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Evidence for Metabolic Inertness of Doxycycline

H. J. C. F. NELIS and A. P. De LEENHEER ˣ

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Abstract □ Several conflicting observations in the literature raised considerable doubt about the metabolic fate of doxycycline, which, like other tetracyclines, has been claimed to be metabolically inert. A double liquid chromatographic approach was used in an attempt to demonstrate the polar metabolites and/or conjugates in excreta of human volunteers who ingested the drug. Both ion-exchange and reversed-phase chromatography failed to reveal significant by-products in urine and feces, except for minor amounts of 4-epidoxycycline. In addition, enzymatic hydrolysis procedures did not present any evidence of the conjugates. Thus, the different excretion behavior of doxycycline, compared to other analogs, cannot be explained in terms of increased metabolism.

Keyphrases □ Doxycycline—tetracycline analog, metabolic fate, analysis, human urine and feces □ Liquid chromatography—analysis, doxycycline in human urine and feces □ Antibacterials—doxycycline, metabolic fate, analysis in human urine and feces

It is generally accepted that tetracycline antibiotics are not metabolized in the human body (1–4). However, considerable doubt still exists concerning the fate of the more lipophilic derivatives (5, 6). A study with ^{3}H-labeled doxycycline failed to reveal by-products in excreta, but a rather poor chromatographic technique had been used (7).

Some investigators noted a dramatic decrease in the half-life of the drug following coadministration of typical enzyme-inducing substances, including barbiturates (8), antiepileptics (9, 10), and ethanol (11). This observation was explained in terms of accelerated doxycycline metabolism and led to speculation concerning the formation of conjugates (10). However, other investigators did not support these views and questioned the reliability of the effect (12). Furthermore, an unidentified, biologically inactive fraction is claimed to occur in feces (13–15) and might be due to intestinally formed metabolites.

The lack of suitable chromatographic techniques has hampered the unambiguous settling of these controversial observations. This paper reports the use of liquid chromatography for detecting possible doxycycline by-products in human urine and feces. In addition, enzymatic hydrolysis was carried out in order not to overlook conjugates. Although a similar approach allowed demonstration of minocycline metabolites in urine (16), strong evidence is presented here for the metabolic inertness of doxycycline.

EXPERIMENTAL

Chromatography—Two liquid chromatographs[1], equipped with a sample valve[2] and a variable-wavelength detector[3] operated at 350 nm, were used. The first column (10 × 0.46 cm) consisted of a strong cation-exchange material[4] and was utilized with a 38:62 (v/v) mixture of ethanol–0.1 *M* citrate buffer (pH 4.6) containing 0.05% edetate disodium as the eluent. A reversed-phase system, previously reported for the quantitative determination of doxycycline in human serum and urine (17), was applied to feces samples as well.

Collection of Excreta—A single 200-mg doxycycline dose was administered orally to human volunteers just after a light breakfast. Urine was collected over the following 24 hr. Three days after administration, feces samples were taken. Blank samples were obtained before drug intake.

Extraction of Urine and Feces—Urine samples were extracted with ethyl acetate as described previously (17).

Feces (0.8 g) were homogenized in 0.2 *M* HCl (12 ml) using a high-speed mixer[5]. After centrifugation, a 1-ml aliquot of the supernate was neutralized with 1 *M* NaOH and buffered with 1 ml of phosphate–sulfite buffer (pH 6) (17). Extraction was performed with 10 ml of ethyl acetate. The organic layer was evaporated to dryness[6], and the residue was redissolved in 1 ml of the chromatographic solvent of either the reversed-phase or the ion-exchange system. Finally, 20–100 μl was injected onto the columns. Alternatively, feces were homogenized in 0.2 *M* acetate buffer (pH 5) and processed in a similar way.

