ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1974, p. 139-142

Vol. 5, No. 2
Printed in U.S.A.

Evaluation of Rifamycin SV and Rifampin Kinetics in Lactating Ewes

G. ZIV AND F. G. SULMAN

Ministry of Agriculture, Kimron Veterinary Institute, Bet Dagan, Israel, and Department of Applied Pharmacology, School of Pharmacy, Hebrew University, Jerusalem, Israel

Received for publication 21 September 1973

Serum and milk concentrations of rifamycin SV and rifampin were determined in lactating ewes after a single intravenous injection, and pharmacokinetic parameters were evaluated by the two-compartment open-system model. Rifampin was distributed throughout a greater volume than rifamycin SV and was eliminated more slowly from the body. The concentrations of the two drugs, both lipophilic weak acids, in milk after intravenous or intramuscular injection were lower than in serum, but rifampin was detected in milk sooner and for longer periods than rifamycin SV. Under constant serum drug concentrations, the observed milk/serum ultrafiltrate concentrations ratios (0.19 to 0.29 for rifamycin SV, and 0.90 to 1.28 for rifampin) were close to the calculated ratios derived from the pH-pK passive diffusion concept.

In the last 20 years many theoretical and experimental studies have been published which have added to our knowledge of the fate of drugs in the body. It has been pointed out (10) that the pharmacokinetics of most drugs and other exogenous substances can be effectively described with the use of the two-compartment open-system model. Examination of these parameters used to describe the distribution and disposition of drugs has led to new concepts of relating the presence of a compound to its effect (4, 11). The utility of a model depends on experimental verification of theoretical considerations. To be acceptable, a theory must predict expected findings. Thus, the single-compartment open model, although useful, is regarded as too simple to explain the phenomena associated with drug administration (11).

This study was undertaken with the objective of deriving certain relationships concerning the concentrations of rifamycin SV and rifampin in serum and milk and to relate theoretical predictions to experimental observations. Although the rifamycins were detected in milk in laboratory and farm animals after parenteral administration (2, 3, 8), no data is available on the mechanism of their penetration into milk.

MATERIALS AND METHODS

Theoretical considerations. For the purpose of this study, it is postulated that the body behaves as a two-compartment open-system model. The problems of intravenous (i.v.) bolus injection in this model system have well known solutions (9, 12, 13, 15). The equation for serum concentration against time has the form $C_1 = A_e^{-\alpha t} + B_e^{-\beta t}$. The linear portion on semilogarithmic plot is evaluated by least square analysis for β and B, where B is the extrapolated zero-time intercept. A second linear portion is found by the method of residuals and this is further analyzed to give α and A. With these hybrid constants, A, B, α, and β, it can be shown that K_{el}, the overall rate constant for drug elimination by various routes, $= \alpha \cdot \beta (A + B)/A \cdot \beta + B \cdot \alpha$. K_{21}, the first-order distribution rate constant between the peripheral compartment (V_2) and the central compartment (V_1), $= A \cdot \beta + B \cdot \alpha/A + B$. K_{12}, the first-order distribution rate constant between the central compartment and the peripheral compartment, $= A \cdot B (\beta - \alpha)^2/(A \cdot \beta + B \cdot \alpha)$. The apparent volume of distribution, given by $V_1 = \text{dose}/A + B$, and the total distribution volume, $V_{D_\beta} = V_1 \cdot K_{el}/\beta$.

On the assumption that at equilibrium the unionized fraction of a partially ionized drug is the same in ultrafiltrates of milk and serum, the ratio between the total concentration of the drug in ultrafiltrates of milk and serum can be calculated. Conversion of the Henderson-Hasselbalch equation gives the following expression for an acid (7): ratio milk ultrafiltrate/serum ultrafiltrate $= 1 + 10 (pH_{milk} - pK_a)/1 + 10 (pH_{serum} - pK_a)$.

Animals. Seventeen lactating Awassi ewes, weighing 48 to 64 kg each, were used in this experiment. The ewes were producing 1.6 to 2.2 kg of normal milk (pH 6.6 to 6.8) daily.

Treatment. Five ewes were given a rapid i.v. injection of an aqueous solution of rifamycin SV (Lepetit, Milan, Italy) at a dose level of 10 mg/kg. Four weeks later, the same ewes were injected i.v. with 10 mg of rifampin per kg (Rifadin, Lepetit). Before injection, the contents of each 300-mg Rifadin capsule were dissolved in 2 ml of 50% aqueous ethanol.

