Riboflavin Distribution and Elimination in Two Functionally Anephric Human Patients

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Abstract □ Pharmacokinetic studies in normal human subjects have suggested that probenecid modifies appreciably the distribution in the body of riboflavin and certain drugs. To exclude the effects of probenecid on renal excretion, the distribution and elimination of riboflavin with and without probenecid were studied in two functionally anephric patients during hemodialysis. A pharmacokinetic analysis of the time course of plasma levels of the vitamin, based on a two-compartment open model, indicates no appreciable effect of probenecid on the distribution and transfer rate constants of riboflavin. Riboflavin was eliminated more slowly by hemodialysis of the patients than by renal excretion in normal subjects. The rate of removal of riboflavin by hemodialysis appears to be primarily a function of the rate of blood flow through the dialyzer.

Keyphrases □ Riboflavin distribution, elimination—anephric patients □ Anephric condition—riboflavin distribution, elimination □ Probenecid effect—riboflavin distribution, elimination □ Hemodialysis—riboflavin elimination

The possible effect of probenecid on the distribution of various compounds in the body has been of recent interest. Gibaldi *et al.* (1, 2) have shown in studies with penicillins that, in addition to the well-known inhibitory effect on the renal excretion of this antibiotic, probenecid also seems to increase significantly the fraction of drug in the apparent body compartment from which elimination occurs. The results of recent studies in this laboratory suggest that probenecid affects also the distribution of riboflavin in the body (3). Since riboflavin is eliminated primarily by renal excretion, it was of interest to pursue further studies in functionally anephric patients in order to exclude the primary renal effect of probenecid. These studies were carried out during the course of hemodialysis of the anephric patients and may also be of nutritional interest since it has been suggested that vitamin deficiencies could develop due to the removal of water-soluble vitamins by dialysis (4).

EXPERIMENTAL

Two functionally anephric[1] male human patients, who were undergoing hemodialysis, served as test subjects. Subject S, age 30, with chronic membranous glomerulonephritis, and Subject K, age 34, with collagen disease, were maintained on a Kiil and a Skeggs-Leonards dialyzer, respectively. Both systems involved dialysate flow of approximately 580 ml./min. and blood flow of about 270 ml./min. Hematocrit values of each subject ranged from 0.22 to 0.24, and the plasma albumin concentration was about 3.2 g. % in each subject.

An intravenous dose of 38.9 mg. riboflavin as riboflavin-5′-phosphate[2] was administered 3 to 5 hr. after the start of hemodialysis. One gram of probenecid[3] in aqueous suspension was given orally, in crossover fashion, 1.5 to 2.0 hr. prior to riboflavin. Blood samples were collected proximal to the arterial shunt 0.5 hr. before and at 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, and 6.0 hr. after injection of riboflavin. Studies in each patient were separated by a 7-day interval.

[1] Less than 3% of normal renal function.
[2] Hyrye Injection, S.F. Durst Co., Philadelphia, Pa.
[3] Benemid, Merck Sharp and Dohme, West Point, Pa.

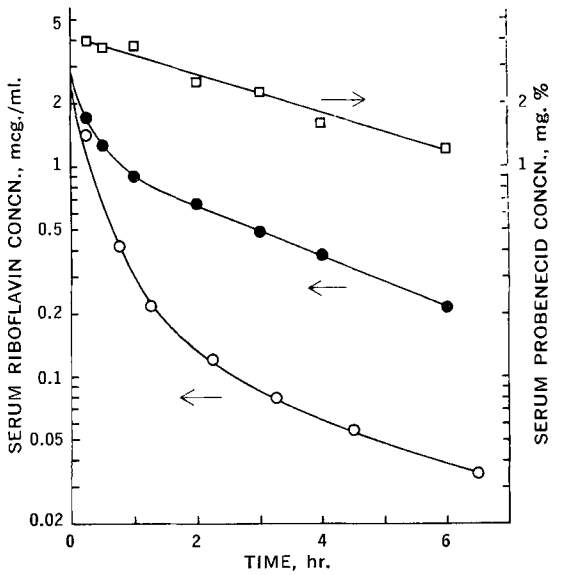

Figure 1—*Time course of total riboflavin (●) plasma concentrations in an anephric subject (S) after intravenous injection of 38.9 mg. riboflavin as riboflavin-5′-phosphate (FMN). Also shown for comparative purposes are riboflavin serum levels in a normal subject (○), 85 kg., who received an i.v. dose of 31 mg. riboflavin as FMN; and (□), probenecid plasma concentrations in the anephric patient after oral administration of 1 g. probenecid at −2 hr.*

Total riboflavin (5) and probenecid (6) concentrations in plasma were determined by methods described previously. No mutual interference in the analyses of the two compounds was found (3). Plasma concentrations were corrected for blank values of samples obtained prior to the administration of either drug.

RESULTS

The time course of total riboflavin and probenecid plasma concentrations in Subject S are shown in Fig. 1. Also depicted for purposes of comparison are riboflavin plasma concentrations obtained in a normal subject. The plasma concentrations of riboflavin (C_p) as a function of time (t) in the anephric subjects were fitted to a biexponential equation:

$$C_p = A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t) \qquad \text{(Eq. 1)}$$

using the "NLIN" digital computer program of Marquardt (7). The distribution (k_{12}, k_{21}) and elimination (k_{el}) rate constants of the typical two-compartment open model (elimination occurring from the central compartment) were obtained from the parameters A, α, B, and β as described by Rescigno and Segre (8). The apparent volume of the central compartment (V_c) was calculated from:

$$V_c = \text{dose}/(A + B) \qquad \text{(Eq. 2)}$$

and an additional "volume" parameter, $(V_d)_\beta$, was determined from:

$$(V_d)_\beta = V_c \cdot k_{el}/\beta \qquad \text{(Eq. 3)}$$

as described by Gibaldi *et al.* (9). The body clearance (Cl_b) of the vitamin was calculated from:

$$Cl_b = k_{el} \cdot V_c \qquad \text{(Eq. 4)}$$

All of the parameters and rate constants described, which were obtained from riboflavin plasma levels in the presence and