Hydrolysis Procedures—Urine samples and feces homogenates were adjusted to pH 5 using acetic acid and 2 *M* NaOH, respectively. Then, they were incubated at 37° with the arylsulfatase–glucuronidase enzyme

[1] Model 8500, Varian Associates, Palo Alto, Calif.
[2] Model CV-6-UHPa-N60, Valco Instruments Co., Houston, Tex.
[3] Varichrom, Varian Associates, Palo Alto, Calif.
[4] Nucleosil SA, 5 μm, Machery & Nagel, Düren, West Germany.
[5] Virtis S 23, Virtis Research Equipment, Gardiner, N.Y.
[6] Rotary Evapo Mix, Büchler Instruments, Fort Lee, N.J.

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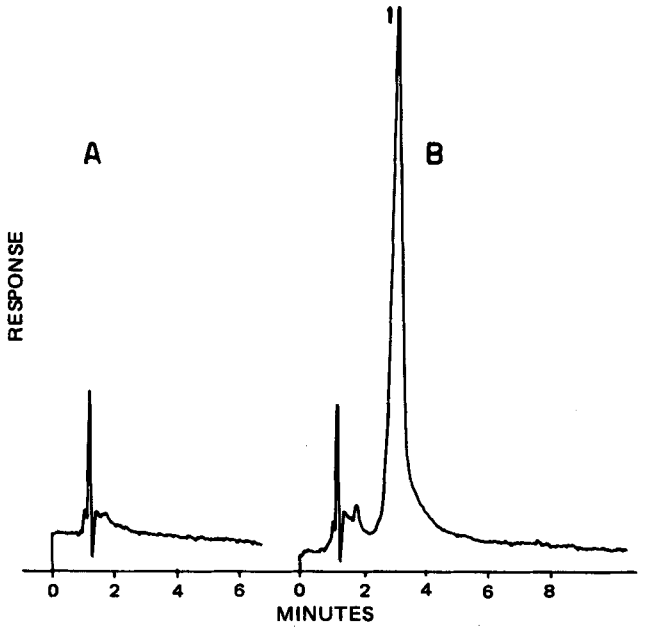

Figure 1—*Chromatograms of a blank urine extract (A) and a urine extract obtained after doxycycline administration (B). Peak 1 is doxycycline. A 10 × 0.46-cm Nucleosil SA (5 μm) column was used, and the mobile phase was ethanol–0.1 M citrate buffer (pH 4.6) with 0.05% edetate disodium (38:62 v/v) at a flow rate of 1 ml/min.*

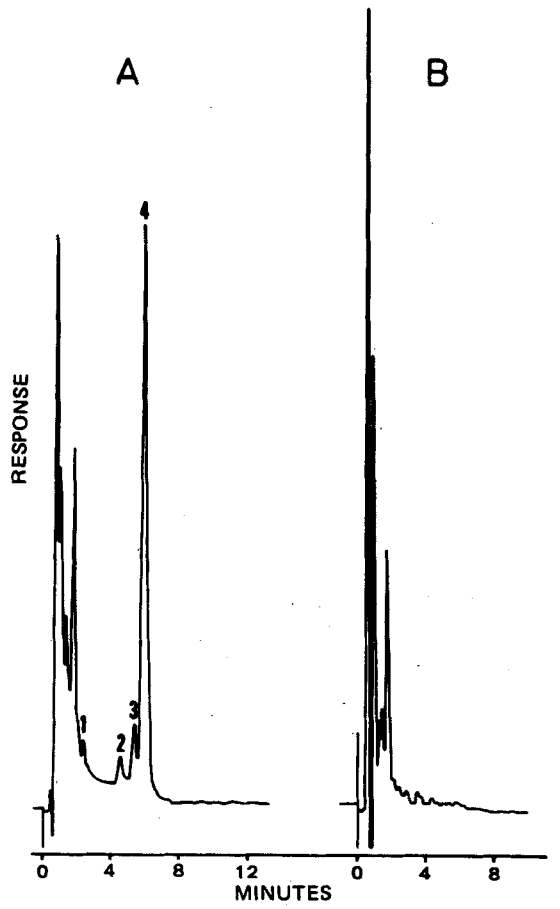

Figure 2—*Chromatograms of urine obtained from a volunteer who ingested 200 mg of doxycycline (A) and a blank urine chromatogram (B). Key: 1, unidentified doxycycline by-product; 2, unidentified peak; 3, 4-epidoxycycline; and 4, doxycycline. A 10 × 0.2-cm Lichrosorb RP8 (5 μm) column was used, and the mobile phase was acetonitrile–0.1 M citric acid (24:76 v/v) at a flow rate of 0.5 ml/min.*

