Biochemical Pharmacology, Vol. 25, pp. 379–382. Pergamon Press, 1976. Printed in Great Britain.

COMPARATIVE EFFECT OF PHENOBARBITAL AND METHAQUALONE ON WARFARIN METABOLISM IN THE RAT

ROBERT L. PROCACCINI, PERSHOTAM P. MATHUR, ROBERT D. SMYTH and NELSON H. REAVEY-CANTWELL

Rorer Research Laboratories, Fort Washington, Pa. 19034, U.S.A.

(*Received* 19 *November* 1974; *accepted* 6 *June* 1975)

Abstract—The effect of methaqualone or phenobarbital pretreatment on the metabolism of [^{14}C]warfarin in the rat was studied in order to ascertain to what extent phenobarbital and methaqualone, a non-barbiturate sedative-hypnotic, affect the pattern of warfarin metabolism. A decrease in the plasma concentration of warfarin was observed 5–24 hr after administration of [^{14}C]warfarin to phenobarbital-pretreated animals. Methaqualone pretreatment did not significantly affect the plasma concentration of intact warfarin. Significantly higher levels of metabolites were found in the plasma of phenobarbital-pretreated animals, as compared to either methaqualone-treated or control groups. These data indicate a marked difference in the ability of phenobarbital and methaqualone to alter warfarin metabolism, even though both drugs have been shown to induce hepatic drug metabolism.

It has been established that phenobarbital exerts its effect upon the metabolic profile and anticoagulant activity of warfarin by induction of hepatic microsomal enzymes responsible for the metabolism of the anticoagulant. Phenobarbital treatment at the sedative-hypnotic dose, caused a decrease in the anticoagulant activity of warfarin [1] and bishydroxycoumarin [2] in man. Ikeda *et al.* [3] demonstrated that pretreatment of rats with phenobarbital resulted in an increase in the conversion *in vitro* of warfarin to its hydroxylated metabolites by hepatic microsomal preparations, requiring both NADPH and molecular oxygen.

A recent report presented evidence that methaqualone [2-methyl-3-*o*-tolyl-4(3H)quinazoline], a non-barbiturate sedative-hypnotic [4, 5], stimulates rat hepatic microsomal enzyme activity when administered for 14 consecutive days [6]. However, the drug has also been shown to elevate aminopyrine *N*-demethylase and aniline hydroxylase after a much shorter pretreatment time [7].

Other workers [8] reported that subacute administration of methaqualone, phenobarbital and glutethimide lowered the plasma levels of bishydroxycoumarin in rats, with phenobarbital being the most potent and methaqualone the least potent of the group. Recent work in this laboratory has shown that pretreatment of rats with methaqualone for 3 days results in maximal induction of hepatic microsomal enzymes, whereas the hypoprothrombinemic action of warfarin was not affected by this treatment [7]. We, therefore, considered it important to establish the pattern of warfarin metabolism in methaqualone-treated rats and to compare this action to that of phenobarbital.

MATERIALS AND METHODS

Animals. Male Wistar rats, weighing 90–110 g, were obtained from Carworth Farms, New York. The animals were housed in individual metabolism cages during the study and were maintained on a standard laboratory diet.

Drug pretreatment. The animals were divided into groups of four and received the following drugs orally for 3 consecutive days: phenobarbital sodium (Gane's Chemical Works, New York), 75 mg/kg, dissolved in water; methaqualone (William H. Rorer, Inc.), dissolved in PEG-200, 60 mg/kg; or polyethylene glycol-200 (PEG-200, J. T. Baker Chemical Co.), 2·5 ml/kg. Animals then received 50 mg/kg, orally, of [^{14}C]warfarin sodium [3-([α-^{14}C]acetonylbenzyl)-4-hydroxycoumarin, Amersham-Searle] 24 hr after the final dose of the inducing drug. The [^{14}C]warfarin (23·5 mCi/m-mole) was diluted with unlabeled warfarin sodium (Abbott Laboratories), so that warfarin of lower specific activity was administered to animals who were to be sacrificed early after administration of the anticoagulant. Conversely, animals to be sacrificed at later time intervals received warfarin of higher specific activity. The specific activity of the [^{14}C]warfarin preparation used in this study ranged from 0·11 to 0·96 mCi/m-mole, at a constant total warfarin dose of 50 mg/kg. This protocol was necessitated by the limited commercial availability of ^{14}C-labeled warfarin. The animals were sacrificed by nitrogen asphyxiation at various time periods after administration of the anticoagulant.

Analytical procedures. Blood samples were collected by cardiac puncture in heparinized containers. A 50-μl aliquot of the plasma sample was digested in NCS solubilizer (Amersham-Searle), 15 ml of 0·7% PPO/toluene mixture was added,* and the samples were counted in a liquid scintillation counter. All ^{14}C counts were corrected for background and counting efficiency by the external standard-channels ratio procedure.

