Cancer Chemother Pharmacol (1985) 15: 63–65

Cancer Chemotherapy and Pharmacology

Evidence for electrophilic properties of N^2-methyl-9-hydroxy ellipticinium acetate (Celiptium) from human biliary metabolites

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Summary. The human biliary metabolism of the antitumor agent N^2-methyl-9-hydroxyellipticinium acetate is described. Three major compounds have been identified by high-performance liquid chromatography and comparison with synthetic reference derivatives: the unchanged drug, the O-glucuronide conjugate and the cysteinyl-ellipticinium adduct. The latter one is the expected detoxification compound of an intermediate electrophilic quinone-imine derivative generated in vivo. This result provides a further evidence that hydroxylated forms of ellipticine derivatives might be activated by a biooxidation route.

Introduction

N^2-Methyl-9-hydroxyellipticinium (9-OH-NME) is one of the most efficient anticancer drugs in the ellipticine series and has consequently been used for clinical purposes [2, 6]. We recently reported that such a compound could be considered as a stable reduced form of a *p*-quinone-imine structure [1]; an electrophilic center might be generated in vivo by an initial biooxidative transformation. This highly electrophilic form, when obtained in vitro, reacts with various nucleophiles, including glutathione (GSH), to form adducts with a covalent C-S bond [8, 9].

Previously, we were able to isolate and identify from rats (i) a bile glutathione conjugate and (ii) a urine *N*-acetylcysteine conjugate as minor metabolites [7, 10]. This indicates that 9-OH-NME is susceptible to a minor metabolic pathway of activation and detoxification.

In human urine, in addition to the glucuronide conjugate of the drug, we have recently detected significant amounts of cysteine and *N*-acetylcysteine conjugates [10]. In this present work, we report the analysis of bile samples collected from a patient with external bile derivation treated with Celiptium and the identification of a cysteine conjugate.

Case report

A 36-year-old male patient being treated for a colon adenocarcinoma was evaluated in this study. He had a percutaneous transhepatic drainage of the biliary tract as symptomatic treatment for neoplastic obstructive jaundice and had exhausted other conventional therapeutic measures. Ten days after insertion of the endoprothesis a clinical and biological improvement was obtained (bilirubin decreased from 254 to 78 μM (normal = 3–20); alkaline phosphatase decreased from 2,175 to 1,077 mU//ml (normal = 0–260); transaminases returned to the normal levels; however, GGT remained at a high level: 503 mU//ml (normal = 0–37); then the patient received 50 mg Celiptium in a 30-min infusion. Bile samples were collected at time 0 (control) and at 1, 2, 3, 4, 5, 7, and 17 h after the beginning of the infusion. Urine was collected for the same period. All these samples were stored at −18 °C.

Materials and methods

Glucuronide, cysteine, *N*-acetylcysteine, and glutathione-ellipticinium reference adducts were prepared according to the procedures previously described [3, 8, 9]. β-Glucuronidase (E. coli type VII) was obtained from Sigma. Chromatographic analysis (HPLC) was performed as for human urine [10].

Results

1. Metabolic profile

The chromatogram of bile for the 3 to 4 h period following drug administration (Fig. 1b) indicates the presence of three new peaks compared with control bile (Fig. 1a). Peaks I, II, and III are respectively identified as unchanged drug, glucuronide conjugate, and cysteine conjugate; after the treatment of bile with β-glucuronidase (Fig. 1c) peak II disappeared and peak I increased, indicating that peak II represents the glucuronide conjugate of the administered drug. Since peak III has the same retention time as the cysteine and glutathione reference conjugates on a C_{18}-μ-Bondapak Waters column, separation was achieved on an ultrasphere ODS Altex column [10]. Consequently, peak III was identified as the cysteine conjugate.

2. Kinetics of biliary excretion

For each collection interval, the unchanged drug and its metabolites were quantified from linear calibration curves (peak areas vs concentrations) of bile samples spiked with known quantities of each reference compound. The biliary excretion of metabolites is shown by collection period in

* Part of this work was done by J. B. as part of his preparation for the degree of Doctor of Medicine in the Centre Claudius Regaud