The drug was then diluted to 0.5% (wt/vol) in sterile physiological saline and approximately 200 ml of the solution were injected i.v. during 3 to 5 min. To four other ewes, rifamycin SV and rifampin were injected intramuscularly (i.m.), each drug at 20 mg/kg, injections given at 3-week intervals.

The partitioning of the drugs between blood and milk was studied in eight additional ewes by a series of multiple intramuscular injections. Rifamycin SV was administered to two ewes, initially at 10 mg/kg and four times at 1-h intervals at 5 mg/kg. Rifamycin SV was administered by the same procedure to two more ewes at half the above doses. Rifampin was injected i.m. to two ewes, twice at 1-h intervals, each time at 10 mg/kg, and to two other ewes, twice at 90-min intervals, each time at 5 mg/kg.

Blood and milk sampling. Blood samples were collected from the vein opposite to the one used for i.v. treatment. Blood was sampled 10, 20, 30, 40, 60, and 90 min, and 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after i.v. injection. Blood was collected at hourly intervals after the i.m. treatments. Each udder was hand-stripped at each blood-sampling period, and milk, pH, and volumes were recorded. The clotted blood and milk samples were centrifuged; portions of serum and skim milk were removed and kept at −18 C until assayed. Ultrafiltrates were prepared of all the serum and milk samples, which were refrigerated overnight (17). These samples were also kept frozen pending their assay.

Assay procedure. Drugs were assayed microbiologically by the cylinder-cup method (1, 2). Concentrations of unbound drug in ultrafiltrates of serum and skim milk were determined using the appropriate standards made in distilled water dialysates of antibiotic-free serum and skim milk adjusted to pH 6.0 with 0.1 M phosphate buffer.

RESULTS

Mean serum and milk concentrations of rifamycin SV and rifampin after a single i.v. injection are presented in Fig. 1. A summary of pharmacokinetic parameters of the two drugs is given in Table 1. During the first 2 h after i.v. injection mean rifamycin SV serum concentrations were higher than those of rifampin, but the former antibiotic disappeared from serum at a rate three times as great as the latter. The $t_{1/2}$ values were 3.3 h and 1.1 h for rifampin and rifamycin SV, respectively. Analysis of the data presented in Table 1 indicate that the K_{el} of rifamycin SV was twice greater than that of rifampin, and that rifamycin SV moved from the central to the peripheral compartment at almost four times the rate of rifampin. Rifampin, however, was calculated to occupy body space greater than that of rifamycin SV and the mean V'_D/V'_1 values were 2.7 for rifamycin SV and 3.5 for rifampin.

After a single i.m. injection of either antibiotic at equal doses, mean peak concentrations in serum were almost the same but were observed 1 h after treatment with rifamycin SV and 3 h after rifampin was administered (Fig. 2). The mean $t_{1/2}$ values were 2 h for the former and 11 h for the latter antibiotic.

Rifampin was first detected in milk 20 min after i.v. injection (Fig. 1) and 1 h after i.m. administration. On the other hand, rifamycin SV was not found in milk until 2 h after i.m. treatment. Mean peak rifampin concentrations were two times higher than those of rifamycin SV, and the disappearance of both antibiotics from milk paralleled their respective disappearance rates from serum.

The partitioning of the two antibiotics between serum and milk during near equilibrium

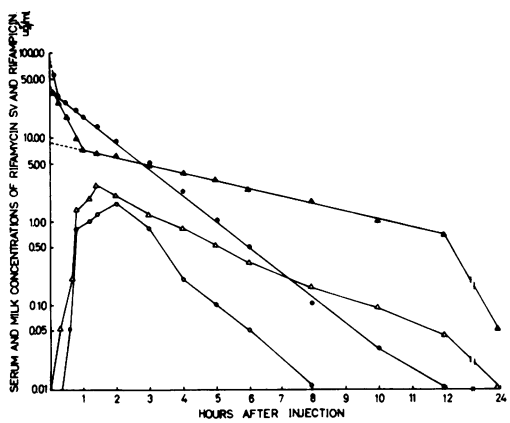

FIG. 1. *Mean rifamycin SV concentrations in serum (●) and milk (○), and mean rifampin concentrations in serum (▲) and milk (△) in a crossover trial involving five lactating ewes after a single i.v. injection of each drug at 10 mg/kg.*

TABLE 1. *Pharmacokinetic parameters for rifamycin SV and rifampin injected i.v. to five ewes in a crossover trial at 10 mg/kg*

