Biochemical Pharmacology, Vol. 32, No. 24, pp. 3887-3890, 1983.
Printed in Great Britain

0006-2952/83 $3.00 + 0.00
Pergamon Press Ltd.

HUMAN AND RAT URINARY METABOLITES OF THE ANTITUMOR DRUG CELIPTIUM ® (N^2-METHYL-9-HYDROXYELLIPTICINIUM ACETATE, NSC 264137). IDENTIFICATION OF CYSTEINE CONJUGATES SUPPORTING THE "BIOOXIDATIVE ALKYLATION" HYPOTHESIS.

Bernard Monsarrat[a], Mohamed Maftouh[a,d], Gérard Meunier[a], Bernard Dugué[a], Jean Bernadou[a,b], Jean-Pierre Armand[b], Claudine Picard-Fraire[c], Bernard Meunier[a,e] and Claude Paoletti[a].

[a]Laboratoire de Pharmacologie et de Toxicologie Fondamentales, CNRS, 205 route de Narbonne, 31400 TOULOUSE, FRANCE, [b] Centre Claudius Regaud, 20-24 rue du Pont Saint-Pierre 31052 TOULOUSE, FRANCE, [c] SANOFI- Recherche, 195 route d'Espagne 31035 TOULOUSE, FRANCE. [d] Present address : see [c], [e] Author to whom correspondence should be addressed.

(*Received* 30 *September* 1983; *accepted* 18 *October* 1983)

Among all the ellipticine derivatives, one of them, the N^2-methyl-9-hydroxyellipticinium or Celiptium® (9-OH-NME, 1), is recently used for cancer therapy (1,2). This drug, bearing an hydroxyl function in the para position of the indolic N-H, is easily oxidized by horseradish peroxidase (HRP) and hydrogen peroxide in an electrophilic quinone-imine (9-Oxo-NME, 1') (3,4). Thus, 9-OH-NME may act not only as an intercalating molecule, as described for ellipticine derivatives (5), but may also be considered as a potential alkylating agent after a two electron oxidation process in vivo (see scheme 1).

Previously, we have demonstrated that the quinone-imine 1' alkylate various models of biological nucleophiles : amino-acids, S-H containing molecules and nucleosides (4,6,7). Among the S-H derivatives, we have prepared via biosynthetic route three different adducts as models of thioether conjugates : the cysteine- ,3, N-acetylcysteine- ,4, and glutathione- ,5, adducts (4,6,8).

Recently, we have been able to identify and isolate from rat bile a glutathione conjugate of 1, identical to the biosynthetic product 5, as a minor metabolite (8,9). This fact can be regarded as an excellent indication that 9-OH-NME is susceptible to previously unrecognized minor metabolic pathway of activation and detoxification analogous to those described for acetaminophen (10,11).

We report here the observation and preliminary characterization of the 9-(O)-glucuronide, 10-(S)-cysteine, and 10-(S)-N-acetylcysteine conjugates in rat and human urines.

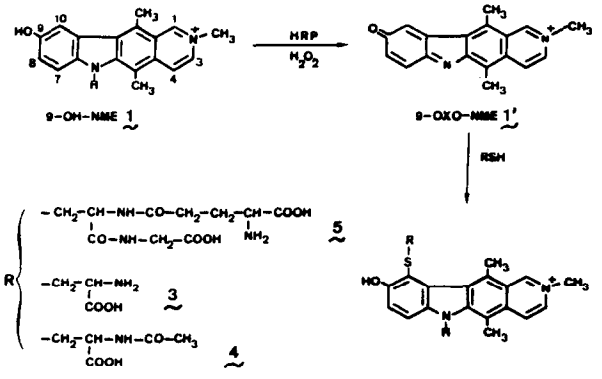
MATERIALS AND METHODS

Materials : 9-OH-NME was obtained from SANOFI Company, cysteine and N-acetylcysteine ellipticinium adducts were prepared according to the previously described procedure (4,6). The 9-(O)-glucuronide conjugate 2 was synthesized by chemical route (8). ß-glucuronidase (E. coli, type VII) was obtained from Sigma. Usual reagents were purchased from Prolabo.

HPLC : High-pressure liquid chromatography analysis were performed on a Waters chromatograph. Analytical separations were performed on C_{18} μ-Bondapack column 10 μ (0.39 x 30 cm) Waters, and reverse phase ultrasphere ODS 5 μ (0.46 x 15 cm) Altex. The C_{18} μ-Bondapack was eluted isocratically using a mixture of methanol/0.05 M ammonium acetate 40/60 (v/v) acidified to pH 4.5 with acetic acid ; the ultrasphere ODS elutions were performed with a non linear gradient (660 solvent programmer Waters, curve 7) from 0 % B to 60 % B in 18 min, the solvents were, A : methanol/0.05 M ammonium biphosphate 34/66 (v/v) acidified to pH 3.15 with trifluoroacetic acid and B : methanol acidified with trifluoroacetic acid : 0.1 %. Flow rate was 1.2 ml/min.