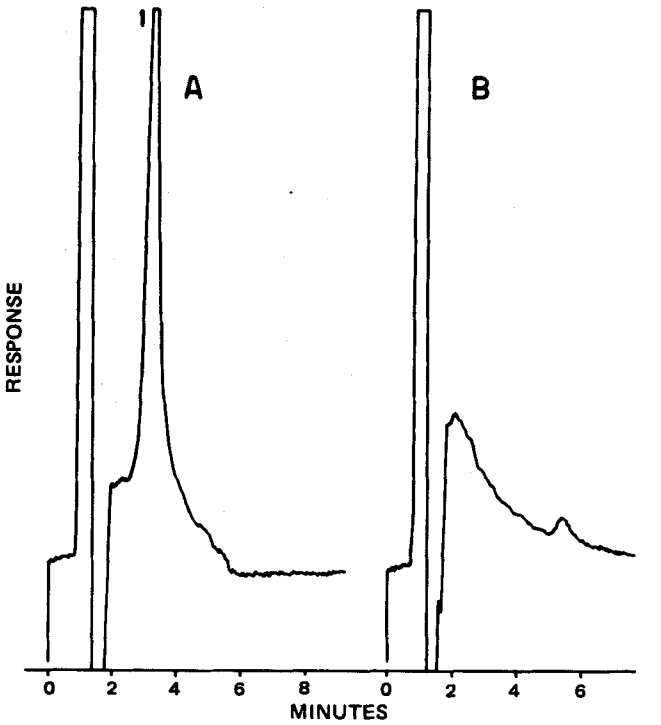

Figure 3—*Chromatograms of a feces extract obtained after doxycycline administration (A) and of a blank feces extract (B). Peak 1 is doxycycline. Conditions were the same as in Fig. 1.*

complex from *Helix pomatia*[7] (200 μl of the enzyme solution/ml) for 48 hr. Reference samples containing no enzyme were kept simultaneously under the same conditions. Feces samples homogenized in the acetate buffer were incubated without pretreatment.

RESULTS

Urine—Injection of urine extracts onto the ion-exchange column yielded one large doxycycline peak (Fig. 1). The chromatographic pattern in the reversed-phase system (Fig. 2) was more composite but also did not show significant additional peaks. The retention time of the principal accompanying peak (peak 3) corresponded to that of 4-epidoxycycline. Unfortunately, its spectrometric characteristics were difficult to determine because of a coeluting compound. For an unknown reason, the epimer eluted as an extremely distorted peak from the ion-exchange column. This could explain its absence in urine chromatograms obtained on this particular column. Direct injection of undiluted urine did not reveal additional doxycycline-related peaks in the chromatograms, nor did detection at other wavelengths (270 and 430 nm).

After incubation with hydrolyzing enzymes, no increase of the doxycycline peak was observed when compared to reference samples.

Feces—A chromatogram of a feces extract obtained on the ion-exchange column showed a single doxycycline peak (Fig. 3). Reversed-phase chromatograms (Fig. 4) again illustrated the absence of major additional peaks. A peak corresponding to 4-epidoxycycline was barely detectable (peak 2). Peak 1 probably was an artifact since it has been observed in serum, urine, and fresh standard solutions as well (17). Results were confirmed by direct injection of the homogenates without extraction.

