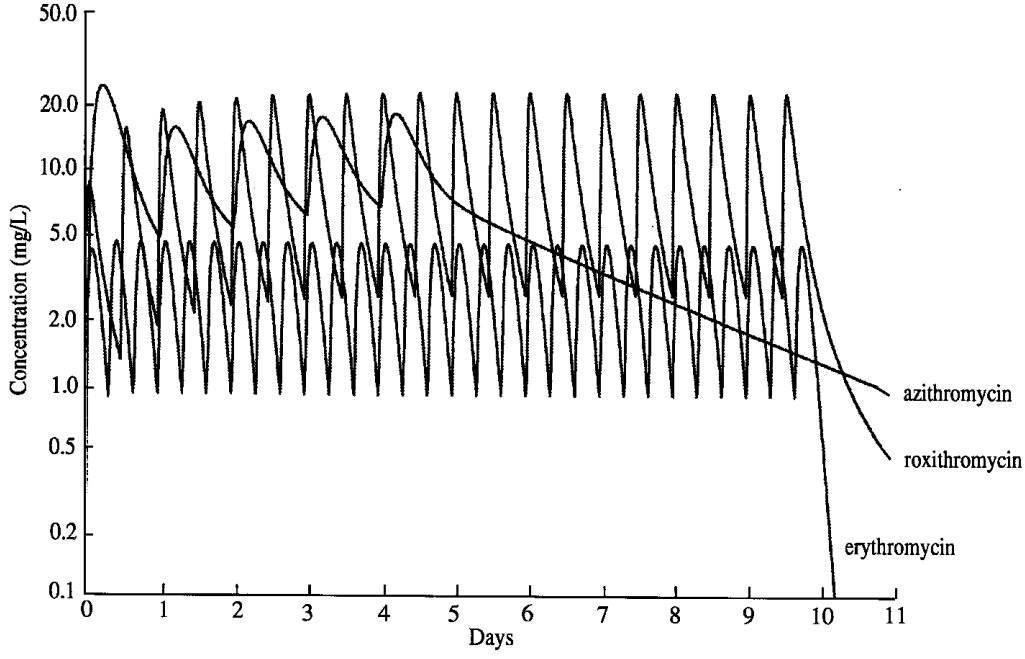

Figure 4. Predicted in-vivo polymorphonuclear concentrations of azithromycin (500 mg + 250 mg od for 4 days), erythromycin (500 mg tds for 10 days) and roxithromycin (150 mg bd for 10 days).

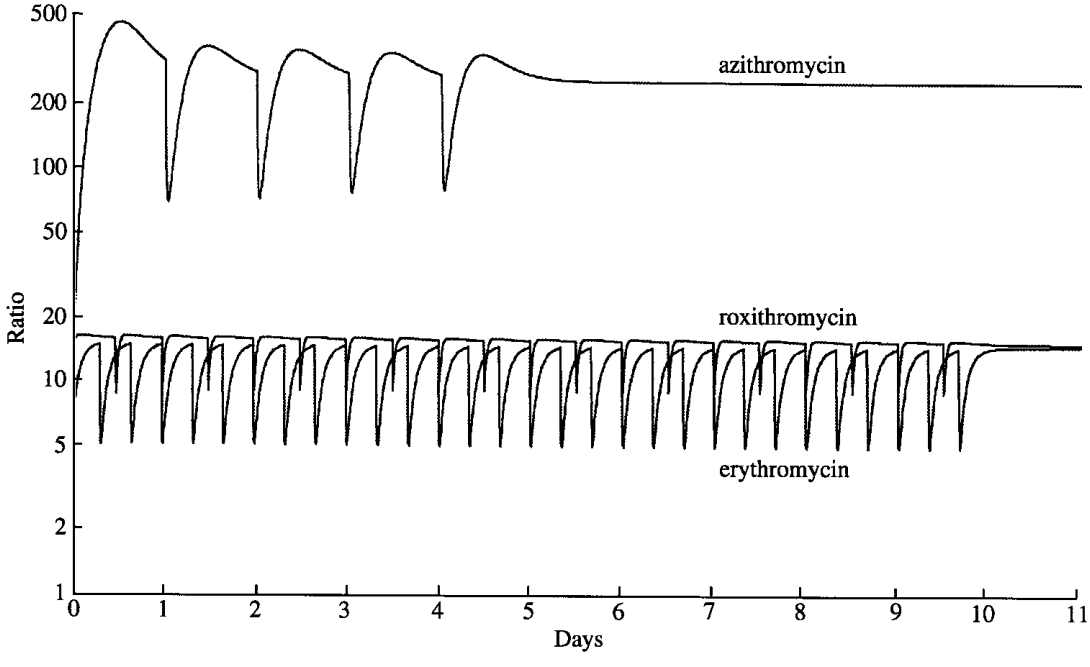

Figure 5. Predicted in-vivo polymorphonuclear/free plasma concentration ratio for azithromycin (500 mg + 250 mg od for 4 days), erythromycin (500 mg tds for 10 days) and roxithromycin (150 mg bd for 10 days).

PMNs. The present simulations suggest that, *in vivo*, the concentrations of roxithromycin and azithromycin in PMNs are likely to be of the same order of magnitude, in spite of marked differences in their cell accumulation and pharmacokinetic characteristics.

The clinical significance of high intracellular concentrations of antibiotics is not clearly established.[12,13] The mechanisms of accumulation of macrolides in phagocytic cells (ion trapping and binding to intracellular membranes) suggest that only a small fraction of the intraphagocytic drug is un-ionized and not bound within the cytoplasm or phagosomes, and thus directly microbiologically active. In this respect, the free antibiotic, which equilibrates between the plasma and the aqueous compartments of the body, may have a major role in determining the level of antibacterial activity.

Table II. The main pharmacokinetic parameters for concentrations within human PMNs and the total and free plasma concentrations

Pharmacokinetic parameter[a]	Total drug			Free drug			Calculated values for PMNs		
	roxithromycin	azithromycin	erythromycin	roxithromycin	azithromycin	erythromycin	roxithromycin	azithromycin	erythromycin
C_{max} (mg/L)	9.3	0.171	1.92	1.45	0.124	0.48	21.4	17.3	4.4
C_{min} (mg/L)	3.35	0.048	0.27	0.17	0.027	0.06	2.6	7.1	0.89
AUC (0–24 h) (h·mg/L)	145	1.93	26	14.5	1.24	6.3	217	279	65
AUC (days 0–5) (h·mg/L)	673	9.12	129	63.2	5.88	31.4	940	1304	321
AUC (days 0–10) (h·mg/L)	1401	12.1	259	137	7.46	62.9	2035	1689	644

C_{max}, peak plasma concentration; C_{min}, trough plasma concentration.
[a]On day 5 unless otherwise stated.

It has been proposed that azithromycin can be transported to the site of an infection by phagocytes 'loaded' with antibiotic.[7] This mechanism may be shared by other macrolides, especially roxithromycin, since the in-vitro data and the pharmacokinetic profiles in humans suggest that the in-vivo concentrations of these two macrolides in PMNs should be similar. Therefore, direct in-vivo measurements of PMN 'transfer' of roxithromycin and azithromycin may, therefore, be warranted, but it is still uncertain whether PMNs can keep the drug intracellularly while moving out of the blood to the site of infection and then fully release it in an active form.

Acknowledgement

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