Treatment of patients : Three patients were included in the present study (2 males and 1 female). Results reported here were obtained following the first administration of the drug as a one hour 100 mg/m^2 infusion at a weekly schedule (2). Urine was collected every 6 hours for a 48 hours period and kept frozen at - 18°C until analysis.

Treatment of animals : Male Sprague-Dawley rats (200-250 g) were used. The drug was administered intravenously to four rats at a dose of 10 mg/kg in sodium acetate buffer 0.1 M (pH 5.0). Urines were collected over a 96 hours period and kept as described above.



Scheme 1.

RESULTS AND DISCUSSION

Aliquots of control urine and urines containing metabolites were directly injected (2-10 μl) onto reverse phase μ Bondapack C_{18} or ultrasphere ODS columns. Preliminary extraction step with various organic solvents was avoided because the high water solubility of several charged metabolites (8,9).

a) Rat urine

The HPLC chromatogram of rat urine for the 6-24 hrs period following the drug administration (figure 1A) indicates the presence of three new peaks (I, II, IV) compared to urine control (figure 1A, dashed line). The peaks I and II co-chromatography respectively with unchanged drug 1 and corresponding synthetic 9-(O)-glucuronide conjugate 2. Moreover, treatment of urine sample with β-glucuronidase supports the identification of peak II as 9-(O)-glucuronide conjugate (peak II disappears to the benefit of peak I).

Previous studies (8,9) pointed out the presence of a glutathione and 9-(O)-glucuronide conjugates in rat bile. Keeping in mind the possible further degradation of this 10-(S)-glutathione conjugate by peptidases (12), the two possible 10-(S)-cysteine 3 and 10-(S)-N-acetylcysteine 4 conjugates were prepared by a biochemical route (6). Comparison of the chromatographic behaviour of metabolite IV (retention times and absorption ratios at 313/254

nm and 313/280 nm) with those of the reference cysteine derivatives 3 and 4 (table 1) contributes to identify it as the 10-(S)-N-acetylcysteine conjugate. No peak corresponding to the cysteine ellipticinium conjugate was detected in rat urine. It is known that the degradation of a glutathione conjugate by peptidases leads to different ratios of cysteine and N-acetylcysteine adducts depending of the N-acetyltransferase activity of the different studied species (13). In the case of rat, this N-acetyltransferase is usely high and so leads to the N-acetylcysteine derivative as the only conjugate in urine.

Table 1. Chromatographic behaviours of 9-OH-NME thioethers conjugates.

	HUMAN			RAT		
	Rt* (min)	Absorption ratios** 313 nm/254 nm	313 nm/280 nm	Rt* (min)	Absorption ratios** 313 nm/254 nm	313 nm/280 nm
Peak III	9.6 (7.6)	1.21	1.40	——	——	——
Reference 3 (cysteine conjugate)	9.6 (7.6)	1.20	1.42	——	——	——
Peak IV	12.6 (11.6)	1.23	1.33	12.6 (11.6)	1.21	1.32
Reference 4 (N-acetylcysteine conj.)	12.6 (11.6)	1.18	1.31	12.6 (11.6)	1.18	1.31

* Rt : Retention time on μ-Bondapack column and in parentheses on ultrasphere ODS column. Absorption ratios were the mean values for metabolite III and IV and reference compounds 3 and 4 measured on HPLC chromatograms.

