Biochemical Pharmacology, Vol. 34, No. 3, pp. 427–428, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

METABOLISM OF THE ANTITUMOR DRUG N^2-METHYL-9-HYDROXYELLIPTICINIUM ACETATE IN ISOLATED RAT KIDNEY CELLS

M. Maftouh, Y. Amiar and C. Picard-Fraire

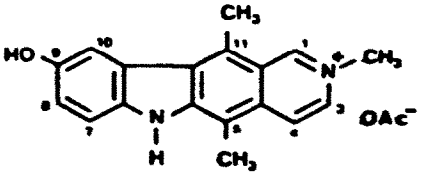
Département de Métabolisme et de Pharmacocinétique, Sanofi Recherche,
195 Route d'Espagne, 31035 Toulouse (France)

Celiptium® (N^2-methyl-9-hydroxyellipticinium acetate, NMHE, see Sch. 1) is an ellipticine derivative currently used in breast cancer (1). Interestingly, antitumor activity against kidney carcinoma, was also reported (2). However, acute renal insufficiency was observed in some of the patients having received a high cumulative dose of the drug (1). These data emphasize that kidneys are targets of the drug.

NMHE was recently shown to be metabolized in vivo via an oxidative activation process into an electrophilic quinone-imine derivative, which was detoxified through sulfhydryl conjugation reactions (3-5) . A similar activation was catalysed in vitro by horseradish peroxidase and the generated quinone-imine alkylate various models of biological nucleophiles such as amino-acids, sulfhydryl derivatives and nucleosides (6). Consequently, it was suggested that biotransformation of NMHE into a reactive metabolite, could be, at least partly, involved in the cytotoxic activity of the drug (3-6).

In addition, there is increasing awareness that nephrotoxicity of a number of drugs can be mediated through the in situ production of reactive and toxic metabolites (7).

These observations led us to consider that antitumor activity at current therapeutic cumulative doses and nephrotoxicity at higher cumulative doses of NMHE, could both rely on the same basic mechanism : intrarenal metabolic drug activation. For this purpose, we have investigated the intrarenal metabolism of NMHE using rat kidney cell preparation.



Sch. 1 : Chemical structure of NMHE

MATERIALS AND METHODS

NMHE was obtained from Sanofi (Sisteron, France). Reference conjugates of NMHE were synthesized as previously described (3) . Collagenase was obtained from Boehringer (Mannheim, GFR).

Kidney cells were isolated using male Sprague-Dawley rats (200-250 g) according to (8) with two minor modifications. Briefly, the collagenase perfusion step was conducted in retrograde direction and in situ.

Incubations were performed at 37°C in siliconized flasks with 10^6 cells/ml and 2 x 10^{-5}M of NMHE, under gentle shaking in a 95 % O_2/5 % CO_2 atmosphere. The incubation medium consisted of Krebs-Henseleit buffer pH 7.4, supplemented with 25 mM of HEPES, 10 % of foetal calf serum and an amino-acide mixture. After 1 hour, 3 ml of methanol were added per ml of incubate to precipitate proteins. The supernatant was used for metabolite analysis.

HPLC analysis was carried out on a Hewlett-Packard 1084 B liquid chromatograph, using a Waters μ-Bondapak C18 column and linear gradient elution from 95 % A/5 % B to 75 % A/25 % B in 7 min, then hold isocratic for 20 min. Solvent A was 5.10^{-2} M ammonium acetate/acetic acid/methanol (79.5/0.5/20) and solvent B methanol. The flow rate was 1.2 ml/min and the UV spectrophotometric detection of NMHE derivatives was monitored at 313 nm.

RESULTS AND DISCUSSION

The results presented in Fig. 1 show the chromatograms of kidney cell incubates with (Fig. 1, middle) and without (Fig. 1, left) NMHE. Four metabolites were identified by comparison with the chromatographic data of synthesized 9-(O)-glucuronide, 10-(S)-glutathione, 10-(S)-cysteine, and 10-(S)-N-acetylcysteine conjugates (Fig. 1, right).

The major metabolite formed during 1 hour incubation was the N-acetylcysteine conjugate. Only small amounts of the glutathione and cysteine conjugates were detected. It was recently reported that a glutathione conjugate of NMHE was present in rat bile, whereas no cysteine or N-acetyl-cysteine conjugates were found (3, 4). By contrast, only the two letter conjugates were recovered in rat and human urines (5). Thus, production of sulfhydryl conjugates after incubation of NMHE with isolated kidney cells from rat, suggests that urinary cysteine and N-acetylcysteine conjugates are cascade metabolites of a glutathione conjugate formed within either liver or kidneys.

