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Effects of Probenecid on the Pharmacokinetics of Allopurinol Riboside

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Allopurinol riboside is an experimental agent for the treatment of leishmaniasis and American trypanosomiasis. Previous studies showed that after oral administration, unexpectedly low levels of allopurinol riboside in plasma are attributable to incomplete absorption and rapid renal clearance. In this randomized, crossover evaluation in healthy volunteers, probenecid reduces the renal clearance of allopurinol riboside, extends the half-life of allopurinol riboside in plasma, and triples the levels of allopurinol riboside in plasma.

Allopurinol riboside is an experimental drug in the treatment of leishmaniasis and American trypanosomiasis (Chagas' disease). These diseases are caused by parasitic protozoa, are potentially fatal, and occur in millions of people on a worldwide basis (17, 18). The drugs currently available are not always curative; furthermore, they are expensive, toxic, and typically must be administered parenterally. The development of safe and orally effective drugs for these diseases has been accorded high priority by the World Health Organization (1).

Allopurinol riboside is a human metabolite of allopurinol (7). Allopurinol riboside is effective against parasites, because a series of enzymes (analogous to those that mediate purine salvage in humans) convert it into 4-aminopyrazolopyrimidine ribonucleoside triphosphate, a cytotoxic product (16). Allopurinol riboside is selectively toxic, because it is not metabolized by the corresponding enzymes in humans (15). Although allopurinol is also selectively cytotoxic (13, 14), it is less suitable for use as an antiparasitic drug, because it is rapidly and nearly completely converted to oxipurinol (7, 15), which has little antiparasitic activity (12).

In a previous study, we found that orally administered allopurinol riboside was safe and well tolerated in 32 healthy male volunteers (21). However, the levels of allopurinol riboside in plasma were low, in part because of brisk renal clearance, which averaged 263 ml/min. The renal tubular secretory transport of a number of drugs (e.g., penicillins and cephalosporins [6], oxipurinol [8], acyclovir [11], and zidovudine glucuronide [9]) is inhibited by probenecid. In this study, we evaluated the effects of probenecid on the pharmacokinetics of allopurinol riboside.

Healthy males were recruited through newspaper advertisements and accepted into the study if a detailed health history, physical examination, serum chemistry analysis, hematologic analysis, urine analysis, and electrocardiogram were normal, if they were within 10% of their ideal body weight for their height, and if they were not chronic users of any drugs and not allergic to allopurinol. Three men were accepted into and completed the study. Two were black, and one was Caucasian; the average age was 33 years. Written informed consent was obtained, and the studies were approved by the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions.

Allopurinol riboside was provided by the Burroughs Wellcome Company as 250-mg gelatin capsules. Probenecid was administered as 500-mg tablets, manufactured by Merck, Sharp and Dohme, and purchased by the Johns Hopkins Hospital Pharmacy.

The study was a randomized, crossover evaluation of allopurinol riboside with and without probenecid, which required 6 weeks to complete: a 6-day treatment period, a 2-week washout and follow-up period, a 6-day crossover treatment period, and a 2-week final follow-up period. The subjects were admitted to the Clinical Research Unit of the Johns Hopkins Hospital for the two treatment periods. Safety monitoring included evaluation of subjective and objective parameters, as detailed previously (21). Allopurinol riboside was given orally in 14 doses of 1,500 mg each; 24 h elapsed between the first and second doses, and the remaining 13 doses were given every 6 h, with the final dose on the morning of the fifth treatment day (see the arrows in Fig. 1). A 2-g loading dose of probenecid was given orally 1 h before the first dose of allopurinol riboside. The subsequent 19 doses of probenecid (500 mg each) were given every 6 h, starting 6 h after the first dose of allopurinol riboside.