DISCUSSION

Application of two liquid chromatographic techniques in combination with hydrolysis procedures strongly suggests the absence of polar doxycycline metabolites and conjugates in urine. Components that are more polar than doxycycline normally will elute before doxycycline from a reversed-phase column. A minor extra peak probably is attributable to

[7] Solution in water (Boehringer, Mannheim, West Germany); glucuronidase activity was 5.2 units/ml (38°, phenolphthalein monoglucuronide as the substrate); arylsulfatase activity was 2.6 units/ml (38°, phenolphthalein disulfate as the substrate).

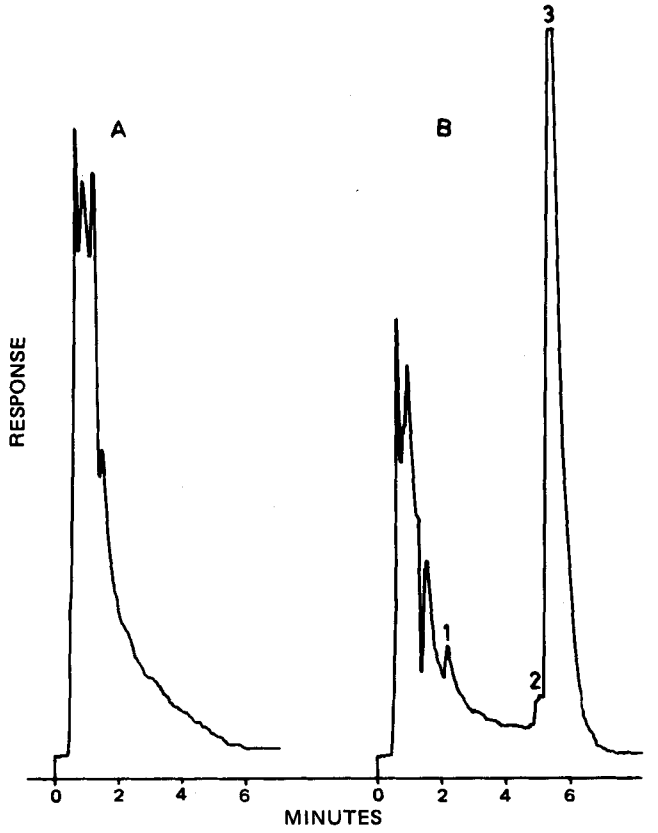

Figure 4—*Chromatograms of a blank feces extract (A) and a feces extract obtained after doxycycline administration (B). Key: 1, unidentified doxycycline by-product; 2, 4-epidoxycycline; and 3, doxycycline. Conditions were the same as in Fig. 2.*

4-epidoxycycline. Its presence is not unlikely because a weakly acidic medium is known to catalyze epimerization (18).

Thus, the lower urinary doxycycline levels, compared to those of the classical tetracyclines, are not due to the presence of biologically inactive metabolites but rather are caused by increased tubular reabsorption (19), which is in agreement with the stronger lipophilic character of the drug.

Doxycycline is claimed to be excreted in the feces by direct diffusion through the intestinal wall (13, 14); biliary excretion seems to be of minor importance (12). Because no extra peaks were detected, the exact nature of the biologically inactive fecal fraction, if present, cannot be elucidated easily using chromatographic techniques. The observation of the present investigators indirectly supports the currently accepted theory of the chelate formation between doxycycline and divalent cations leading to partial biological deactivation (20). Homogenization of feces in hydrochloric acid releases doxycycline from the complex. The need for a relatively strong acid to recover all fecal doxycycline is documented (13). The quasi-absence of the epimeric form can be rationalized in terms of the less favorable pH medium in the intestine. The possibility of hydrolysis of labile conjugates in the strong acid prior to enzymatic attack was not considered because homogenization in the less aggressive acetate buffer yielded the same results.

Because the existence of metabolites was not indicated, the interaction between doxycycline and certain drugs cannot be explained in terms of enzyme induction. On the other hand, displacement of the drug from plasma proteins might be the mechanism involved. Although doxycycline, unlike other analogs, is excreted *via* an alternative intestinal pathway, metabolism is not a supplementary factor distinguishing the drug from the classical tetracyclines.

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