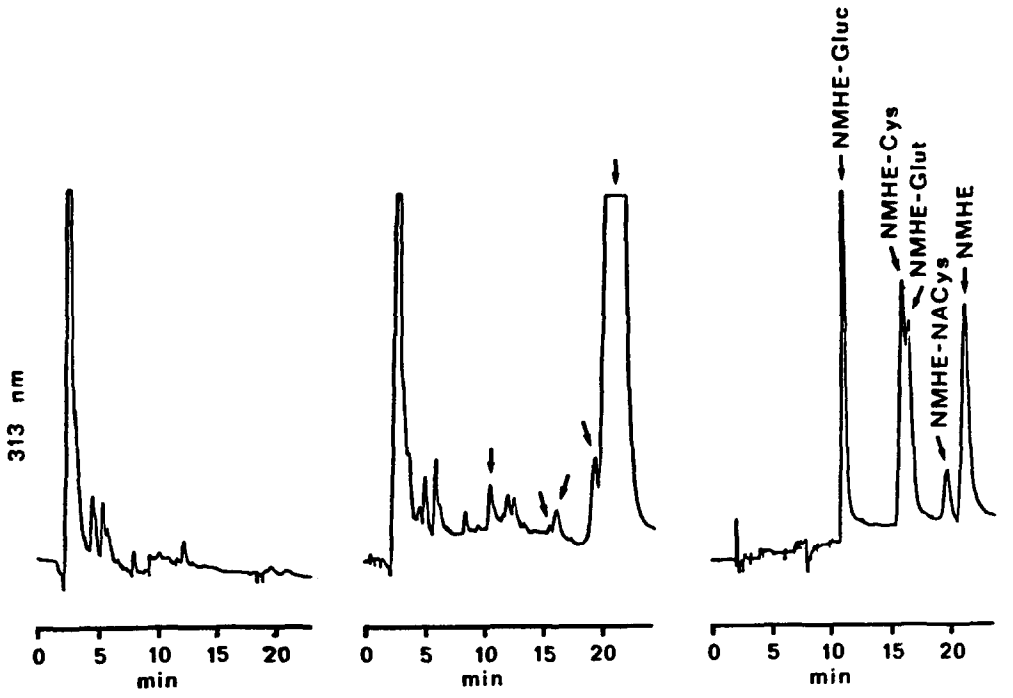


Fig 1 : HPLC chromatograms of rat kidney cell incubates without (left), with (middle) NMHE and of reference compounds : NMHE ; NMHE-Gluc, 9-(O)-glucuronide conjugate ; NMHE-Glut, 10-(S)-glutathione conjugate ; NMHE-Cys, 10-(S)-cys-teine conjugate and NMHE-NACys, 10-(S)-N-acetylcysteine conjugate.

The discovery of NMHE-sylfhydryl conjugates following incubation of NMHE with rat kidney cells, provides evidence that a oxidative activation of the drug into a electrophilic intermediate is possible in kidneys. We suggest that such a reactive intermediate may be involved via an "biooxidative alkylation" process in the observed antitumor activity of NMHE towards kidney tumors (2) . On the other hand, the possibility exists that this reactive metabolite, might be responsible for the few cases of nephrotoxicity previously reported (1) in the patients having received a high cumulative dose of NMHE.

REFERENCES

1 - P. Juret, J.F. Heron, J.E. Couette, T. Delozier and J.Y. Le Talaer, Cancer Treat. Rep., 66, 1909 (1982).
2 - A.L. Harris and D. Spence, Ann. Intern. Med., 95, 125 (1981).
3 - M. Maftouh, G. Meunier, B. Dugué, B. Monsarrat, B. Meunier and C. Paoletti, Xenobiotica, 13, 303 (1983).
4 - M. Maftouh, B. Monsarrat, R.C. Rao, B. Meunier and C. Paoletti, Drug Metab. Disp., 12, 111 (1984).
5 - B. Monsarrat M. Maftouh, G. Meunier, B. Dugué, J. Bernadou, J.P. Armand, C. Picard-Fraire, B. Meunier and C. Paoletti, Biochem. Pharmacol., 32, 3887 (1983).
6 - B. Meunier, C. Auclair, J. Bernadou, G. Meunier, M. Maftouh, S. Cros, B. Monsarrat and C. Paoletti, in "Structure-activity relationships of antitumor agents", Eds, D.N. Reinhoud, T.A. Connors, H.M. Pinedo and K.W. Van de Pool (Martinus Nijhoff, the Hague), pp. 149-181 (1983).
7 - J.R. Mitchell, R.J. Mc Murtry, C.N. Statham and S.D. Nelson, Am. J. Med., 62, 518 (1977).
8 - D.P. Jones, G.B. Sundby, K. Ormstad and S. Orrenius, Biochem. Pharmacol., 28, 929 (1979).