Venous blood samples for drug and metabolite determinations were drawn before the first dose of probenecid (if given) and just before the first dose of allopurinol riboside. Subsequent samples were taken at 30, 60, 80, 100, and 120 min and 3, 4, 6, 8, 10, and 12 h after the first dose of allopurinol riboside. On the mornings of the next 4 days, blood samples were drawn just before the 2nd, 6th, 10th, and 14th doses of allopurinol riboside. After the 14th (final) dose of allopurinol riboside, blood samples were drawn at 30, 60, 80, 100, and 120 min and 3, 4, 6, 8, 10, 12, and 24 h. A baseline urine sample was taken before drug administration, and urine samples were collected from 0 to 2, 2 to 4, 4 to 8, and 8 to 24 h after the first and last doses of allopurinol riboside. Twenty-four-hour urine collections were made on days 2, 3, and 4 of treatment. Blood and urine samples were collected, and assayed by a high-pressure liquid chromatographic method previously described in detail (21). The assay was sensitive for allopurinol riboside at levels of ≤ 0.1 μg/ml and was linear from ≤ 0.2 to ≥ 50 μg/ml. In more than 100 determinations, the standard deviations were within 15 and 2% of the means at 0.2 and 50 μg/ml, respectively. Probenecid was not detected in the assay and did not

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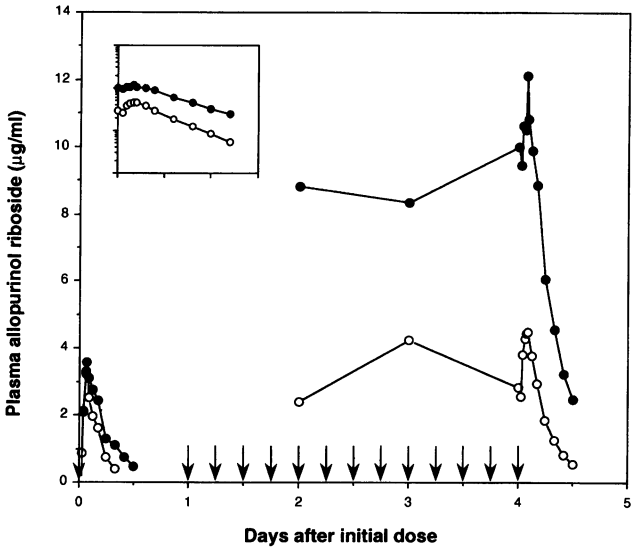

FIG. 1. Effects of probenecid on the levels of allopurinol riboside in plasma in subject 1. Fourteen doses of allopurinol riboside (500 mg each) were administered orally (indicated by the arrows). A 2-g loading dose of probenecid was given 1 h before the first dose of allopurinol riboside; 500-mg maintenance doses were given 6 h after the initial dose of allopurinol riboside and every 6 h thereafter. Plasma allopurinol riboside concentrations with probenecid (●) are more than twice those without probenecid (○). After the final dose, the half-life of allopurinol riboside in plasma is increased by probenecid (insert, data plotted on log scale).

interfere with measurement of allopurinol riboside or its metabolites.

Previous studies indicated that in humans, the terminal portion of the single dose disposition curve of allopurinol riboside in plasma is monoexponential (21). Therefore, in this study, the half-life in plasma after the first and last doses was calculated by linear regression of the logarithmically transformed values of the concentrations in plasma from 2 to 12 h after dosing (mean r^2, 0.982; see Fig. 1 insert). Areas under the concentration-time curves were calculated by the trapezoidal rule. Renal clearance was determined from the amount of drug or metabolite excreted into urine in each collection period and from the area under the concentration-time curve in the same time period. Estimates of creatinine clearance were computed by the equation of Cockcroft and Gault (5).

In the first treatment period, one subject received allopurinol riboside alone and two subjects received allopurinol riboside plus probenecid. The order of treatment made no obvious difference in the kinetics, and drug or metabolites were not detectable in plasma or urine samples after the 2-week washout period. The only significant laboratory changes were the expected decreases in uric acid levels in serum, in some cases to less than 1 mg/dl (normal levels, 4.2 to 8.8 mg/dl).

For allopurinol riboside, peak concentrations in plasma, area under the concentration-time curve, and plasma half-life are increased by coadministration of probenecid (Fig. 1 and Table 1). These alterations are apparent after the first and after the final dose of allopurinol riboside. Probenecid increases the trough concentrations of allopurinol riboside (means for days 3 to 5 are 3.7 μg/ml without versus 9.0 μg/ml with probenecid). Renal clearance of allopurinol riboside is halved by probenecid (Table 2). By day 4 the total amount of allopurinol riboside excreted in the urine is the same with or without probenecid (Table 2), indicating allopurinol riboside has reached steady state. Probenecid also alters the kinetics of oxipurinol. The peak concentrations, area under the plasma concentration-time curve, and half-life in plasma of oxipurinol are all reduced (Table 1), and renal clearance is more than doubled (Table 2).