** Standard deviations are in the range 0.03 - 0.05.

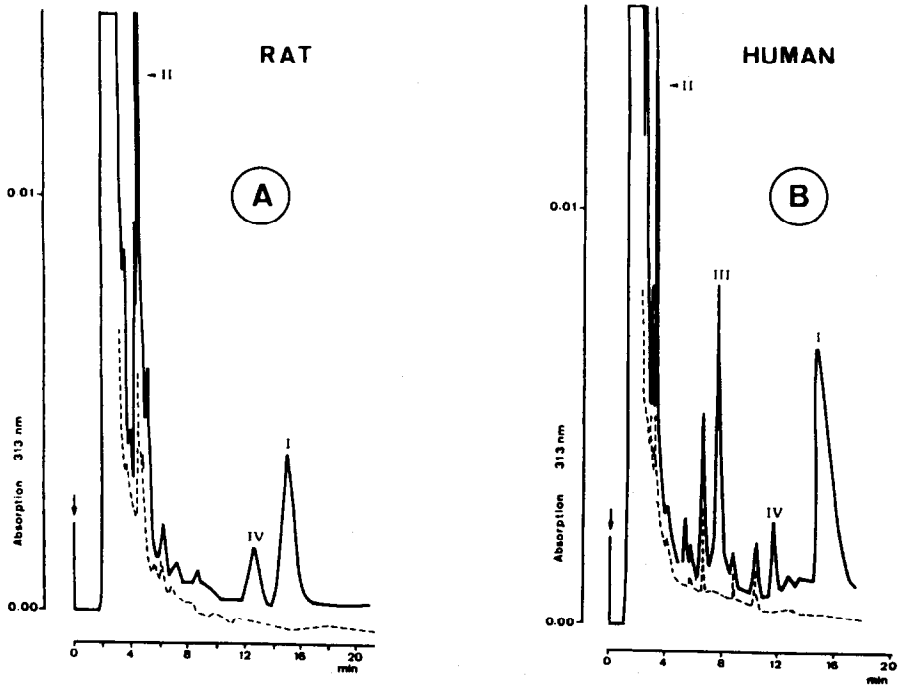


Figure 1 : HPLC profiles of urinary metabolites. (A) : Rat urine metabolites onto C_{18} μ-bondapak column ; dashed line : control urine. (B) : Human urine metabolites onto ultrasphere ODS column ; dashed line : control urine.

b) Human urine

The chromatogram of human urine for the 18-24 hrs period following the drug administration (see figure 1B) indicates the presence of four peaks. Peaks I, II and IV are respectively identified as the unchanged drug, the glucuronide and the N-acetylcysteine conjugates (table 1). The extra peak (peak III, figure 1B), detected in human urine only, corresponds to the 10-(S)-cysteine conjugate (table 1). The HPLC separation of human urine have been done onto an ultrasphere ODS Altex column to obtain the separation of the cysteine adduct from the glutathione adduct (The separation of these two adducts was not possible on C_{18} μ-bondapak waters column).

So, it has to be noted that, despite previous report (14), the main urine metabolite of the drug is the 9-(O)-glucuronide conjugate. Previously, we have described the presence of the glutathione-ellipticinium conjugate in rat bile, this metabolite resulting from the deactivation of an electrophilic form of the administered drug obtained by an oxidative bioactivation process. Such glutathione conjugates are known to be excreted in urine as mercapturic derivatives since the glutathione adducts are cleaved by peptidases (12). Experiments are in progress to isolate, purify and confirm by spectroscopic methods the structure of the S-conjugates of this ellipticine derivative from rat and human urines.

In this work, the preliminary identification of these S-conjugates in vivo suggests that a "biooxidative alkylation" process has to be considered as an hypothesis to explain in part, at least, the cytotoxic activity of this 9-hydroxylated form of ellipticine.

REFERENCES

1. C. PAOLETTI, J.B. LE PECQ, N. DAT-XUONG, P. JURET, H. GARNIER, J.L. AMIEL and J. ROUESSE, Recent Results in Cancer Res., 74, 107-123 (1980).
2. P. JURET, J.F. HERON, J.E. COUETTE, T. DELOZIER and J.Y. LE TALAER, Cancer Treat. Rep., 66, 1909-1916 (1982).
3. C. AUCLAIR and C. PAOLETTI, J. Med. Chem., 24, 289-295 (1981).
4. G. MEUNIER, Thèse de 3ème cycle. Toulouse University, June 1982.
5. J.B. LE PECQ, N. DAT-XUONG, C. GOSSE and C. PAOLETTI, Proc. Natl. Acad. Sci. USA, 71, 5078-5082 (1974).
6. G. MEUNIER, B. MEUNIER, C. AUCLAIR, J. BERNADOU and C. PAOLETTI, Tetrahedron Letters, 365-368 (1983).
7. B. MEUNIER, C. AUCLAIR, J. BERNADOU, G. MEUNIER, M. MAFTOUH, S. CROS, B. MONSARRAT and C. PAOLETTI, in "Structure-activity relationships of antitumors agents", Eds, Reinhoud, D.N., Connors, T.A., Pinedo, H.M. and Van de Pool, K.W. (Martinus Nijhoff, the Hague), pp. 149-181 (1983).
8. M. MAFTOUH, G. MEUNIER, B. DUGUE, B. MONSARRAT, B. MEUNIER and C. PAOLETTI, Xenobiotica, 13, 303-310 (1983).
9. M. MAFTOUH, B. MONSARRAT, R.C. RAO, B. MEUNIER and C.PAOLETTI, Drug Metab. Disp., accepted for publication (1983).
10. I.C. CALDER, S.J. HART, K. HEALEY and K.N. HAM, J. Med. Chem., 24, 988-993 (1981) .
11. J.A. HINSON, T.J. MONKS, M. HONG, R.J. HIGHET and L.R. POHL, Drug. Metab. Disp., 10, 47-50 (1982).
12. P. JENNER and B. TESTA, Concepts in Drug Metabolism, Part A, pp 222-225, Marcel Dekker, New York (1980).
13. W.B. JAKOBY, Enzymatic basis of detoxication, Vol. II, pp 113, Academic Press, New York (1980).
14. N. VAN BAC, C. MOISAND, A. GOUYETTE, G. MUZARD, N. DAT-XUONG, J.B. LE PECQ and C. PAOLETTI, Cancer Treat. Rep., 8-9, 879-887 (1980).