Bioequivalence of allopurinol preparations: to be assessed by the parent drug or the active metabolite?*

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Summary. Allopurinol is converted almost completely into a single active metabolite, oxipurinol, which has the same therapeutic pattern but a much longer elimination half-life than the parent compound. Therefore both allopurinol and oxipurinol were evaluated in our bioequivalence study in healthy volunteers comparing two allopurinol brands. Bioequivalence determination was based on the 90% confidence intervals (CI) of the area under the plasma concentration time curve from time zero to infinity ($AUC_{0-\infty}$), of the area from time zero to the last measurable plasma concentration ($AUC_{0-t\ (last)}$), and $C_{max}$. Because of the lack of compound-specific criteria we used conventional limits for the bioequivalence range. Under these conditions the brand chosen as test preparation was judged to be bioequivalent to the reference form with respect to the extent of bioavailability, $AUC_{0-\infty}$, and $AUC_{0-t\ (last)}$ of the parent drug. The CI of $C_{max}$ of allopurinol slightly exceeded the upper limit of 130%, so that bioequivalence was not confirmed with regard to the rate of bioavailability of the parent compound. The CI values of both AUC and $C_{max}$ of the active metabolite were tighter than those of allopurinol. In addition, the CI values of $C_{max}$ of oxipurinol were smaller than those of the corresponding AUC. As a consequence the test drug can clearly be accepted as bioequivalent, based on metabolite data. Since the active metabolite is of greater therapeutic significance than the parent drug, assessment of the bioequivalence of allopurinol preparations needs to be based on oxipurinol rather than allopurinol. Our data provide further evidence that establishing compound-specific criteria is required for bioequivalence evaluation in drugs with a single active metabolite.

Key words: Allopurinol – Oxipurinol – Single active metabolite – First-pass metabolism – Pharmacokinetics – Criteria for bioequivalence

Allopurinol worldwide is the main drug used for treating hyperuricemia. It is almost completely converted into a single major metabolite, oxipurinol, which itself lowers serum uric acid due to inhibition of uric acid formation. The therapeutic effect of allopurinol to a large extent is attributed to its active metabolite oxipurinol [13]. Various production techniques for allopurinol were patented more than 20 years ago, so that analogous brands became available before numerous generic preparations were marketed. As in generics, bioequivalence needs to be shown for such brands.

In general, bioequivalence is assessed by comparing the plasma concentrations of the parent drug after application of various preparations. However, the conventional method of bioequivalence testing, relying on the parent drug, is insufficient in a compound such as allopurinol, which is rapidly converted into an active metabolite and can be regarded as a prodrug. Because of its therapeutic relevance, the active metabolite needs to be evaluated [3]. The adequate role of oxipurinol for assessment of bioequivalence in allopurinol preparations has not so far been clearly defined. The aim of this investigation was to compare two allopurinol brands by means of the parent drug and the active metabolite and to explore the validity of allopurinol and oxipurinol for assessing bioequivalence.

Abbreviations: $AUC_{0-t\ (last)}$ = area under the plasma concentration time curve from time zero to the last measurable plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration time curve from time zero to infinity; $AUC_{"m"}$ = area under the plasma concentration time curve from the last measurable concentration to infinity; $C_{max}$ = maximum plasma concentration; $t_{max}$ = time to reach maximum plasma concentrations; $t_{1/2}$ = plasma elimination half-life; CI = confidence interval; T = test preparation; R = reference preparation; ANOVA = analysis of variance

* Dedicated to Prof. Dr. N. Zöllner on the occasion of his 70th birthday
Materials and methods

Subjects

Eighteen healthy male volunteers were included in the study. Mean age was 29.1 ± 3.9 years, height 180.7 ± 8.1 cm, and weight 75.1 ± 9.8 kg. Their individual characteristics are given in Table 1. History, physical examination, clinical chemistry, hematology, urine analysis, and ECG revealed no abnormalities. Body weight was stable during the last 4 weeks before entering the investigation. All subjects were nonsmokers and free of drugs for at least 1 week prior to and during the entire period of study. Alcoholic beverages were avoided from 48 h prior to allopurinol administration until the end of the sampling period. Unusual physical activities and taking a sauna also were prohibited during this time. Signed informed consent was obtained from all participants before the investigation. The study protocol was approved by the Ethics Committee of the University of Heidelberg Medical Faculty.

Experimental procedures

The investigation was designed as a randomized one-way crossover experiment. The study drugs were Zyloric 300, commercial lot no. B 5714 A, used as the reference preparation (R), and Foligan 300, commercial lot no. 43140 E, as test preparation (T). Because of the slow elimination of the active metabolite oxipurinol a washout period of 2 weeks between administration of the two drugs was allowed. After an overnight fast, at 8:00 A.M. one tablet of T or R was ingested with 200 ml mineral water (Fachinger). A standard breakfast was served after 2 h. All food was eaten within 15 min. Four hours after drug administration the regular lunch available in the hospital was given.

For determining plasma concentrations of allopurinol and oxipurinol, 10-ml blood samples were drawn using a plastic needle inserted in a superficial forearm vein before and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 h after drug administration. Additional samples were collected at 24, 34, 48, 72, 96 h after allopurinol ingestion. The blood was drawn into heparinized plastic tubes and centrifuged immediately at 3000 rpm. The plasma was frozen at −23°C until analysis. We collected 24-h urine 0–24, 24–48, 48–72, and 72–96 h after allopurinol administration. After measuring the total urine volume two 10-ml samples of the urine were frozen until analysis.

Analytical techniques

Allopurinol and oxipurinol were determined simultaneously by high-pressure liquid chromatography after purifying plasma samples by ultrafiltration and pretreating urine with anion exchange resins. Reversed-phase column and ultraviolet detection were used for separation and quantitation of both compounds. Calibration curves are linear in the range of 0.1–20 μg/ml. The detection limit is 0.1 μg/ml for each substance; the method is thus adequately sensitive for application in pharmacokinetic studies. The precision is 3–5%. The assay was validated by liquid chromatography with photodiode array detection [5].

Pharmacokinetic calculations and biometric evaluation

All pharmacokinetic calculations were performed with a model-independent approach. The area under the plasma concentration time curve covering the time between drug application and the last plasma concentration value above or equal to the determination limit (AUC0–t(last)) was calculated for allopurinol and oxipurinol according to the linear trapezoidal rule. The area under the curve from dosing to infinity (AUC0–∞) comprises AUC0–t(last) and a remainder which is not covered by measured values (AUC(last); the latter is estimated from the elimination rate constant ke, which was derived from the last three concentration time points by log linear regression. AUC0–t(last) must account for more than 80% of AUC0–∞ to ensure