The extraction of [^{14}C]warfarin from frozen plasma was carried out essentially according to Lewis *et al.* [9]. Approximately 300–400 μl of recentrifuged

* PPO = 2,5-diphenyloxazole.

BCP 25/4—B

plasma was pipetted into 2 ml of distilled water, and the samples were acidified by the addition of 1 ml of 3 N HCl. The acidified samples were mixed on a Vortex mixer for 10 sec prior to the addition of 20 ml ethylene dichloride (EDC; Baker Chemical). The samples were extracted by mechanical shaking for 30 min, and then centrifuged for 10 min at 3500 g at room temperature.

The entire aqueous phase was transferred into a fresh series of tubes. 10 ml diethyl ether was added, and the samples were re-extracted for an additional 18–24 hr. Use of both EDC and ether in the extraction procedure resulted in higher ^{14}C recoveries (> 90 per cent) than with EDC alone. The EDC and ether extracts were combined and transferred to conical glass tubes and evaporated to dryness at 55°. The residue was dissolved in exactly 20 μl EDC and 10–15 μl was spotted on a Silica gel GF thin-layer chromatography (t.l.c.) plate (Analtech, 20 × 20 in; 250 μm). The plates were developed in EDC–acetone (4:1) to a height of approximately 14 cm (about 35 min). Authentic samples of various warfarin metabolites (6-hydroxy, 7-hydroxy warfarin, warfarin alcohols [3-(α-propane-2-ol) benzyl-4-hydroxycoumarin], or warfarin, chromatographed along with the plasma extracts, were employed as an internal control to check the separation of warfarin from its metabolites in the system. In addition, [^{14}C]warfarin was added to a sample of control plasma and carried through the extraction procedure in order to account for any alterations occurring during the extraction procedure.

After development, the plates were air-dried and examined under shortwave ultraviolet light in order to locate the position of warfarin and its metabolites. The plates were then scanned with a Berthold radioscanner equipped with a Varian ratemeter-integrator (Varian Aerograph, Walnut Creek, Calif.), at a counting efficiency of approximately 22%. This system measures net radioactivity of each radioactive locus on the t.l.c. plates. Two peaks of radioactivity were resolved, one peak corresponding to intact warfarin (R_f 0·53) and a second peak representing total warfarin metabolites (R_f 0·42). The total amount of radioactivity which had migrated on the plate and the net radioactivity under the locus corresponding to unchanged warfarin or warfarin metabolites were determined. The data are expressed as the concentration (nmoles/ml of plasma) of unmetabolized warfarin or per cent of total radioactivity as metabolites. Student's t-test was used for the determination of significant differences between means [10].

RESULTS

Following oral administration of [^{14}C]warfarin sodium, plasma levels of the unmetabolized drug rose to apparent maximal concentrations approximately 3 hr after drug administration in all three groups (Fig. 1). Maximal plasma warfarin concentrations, as determined by t.l.c. radiochromatography for control (PEG-200), phenobarbital and methaqualone-pretreated groups, were: 369 ± 27, 222 ± 4, and 349 ± 2 nmoles/ml respectively.

These data indicate that the maximal plasma warfarin concentration in phenobarbital-pretreated animals was significantly less than in either control or

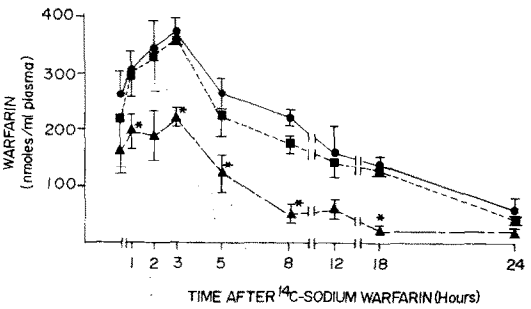

Fig. 1. Effect of methaqualone and phenobarbital pretreatment on plasma warfarin concentration in the rat. Each point represents the mean ± S. E. M. from three to four animals. The animals were pretreated for 3 days with oral doses of (●) PEG-200, 2·5 ml/kg; (■) methaqualone, 60 mg/kg; or (▲) phenobarbital, 75 mg/kg. [^{14}C]warfarin was administered orally 24 hr after the last dose of the test drug. The asterisk (*) indicates $P \leq 0{\cdot}05$ compared to PEG-200.

methaqualone-pretreated animals. In contrast, warfarin levels in the methaqualone-pretreated animals were similar to those observed in control animals during the initial 24 hr after warfarin administration. In order to determine whether this difference was due to an alteration in warfarin absorption [11], the amount of radioactivity remaining in the gastrointestinal tract was analyzed. The amount of radioactivity recovered after 2 hr from the gastrointestinal tract was 4·6 ± 1·0, 9·2 ± 0·8 and 6·5 ± 1·5 per cent of the total dose of [^{14}C]warfarin administered to control, phenobarbital and methaqualone-pretreated animals respectively. These data demonstrate that warfarin absorption was essentially complete in all groups at 2 hr post-drug. Therefore, the lower plasma levels of [^{14}C]warfarin in phenobarbital-pretreated animals seen at this time (Fig. 1) were not simply a reflection of decreased warfarin absorption.