Pharmacokinetic parameters	Rifamycin SV		Rifampin	
	Mean	SD[a]	Mean	SD
A, μg/ml	82.5	17.6	43.8	10.4
B, μg/ml	35.0	9.2	9.0	3.8
α, h^{-1}	3.3	0.3	1.0	0.2
β, h^{-1}	0.28	0.06	0.1	0.01
Cp°, μg/ml	117.5	26.8	52.8	14.2
K_{12}, h^{-1}	1.62	0.20	0.40	0.04
K_{21}, h^{-1}	1.18	0.25	0.23	0.03
K_{el}, h^{-1}	0.78	0.10	0.35	0.10
$t_{1/2}$, h	1.10	0.10	3.30	0.20
V'_1, % body weight	8.51	3.73	18.92	7.04
V'_D % body weight	23.65	10.36	66.15	24.51

[a] SD, Standard deviation.

is shown in Table 2. At the concentrations tested, rifamycin SV and rifampicin were 72% and 84%, respectively, bound to serum and both antibiotics were about 20% bound to milk. The observed ratios of milk ultrafiltrate to serum ultrafiltrate concentrations were close to the calculated ones. The ratios were not influenced by as much as fourfold differences in the concentrations of the drugs in serum.

DISCUSSION

The two chemically and structurally related rifamycins studied in ewes presented distinct pharmacokinetic patterns which were quite evident when data were analyzed according to the two-compartment open-system model. Although rifamycin SV was found to move from the central to the peripheral compartment much faster than did rifampin, this was not reflected in a higher distribution volume since its K_{21} and K_{el} were also substantially greater than the corresponding values for rifampicin. It was suggested (5, 17) that extensive serum-protein binding can limit the distribution of drugs in the body, particularly for drugs like rifampin which are more than 80% bound. This did not appear to be the case in our studies where the K_{12}/K_{21} for rifampin was 1.70, whereas a lower ratio, i.e., 1.37, was determined for rifamycin SV. The existence of an enterohepatic circulation, which greatly extends the length of time that the drug remains in the body, was documented for the rifamycins (3, 6). It appears that at least in the ovine species rifampin is better reabsorbed from the gastrointestinal tract and is better distributed throughout the body than rifamycin SV.

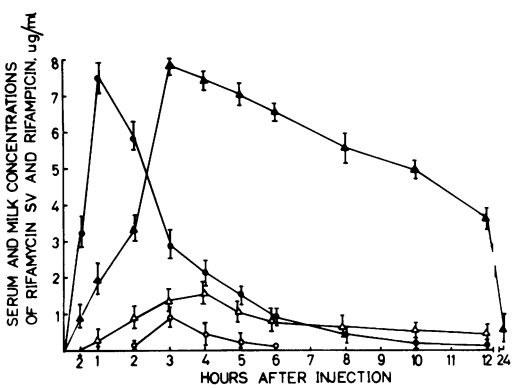

FIG. 2. *Rifamycin SV concentrations in serum (●) and milk (○) and rifampin concentrations in serum (▲) and milk (△) in a crossover trial involving four lactating ewes after a single i.m. injection of each drug at 20 mg/kg (mean ± SD).*

When pharmacokinetically evaluating serum and milk levels of several penicillin and cephalosporin derivatives in cows and ewes, it was noted (16) that derivatives showing greatest distribution volumes were also more rapidly appearing in milk, and that the extent of passage into milk was influenced by the drug's ionization in serum and its lipid solubility. The rifamycins are weak organic acids and it was calculated that in serum (pH 7.4), rifamycin SV with a pK_a of 2.8 and 6.7 (14) is 0.22% unionized, whereas rifampin, which is a much weaker acid, with a pK_a of 7.9 (6) is 76% unionized. Although the rifamycins are highly lipid soluble, the octanol to phosphate buffer partition coefficient at pH 7.4 for rifamycin SV is lower than that of rifampin (6). The lipid-solubility characteristics of the predominantly unionized rifampin probably accounted for the rapid penetration of the drug into milk. Penetration of both rifamycins into milk appeared to follow the pH-pK passive diffusion concept as the observed milk

TABLE 2. *Concentration of rifamycin SV and rifampin in serum and milk and in their ultrafiltrates during near equilibrium*