Table I—Pharmacokinetic Parameters[a] of Riboflavin Distribution and Elimination in Anephric Subjects in the Presence and Absence of Probenecid

	Subject[b]			
	S		K	
	Probenecid,[c] g.			
	0	1	0	1
Parameter				
A, mcg./ml.	1.52	1.88	1.38	1.23
B, mcg./ml.	1.13	0.971	0.740	0.757
α, hr.$^{-1}$	3.39	4.81	2.92	2.34
β, hr.$^{-1}$	0.275	0.211	0.0534	0.142
CD[d]	0.9999	0.9997	0.9988	0.9990
k_{12}, hr.$^{-1}$	1.48	2.67	1.77	1.16
k_{21}, hr.$^{-1}$	1.60	1.78	1.05	0.978
k_{el}, hr.$^{-1}$	0.581	0.571	0.148	0.339
V_c, l.	14.7	13.6	18.3	19.5
V_c, percent of body weight	26.1	24.2	25.1	26.7
V_d-β, l.	31.1	36.7	50.8	46.5
V_d-β, percent of body weight	55.5	65.5	69.6	63.7
Cl_b, ml./min.	142	129	45	110

[a] Symbols are defined in the text. [b] Body weights: S, 56 kg.; K, 73 kg. [c] Plasma concentration range of probenecid: Subject S: 2.47 to 0.95 mg. %; Subject K: 3.96 to 1.22 mg. %. [d] Coefficient of determination: $(\Sigma obs.^2 - \Sigma dev.^2)/\Sigma obs.^2$

absence of probenecid, are listed in Table I. The coefficient of determination (CD) for each of the four sets of data is close to unity, indicative of the excellent fit of the experimental data to the curve calculated from Eq. 1.

It was also of interest to determine if blood flow through the dialyzer or diffusion of riboflavin across the dialysis membrane may be rate limiting in the elimination of the vitamin by hemodialysis. The effective plasma flow rate (Q_E) through the dialyzer can be calculated from the relationship:

$$Q_E = Q \cdot (1 - \text{HCT}) \cdot F_f \qquad \text{(Eq. 5)}$$

where Q is the actual blood flow rate (about 270 ml./min.), HCT is the hematocrit (0.23), and F_f is the fraction of nonprotein-bound vitamin in the plasma. The value of the latter was calculated to be about 0.5 based on the actual albumin concentration in the plasma (3.2 g. %) and the previously determined (10) association constants of the vitamin with human albumin. The effective plasma flow rate thus estimated from Eq. 5 is about 110 ml./min., which is quite similar to the body clearance values observed in three of the four studies in the anephric patients (Table I). It was not possible to determine dialysance directly due to the very rapid rate of flow of the dialysis fluid (580 ml./min.) which resulted in such extensive dilution of riboflavin, making its determination in the dialysis fluid impossible.

DISCUSSION

The distribution parameters V_c and $(V_d)_\beta$ are very similar in all four tests when expressed as a percent of body weight (Table I). Probenecid, therefore, had no apparent effect on the distribution of riboflavin in the body under the experimental conditions. The rate constants k_{12} and k_{21} were somewhat more variable but also showed no consistent differences as a function of probenecid administration. It may be concluded, therefore, that the apparent effect of probenecid on riboflavin distribution in a normal subject (3) is probably an indirect result of its effect on the renal excretion of the vitamin.

The distribution parameter V_c ranged from 24.2 to 26.7% of body weight in the present study which is appreciably larger than the 16% value reported previously for a normal subject (3). This difference is consistent with the low hematocrit and low plasma albumin concentration in the anephric patients.

The similarity of three of the four body clearance (Cl_b) values to the calculated effective plasma flow rate through the dialysis system suggests that blood flow through the dialyzer is either the rate-limiting step or at least one of the rate-determining processes in the elimination of riboflavin by hemodialysis. This assumes that metabolism and extrarenal excretion (other than hemodialysis) contribute little to the elimination of the vitamin in anephric patients as is the case in normal subjects (1, 3).

Renal clearance of riboflavin in normal subjects involves glomerular filtration, renal tubular secretion, and renal tubular reabsorption by a specialized saturable process (11). Clearance is, therefore, concentration dependent, ranging from as high as 400 ml./min. at high plasma concentrations of riboflavin to 150 ml./min. or less at low plasma concentrations in normal adults (11). On the other hand, body clearance of riboflavin is not concentration dependent in functionally anephric patients due to the absence of a renal reabsorption process. A direct comparison of renal clearance in normal subjects with body clearance in hemodialyzed patients is therefore impossible.

The pharmacokinetics of riboflavin elimination in anephric patients and normal subjects differ also in that the time course of riboflavin concentration decline in the plasma of the patients is describable by a biexponential expression while a triexponential expression is required for normal subjects (11). This appears to be due to the absence of the renal reabsorption capability in the patients. A similar effect has been found in newborn infants whose renal mechanisms are immature (12).

It is of interest that probenecid, which is eliminated in normal subjects almost exclusively by biotransformation and which because of its high affinity to plasma proteins (6) is not likely to be removed effectively by hemodialysis, was as rapidly eliminated in the two patients (half-life of 2 to 4 hr.) as in normal subjects at a comparable dosage level (3, 6). On the other hand, drugs that are eliminated solely or primarily by renal excretion have a much longer half-life in anephric patients than in normal subjects (13, 14).

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