Although allopurinol riboside is not an organic acid, probenecid has a clear and striking effect on its pharmacokinetics. Probenecid reduces renal clearance of allopurinol

TABLE 1. Pharmacokinetic parameters of allopurinol riboside and oxipurinol in plasma

Compound, dose, and subject no.	Peak concn (μg · ml^{-1})			AUC (μg · h · ml^{-1})[a]			$t_{1/2}$[b] (h)		
	Without probenecid	With probenecid	Ratio[c]	Without probenecid	With probenecid	Ratio	Without probenecid	With probenecid	Ratio
Allopurinol riboside									
After initial dose									
Subject 1	3.4	3.7		12.7	21.5		2.2	3.3	
Subject 2	3.5	4.9		24.3	41.1		3.9	6.6	
Subject 3	3.4	4.0		22.9	43.1		4.5	6.3	
Mean	**3.4**	**4.2**	**1.2**	**20.0**	**35.2**	**1.8**	**3.6**	**5.4**	**1.5**
After final dose									
Subject 1	4.5	12.1		20.0	60.5		3.7	4.5	
Subject 2	9.1	12.6		38.9	62.3		3.7	6.6	
Subject 3	3.8	10.3		16.6	39.4		4.4	5.0	
Mean	**5.8**	**11.7**	**2.0**	**25.2**	**54.1**	**2.1**	**3.9**	**5.4**	**1.4**
Oxipurinol									
Subject 1	17.2	9.6		97.5	49.1		29.7	34.2	
Subject 2	26.3	20.0		147.6	91.6		38.4	20.0	
Subject 3	13.1	12.4		80.1	56.9		24.8	13.4	
Mean	**18.9**	**14.0**	**0.7**	**108.4**	**65.9**	**0.6**	**31.0**	**22.5**	**0.7**

[a] AUC, area under the concentration-time curve. For allopurinol riboside, this parameter was measured 0 to 24 h after the initial dose and 0 to 6 h after the final dose; for oxipurinol, it was measured 0 to 6 h after the final dose of allopurinol riboside.
[b] $t_{1/2}$, half-life.
[c] Ratio of the value with probenecid to that without probenecid.

TABLE 2. Renal excretion

Compound and subject no.	Clearance[a] (ml · min^{-1})			Amt (mg) excreted into urine		
	Without probenecid	With probenecid	Ratio[b]	Without probenecid	With probenecid	Ratio
Allopurinol riboside						
Subject 1	232	112		1,017	1,479	
Subject 2	204	81		2,324	1,457	
Subject 3	176	96		908	921	
Mean	**204**	**96**	**0.5**	**1,416**	**1,285**	**1.1**
Oxipurinol						
Subject 1	14.2	32.4		385	465	
Subject 2	9.4	22.7		398	557	
Subject 3	15.9	37.0		351	457	
Mean	**13.2**	**30.7**	**2.3**	**378**	**493**	**1.3**
Allopurinol						
Subject 1				45.0	38.1	
Subject 2				36.0	42.0	
Subject 3				31.3	30.7	
Mean	—[c]	—		**37.4**	**36.9**	**1.0**
Creatinine[d]						
Subject 1	137.5	125.4				
Subject 2	99.0	94.7				
Subject 3	99.0	88.8				
Mean	**111.8**	**103.0**	**0.9**			

[a] 0 to 24 h after final dose of allopurinol riboside; in the two treatment periods, the urine volumes for each volunteer varied by less than 15%. The mean values of urine pH were 6.2 and 5.7 (with and without probenecid, respectively).
[b] Ratio of the mean value with probenecid to that without probenecid.
[c] —, drug levels in plasma too low for reliable calculation of area under the concentration-time curve.
[d] Calculated from six daily values.

riboside to the rate of glomerular filtration (Table 2), suggesting complete inhibition of renal tubular secretion and maximum benefit from blockade of renal excretion. As a consequence, the levels of allopurinol riboside in plasma are more than doubled (Fig. 1 and Table 1). Although this study involves a small number of subjects, each subject served as his own control, and the kinetic changes attributable to probenecid are obvious in each of the subjects. Furthermore, the pharmacokinetic data obtained with allopurinol riboside alone compare well with values we found previously (21), suggesting that these volunteers are reasonably representative.