The effect of drug pretreatment on the relative proportion of metabolized warfarin present in plasma is presented in Fig. 2. The relative concentration of metabolized warfarin in the plasma of control rats did not exceed 20% of the total plasma radioactivity throughout the initial 24 hr after administration of

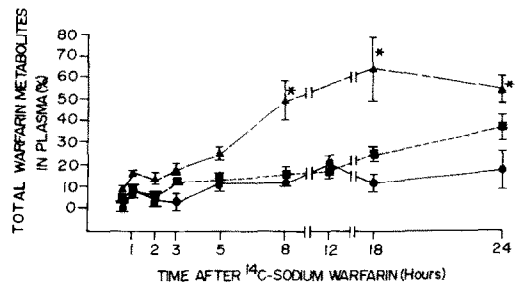

Fig. 2. Effect of methaqualone and phenobarbital pretreatment on plasma warfarin metabolites in the rat. The results are expressed as per cent of total ^{14}C present in plasma as warfarin metabolites. Each point represents the mean ± S. E. M. from three to four animals. The animals were pretreated for 3 days with oral doses of (●) PEG-200, 2·5 ml/kg; (■) methaqualone, 60 mg/kg; or (▲) phenobarbital, 75 mg/kg. [^{14}C]warfarin was administered orally, 24 hr after the last dose of test drug. The asterisk (*) indicates $P \leq 0{\cdot}05$ compared to PEG-200.

the anticoagulant. The temporal pattern of metabolized warfarin in the plasma of methaqualone-pretreated rats closely paralleled that of the control group for the initial 18 hr after warfarin. In contrast, approximately 50 per cent of the total radioactivity found in the plasma of phenobarbital-pretreated rats was in the form of warfarin metabolites as early as 8 hr after warfarin administration.

DISCUSSION

The ability of several sedative-hypnotic drugs to alter the metabolism of the anticoagulant drugs, bishydroxycoumarin and warfarin, is well documented [12–16]. However, little is known of the effects of methaqualone on the metabolism of these agents. This is of special importance since methaqualone has been used as a sedative-hypnotic in geriatric patients [17, 18], many of whom may also be on chronic anticoagulant therapy. The elevation in the rate of conversion of warfarin to its hydroxy metabolites by pretreatment with phenobarbital leads to a reduction in warfarin plasma levels and subsequent diminution in the hypoprothrombinemic response. The hydroxy metabolites of warfarin are relatively inactive except for the 4′-hydroxy derivative, which is one-fourth as active as warfarin [19]. Therefore, any agent stimulating the conversion of warfarin to its metabolites will necessarily decrease both the intensity and duration of its anticoagulant activity.

In our previous studies, three daily oral doses of 60 mg/kg of methaqualone had no effect upon the hypoprothrombinemic action of warfarin [20]. However, pretreatment with phenobarbital, 75 mg/kg, significantly decreased the prothrombin time in warfarin-treated rats from 73 ± 4·4 sec in controls to 32 ± 2·5 sec, as compared to 75 ± 2·7 sec in methaqualone-pretreated animals.

It should be emphasized that maximal levels of hepatic microsomal enzyme induction are attained after only 3 days of treatment with either methaqualone [7] or phenobarbital [21]. In a recent study [6], pretreatment of rats with 100 mg/kg of methaqualone, given orally for 14 days, resulted in an induction response which was less than that observed after only 3 days of pretreatment at a 60 mg/kg dose.

The levels of intact warfarin in control and methaqualone-pretreated animals throughout the initial 24 hr after warfarin administration were found to be equivalent. In addition, peak plasma levels of the anticoagulant 3 hr after warfarin administration are quite similar in control and methaqualone-pretreated animals. The finding that the peak plasma levels of warfarin in the phenobarbital-pretreated groups were much lower than in either control or methaqualone groups (Fig. 1) is attributed to the relatively high rate of metabolism of the anticoagulant. This was also confirmed by the presence of significantly higher levels of [^{14}C]warfarin metabolites in the plasma of phenobarbital-treated rats as early as 5 hr after warfarin administration (Fig. 2).