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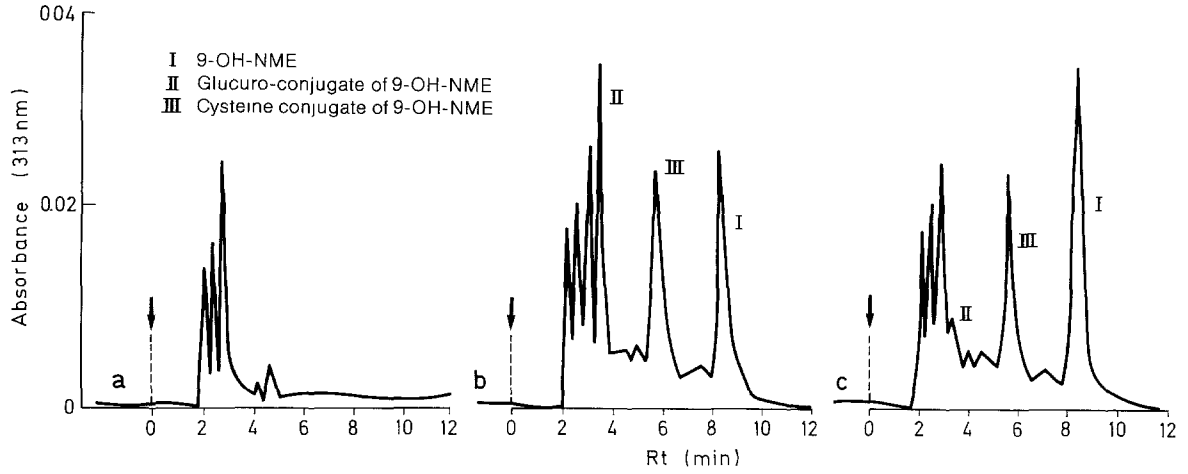

Fig. 1 a–c. HPLC chromatograms: **a** Control bile collected before drug administration; **b** metabolic profile of bile aliquot collected 4 h after administration of Celiptium; **c** identical bile aliquot after treatment with β-glucuronidase

Fig. 2. A metabolite total of 3% with respect to the administered dose (cysteine conjugate 2.5%, glucuronide conjugate 0.5%) and 4.8% of unchanged drug were recovered from the bile samples.

Discussion

The main bile metabolite of Celiptium is the cysteine conjugate. Since (i) glutathione is the major source of the cysteine moiety for S conjugates [5] and (ii) glutathione conjugates can be converted to cysteine conjugates by liver γ-glutamyl transpeptidase (GGT) and amino peptidases [11], the presence of such a metabolite involves conjugation of an electrophilic form of 9-OH-NME, with the endogenous glutathione as a nucleophile. This supports the hypothesis of an initial biooxidative transformation of the drug leading to a highly electrophilic quinoneimine.

The absence of glutathione conjugate in human bile, in contrast to rat bile samples [7], may probably be correlated to the high plasmatic level of γ-glutamyl transpeptidase (503 mU//ml) due to the persistent biliary obstruction and not to the slight difference in GGT level usually observed between human and rat [4, 11].

Furthermore, in the urine samples obtained during bile collection no cysteine or *N*-acetylcysteine conjugates have been detected. This strongly suggests that the cysteine-ellipticinium conjugates previously observed in human urines of patients without bile derivation are the result of a drug detoxification process by glutathione, which mainly occurs in liver.

In conclusion, it seens that hydroxylated forms of ellipticine derivatives might be activated in vivo to an electrophilic species, and this will be considered in future studies of the cytotoxic mechanism of ellipticine compounds.

Acknowledgements. We thank G. Meunier and B. Dugué for the preparation of reference compounds.

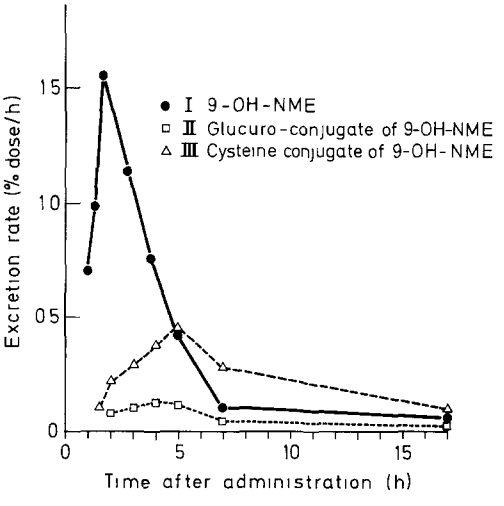

Fig. 2. Biliary excretion rate of Celiptium metabolites. In each bile collection period, the unchanged drug I and metabolites II and III were determined individually by analytical HPLC and expressed as percentages of the total administered dose.

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Received June 18, 1984/Accepted October 31, 1984