When coadministered with probenecid, the mean peak and trough levels of allopurinol riboside in plasma (11.7 and 9.0 μg/ml, respectively) are within the therapeutic range predicted from in vitro studies with *Leishmania donovani* (90% inhibition of growth at 0.6 to 20 μg/ml) and *Trypanosoma cruzi* (causative organism of Chagas' disease, 50% inhibition of growth at 5 μg/ml) (2, 16). Patients with cutaneous leishmaniasis have been treated with allopurinol riboside alone or with probenecid (20). Although the groups were small and pharmacokinetics were not evaluated, patients receiving allopurinol riboside plus probenecid had a higher cure rate than did those who received allopurinol riboside alone, suggesting that efficacy may be enhanced by higher levels of allopurinol riboside in plasma. In another phase II study, 3 of 10 Kenyan children with visceral leishmaniasis were cured with a combination of allopurinol riboside plus probenecid; correlation of efficacy with drug levels is pending (23).

We proposed a model to account for the unexpected appearance of allopurinol and oxipurinol in volunteers who receive allopurinol riboside (21). Briefly, the drug is incompletely absorbed, perhaps because a gut transport mechanism is saturated. Increasing doses of allopurinol riboside lead to increasing levels of allopurinol in plasma, which may be generated from unabsorbed allopurinol riboside by enteric flora, absorbed, and converted to oxipurinol. In this study, the appearance of allopurinol in plasma and urine, and the 1:10 ratio (on a milligram basis) of allopurinol to oxipurinol excreted into urine (Table 2) is similar to that found in patients given allopurinol (15, 22) and supports the proposed model. As expected (6), probenecid blocks the renal tubular reabsorption of oxipurinol (Table 2) and thereby reduces levels of this inactive and potentially toxic metabolite in plasma.

Allopurinol riboside appears to be safe and well tolerated in healthy volunteers (21) and in patients with leishmaniasis (20, 23). Furthermore, clear evidence of antiparasitic activity in patients strongly suggests that the purine salvage pathway is a promising target for rational therapy. Although the levels of allopurinol riboside in plasma are substantially improved by coadministration of probenecid, these levels remain at the threshold required to cure most infections. In view of the pressing need for safe, orally effective, new antileishmanial and antitrypanosomal agents (1), structural modifications to improve drug absorption should be evaluated. For example, modified purine nucleoside analogs, such as 6-deoxyacyclovir (10), or the diacetyl derivative (BRL 42810) of 6-deoxy-9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine (BRL 39123) (3), increase the levels of active antiviral agent in plasma up to 10-fold after oral administration of the prodrug (4, 19).