The difference in the effect of these two microsomal enzyme inducers on warfarin metabolism may be explained in terms of their specificity or potency as inducers of hepatic drug-metabolizing enzymes. Although phenobarbital and methaqualone both induce hepatic microsomal enzymes, this does not imply that they induce the same spectrum of enzymes. The pertinent literature involving differences between the two classic inducers, phenobarbital and 3-methylcholanthrene (3-MC), will elucidate this point. Phenobarbital stimulates a greater variety of hepatic enzymes than does 3-methylcholanthrene, and certain enzymes which are not affected by one type of inducer are stimulated by the other [21]. In addition, the enzymes induced by both phenobarbital and 3-methylcholanthrene sometimes differ in their sensitivity to SKF-525A inhibition [22]. Therefore, the results of our study might be explained by assuming that phenobarbital, but not methaqualone, stimulates the enzyme(s) responsible for the metabolism of warfarin. An alternative explanation is simply that methaqualone is a much less potent inducer of microsomal enzymes than is phenobarbital, and hence does not interfere with warfarin metabolism to nearly the same degree as does phenobarbital. In either case, this study and our previous finding that methaqualone had no effect on prothrombin times in warfarin-treated rats strongly suggest that methaqualone does not affect warfarin metabolism in rats.

A recent clinical report lends further support to our findings in rats. Udall [23] measured prothrombin times for 4 weeks in human volunteers after a 4-week daily treatment with either phenobarbital, secobarbital, chloral hydrate, glutethimide or methaqualone. The patients were maintained on a constant dose of warfarin throughout a 12-week test period. Of the drugs tested, only chloral hydrate (0·5 g/day) and methaqualone (0·3 g/day) did not significantly affect prothrombin times either during or after the drug treatment period. In contrast, phenobarbital (100 mg/day) significantly depressed prothrombin times in warfarin-treated patients, thereby interfering with anticoagulant therapy.

Acknowledgements—The authors wish to thank Dr. William Trager, University of Washington, for his gift of warfarin alcohols; Dr. Collin Schroeder, Wisconsin Alumni Research Foundation, Inc., for 6-hydroxy and 7-hydroxy warfarin; and Dr. A. O. Geiszler, Abbott Laboratories, Chicago, for warfarin. We also wish to thank Dr. Allyn F. DeLong, William H. Rorer, Inc., for his technical guidance.

REFERENCES

1. D. S. Robinson and M. G. MacDonald, *J. Pharmac. exp. Ther.* **153**, 250 (1966).
2. S. A. Cucinell, A. H. Conney, M. Sansur and J. J. Burns, *Clin. Pharmac. Ther.* **6**, 420 (1965).
3. M. Ikeda, A. H. Conney and J. J. Burns, *J. Pharmac. exp. Ther.* **62**, 338 (1968).
4. Y. Duchastel, *Can. med. Ass. J.* **86**, 586 (1962).
5. A. Saulairac and C. Gottesmann, *Life Sci.* **6**, 1229 (1967).
6. D. S. Platt and B. L. Cockrill, *Biochem. Pharmac.* **18**, 459 (1969).
7. P. P. Mathur, R. D. Smyth, T. Herczeg, J. A. Boren and N. H. Reavey-Cantwell, *Pharmacologist* **16**, 240 (1974).
8. J. Zaroslinski, R. Brown and I. Possley, *Archs int. Pharmacodyn. Thér.* **195**, 185 (1972).
9. R. J. Lewis, I. P. Ilnicki and M. Carlstrom, *Biochem. Med.* **4**, 376 (1970).

10. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th Edn. p. 59. Iowa State University Press, Ames (1967).
11. R. A. O'Reilly and P. M. Aggeler, *Pharmac. Rev.* **22**, 35 (1970).
12. P. G. Dayton, Y. Tarcan, T. Chenkin and M. Weiner, *J. clin. Invest.* **40**, 1797 (1961).
13. M. Corn and J. F. Rockett, *Med. Ann. Distr. Columbia* **34**, 578 (1965).
14. S. A. Cucinell, I. Odessky, M. Weiss and P. G. Dayton, *J. Am. med. Ass.* **197**, 366 (1966).
15. J. Koch-Wesser and E. M. Sellers, *New Engl. J. Med.* **285**, 487 (1971).
16. R. P. Beliles and G. U. Foster, *Toxic. appl. Pharmac.* **27**, 225 (1974).
17. H. A. Cromwell, *J. Am. geriat. Soc.* **16**, 814 (1968).
18. C. H. Kramer, *J. Am. geriat. Soc.* **15**, 455 (1967).
19. J. N. Eble, *Ph. D. Thesis*, University of Wisconsin (1953).
20. P. P. Mathur, R. D. Smyth, R. L. Procaccini and N. H. Reavey-Cantwell, *Clin. Res.* **22**, 688A (1974).
21. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
22. N. E. Sladek and G. J. Mannering, *Fedn Proc.* **25**, 418 (1966).
23. J. A. Udall, *Am. J. Cardiol.* **35**, 67 (1975).