Ewe no.	Treatment	Minimal to maximal concn in			Ratio of milk ultrafiltrate to serum ultrafiltrate	
		Milk (µg/ml)	Serum (µg/ml)	Serum ultrafiltrate (µg/ml)	Observed minimal to maximal	Calculated
10	Rifamycin SV	0.22–0.28	3.2–3.6	0.92–0.98	0.20–0.24	0.25
11	Rifamycin SV	0.30–0.38	3.4–4.0	0.95–1.10	0.26–0.29	0.25
12	Rifamycin SV	0.58–0.78	0.8–1.3	0.25–0.28	0.19–0.23	0.25
13	Rifamycin SV	0.60–0.94	0.9–1.2	0.23–0.29	0.22–0.28	0.25
14	Rifampin	1.32–1.54	4.3–5.0	0.88–1.00	1.23–1.28	1.00
15	Rifampin	0.54–0.92	3.8–4.2	0.58–0.70	0.95–1.10	1.00
16	Rifampin	0.22–0.34	1.0–1.3	0.20–0.24	0.90–1.22	1.00
17	Rifampin	0.20–0.40	0.8–1.2	0.18–0.22	0.95–1.15	1.00

to serum ultrafiltrate concentration ratios were in close agreement with the calculated ratios, and for each drug the ratios were independent of serum drug concentrations.

ACKNOWLEDGMENT

Thanks are due to R. Haber, ABIC Ltd., Ramat Gan, Israel, for the liberal supply of the rifamycins used in this study.

LITERATURE CITED

1. Arret, B., D. P. Johnson, and A. Kirshbaum. 1971. Outline of details for microbiological assays of antibiotics: second revision. J. Pharm. Sci. **60**:1689-1694.
2. Bergamini, S., and G. Fowst. 1965. Rifamycin SV. A review. Arneimittel-Forschung **15**:951-1002.
3. Furez, S., R. Scotti, R. Pallanza, and E. Mapelli. 1967. Rifampicin. III. Absorption, distribution and elimination in man. Arneimittel-Forschung **17**:534-537.
4. Gibaldi, M. 1969. Effect of mode of administration on drug distribution in a two compartment open system. J. Pharm. Sci. **58**:327-331.
5. Keen, P. 1971. Effect of binding to plasma proteins on the distribution, activity and elimination of drugs, p. 213-233. *In* B. B. Brodie and J. R. Gillette (ed.), Handbook of experimental pharmacology, vol. 28, part 1, Springer, Berlin.
6. Maggi, N., C. R. Pasqualucci, R. Ballota, and P. Sensi. 1966. Rifampicin, a new orally active rifamycin. Chemotherapia **11**:285-287.
7. Rasmussen, F. 1966. Studies on the mammary excretion and absorption of drugs, p. 1-87. Carl Fr. Mortensen, Publ., Copenhagen.
8. Redaelli, G., S. Nani, G. Ruffo, A. Socci, and L. Guallini. 1971. The use of rifamycin SV in the treatment of bovine mastitis. Vet. Rec. **88**:297-304.
9. Resingo, A., and G. Serge. 1966. Drug and tracer kinetics, p. 24-43. Blaisdell Publ. Co., Waltham, Mass.
10. Riegelman, S., J. Loo, and M. Rowland. 1968. Shortcomings in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment. J. Pharm. Sci. **57**:117-123.
11. Riegelman, S., J. Loo, and M. Rowland. 1968. Concepts of a volume distribution and possible errors in evaluation of this parameter. J. Pharm. Sci. **57**:128-133.
12. Riggs, D. S. 1963. Mathematical approach to physiological problems, p. 193-220. Williams & Wilkins, Co., Baltimore, Md.
13. Teorell, T. 1937. Kinetics of distribution of substances administered to the body. Arch. Intern. Pharmacodyn. **57**:205-240.
14. Umezawa, H. 1967. Index of antibiotics from Actinomycetes, p. 564. University of Tokyo Press.
15. Wagner, J. G., and J. Northam. 1967. Estimation of volume of distribution and half-life of a compound after rapid intravenous injection. J. Pharm. Sci. **56**:529-531.
16. Ziv, G., J. Shani, and F. G. Sulman. 1973. Pharmacokinetic evaluation of penicillin and cephalosporin derivatives in serum and milk of lactating cows and ewes. Amer. J. Vet. Res. **34**:1561-1566.
17. Ziv, G., and F. G. Sulman. 1972. Binding of antibiotics to bovine and ovine serum. Antimicrob. Ag. Chemother. **2**:206-213.