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REFERENCES

1. **Anonymous.** 1988. Tropical disease research: science at work, 2nd ed., p. 31–38. UNDP/World Bank/WHO, Geneva, Switzerland.
2. **Berens, R. L., J. J. Marr, D. J. Nelson, and S. W. LaFon.** 1980. Antileishmanial effects of allopurinol and allopurinol riboside on intracellular forms of *Leishmania donovani*. Biochem. Pharmacol. **29:**2397–2398.
3. **Boyd, M. R., T. H. Bacon, D. Sutton, and M. Cole.** 1987. Antiherpesvirus activity of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) in cell culture. Antimicrob. Agents Chemother. **31:**1238–1242.
4. **Boyd, M. R., R. Boon, S. E. Fowles, K. Pagano, D. Sutton, R. A. Vere Hodge, and B. D. Zussman.** 1988. Some biological properties of BRL 42810, a well absorbed oral prodrug of the antiherpesvirus agent BRL 39123. Antiviral. Res. **9:**146.
5. **Cockcroft, D. W., and M. H. Gault.** 1976. Prediction of creatinine clearance from serum creatinine. Nephron **16:**31–41.
6. **Cunningham, R. F., Z. H. Israili, and P. G. Dayton.** 1981. Clinical pharmacokinetics of probenecid. Clin. Pharmacokinet. **6:**135–151.
7. **Elion, G. B., A. Kovensky, and G. H. Hitchings.** 1966. Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. Biochem. Pharmacol. **15:**863–880.
8. **Elion, G. B., T. E. Yu, A. B. Gutman, and G. H. Hitchings.** 1968. Renal clearance of oxipurinol, the chief metabolite of allopurinol. Am. J. Med. **45:**69–77.
9. **Kornhauser, D. M., B. G. Petty, C. W. Hendrix, A. S. Woods, L. J. Nerhood, J. G. Bartlett, and P. S. Lietman.** 1989. Probenecid and zidovudine metabolism. Lancet **ii:**473–475.
10. **Krenitsky, T. A., W. W. Hall, P. deMiranda, L. M. Beauchamp, H. J. Schaeffer, and P. D. Whiteman.** 1984. 6-Deoxyacyclovir: a xanthine oxidase-activated prodrug of acyclovir. Proc. Natl. Acad. Sci. USA **81:**3209–3213.
11. **Laskin, O. L., P. deMiranda, D. H. King, D. A. Page, J. A. Longstreth, and P. S. Lietman.** 1982. Effects of probenecid on the pharmacokinetics and elimination of acyclovir in humans. Antimicrob. Agents Chemother. **21:**804–807.
12. **Marr, J. J., and R. L. Berens.** 1977. Antileishmanial effect of allopurinol. J. Infect. Dis. **136:**724–732.
13. **Marr, J. J., R. L. Berens, and D. J. Nelson.** 1978. Antitrypanosomal effect of allopurinol: conversion in vivo to aminopyrazolopyrimidine nucleotides by *Trypanosoma cruzi*. Science **201:**1018–1020.
14. **Nelson, D. J., C. J. L. Bugge, G. B. Elion, R. L. Berens, and J. J. Marr.** 1979. Metabolism of pyrazolo(3,4-d)pyrimidines in *Leishmania braziliensis* and *Leishmania donovani*. J. Biol. Chem. **254:**3959–3964.
15. **Nelson, D. J., S. W. LaFon, G. B. Elion, J. J. Marr, and R. L. Berens.** 1980. Comparative metabolism of a new antileishmanial agent, allopurinol riboside, in the parasite and the host cell, p. 7–12. *In* A. Rapado and R. W. E. Watts (ed.), Purine metabolism in man III. Plenum Press, New York.
16. **Nelson, D. J., S. W. LaFon, J. V. Tuttle, W. H. Miller, R. L. Miller, T. A. Krenitsky, G. B. Elion, R. L. Berens, and J. J. Marr.** 1979. Allopurinol ribonucleoside as an antileishmanial agent: biological effects, metabolism, and enzymatic phosphorylation. J. Biol. Chem. **254:**11544–11549.
17. **Neva, F., and D. Sacks.** 1990. Leishmaniasis, p. 296–308. *In* K. S. Warren and A. A. F. Mahmoud, (ed.), Tropical and geographical medicine. McGraw-Hill Book Company, New York.
18. **Nogueira, N., and J. R. Coura.** 1990. American trypanosomiasis (Chagas' disease), p. 281–296. *In* K. S. Warren and A. A. F. Mahmoud (ed.), Tropical and geographical medicine. McGraw-Hill Book Company, New York.
19. **Petty, B. G., R. J. Whitley, S. Liao, H. C. Krasny, L. E. Rocco, L. G. Davis, and P. S. Lietman.** 1987. Pharmacokinetics and tolerance of desciclovir, a prodrug of acyclovir, in healthy human volunteers. Antimicrob. Agents Chemother. **31:**1317–1322.
20. **Saenz, R. E., H. M. Paz, C. M. Johnson, J. J. Marr, D. J. Nelson, K. H. Pattishall, and M. D. Rogers.** 1989. Treatment of American cutaneous leishmaniasis with orally administered allopurinol riboside. J. Infect. Dis. **160:**153–158.
21. **Shapiro, T. A., J. B. O. Were, K. Danso, D. J. Nelson, R. E. Desjardins, and C. L. Pamplin.** 1991. Pharmacokinetics and metabolism of allopurinol riboside. Clin. Pharmacol. Ther. **49:**506–514.
22. **Simmonds, H. A.** 1969. Urinary excretion of purines, pyrimidines and pyrazolopyrimidines in patients treated with allopurinol or oxipurinol. Clin. Chim. Acta **23:**353–364.
23. **Were, J. B. O.** Unpublished data.