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METABOLISM OF A POTENTIAL 8-AMINOQUINOLINE ANTILEISHMANIAL DRUG IN RAT LIVER MICROSOMES*

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Abstract—The metabolism of the 8-aminoquinoline, 8-(6-diethylaminohexylamino)-6-methoxy-lepidine dihydrochloride (WR 6026·2HCl), was studied in a rat hepatic microsomal system. The results show that WR 6026·2HCl was metabolized into two more polar compounds. The structures of these metabolites as proven by gas chromatography-mass spectrometry, ultraviolet absorption, and high performance liquid chromatography were: 8-(6-ethylaminohexylamino)-6-methoxy-lepidine (metabolite 1) and 8-(6-diethylaminohexylamino)-6-methoxy-4-hydroxymethyl quinoline (metabolite 2). The formation of both metabolites was NADPH dependent and also linearly dependent on incubation time and microsomal protein concentration at 0.24 mM WR 6026·2HCl. Studies on the effects of pretreatment of animals with either phenobarbital or Aroclor 1254 suggest that cytochrome P-450 isozymes catalyzed both N-deethylation and hydroxylation reactions. N-deethylase activity was induced by either pretreatment; however, hydroxylase activity was unaffected by phenobarbital pretreatment and significantly elevated by Aroclor 1254 pretreatment. These results suggest that these two reactions are catalyzed by different cytochrome P-450 isozymes. The formation of these two metabolites *in vivo* may play an important role in the antileishmanial activity of WR 6026·2HCl.

The leishmaniases are parasitic diseases affecting perhaps 100,000,000 people in the tropical and semi-tropical world [1]. The major forms of human disease are papular ulcerative cutaneous disease (cutaneous disease), erosive oral, nasal or pharyngeal disease (mucocutaneous disease), or hepatosplenomegaly (visceral disease). The leishmaniases result from infection of the macrophages of the respective organs by the amastigote form of the parasite. The leishmaniases are initially treated with pentavalent antimonials such as sodium stibogluconate [Pentostam] (Burroughs Wellcome) or *N*-methylglucamine [Glucantime (Rhodia)] [2–4]. The 10–25% of cases that are antimony-resistant [4] are treated with more or higher doses of antimony, with pentamidine or with amphotericin B [2–4]. These compounds are parenterally administered and have low therapeutic indices.

The Walter Reed Army Institute of Research has screened more than three thousand compounds as possible antileishmanial drugs against visceral leishmaniasis in hamsters [5] and found that a number of 8-aminoquinoline derivatives were very active against *Leishmania donovani*. The most active compound which has been tested thus far is 8-(6-diethylaminohexylamino)-6-methoxy-lepidine dihydrochloride (WR 6026·2HCl) whose structure is shown in Fig. 1 (4-methylquinoline is a lepidine). WR 6026·2HCl is 400–700 times more active than

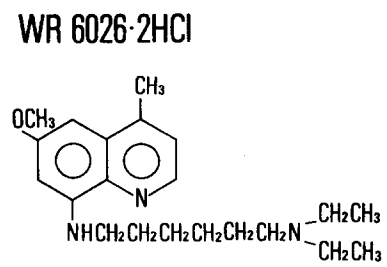

Fig. 1. Structure of WR 6026·2HCl [8-(6-Diethylaminohexylamino)-6-methoxy-lepidine dihydrochloride].

Glucantime in the hamster screening test [6] and is more active by oral administration than by intramuscular administration [6]. The observations that WR 6026 is relatively ineffective against amastigotes within macrophages *in vitro* [7] strongly suggest that WR 6026·2HCl is biotransformed into more potent antileishmanial compounds *in vivo*. WR 6026·2HCl is approximately 200 times more active than the antimalarial primaquine *in vivo* in the hamster suppressive test [7], but only twice as active as primaquine *in vitro* [6]. The potential of WR 6026·2HCl to be an antileishmanial agent effective after oral administration has resulted currently in Phase I clinical pharmacology studies with this drug. Therefore, it is important to understand the role of metabolism in the toxicity and efficacy of this compound and identify the intermediates formed during the excretion of this drug.

This study is the first of a series of studies designed to generate, isolate, and identify metabolic inter-

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mediates of WR 6026·2HCl. In this paper we report on the metabolism of WR 6026·2HCl in an *in vitro* rat liver microsomal preparation. The rat was chosen as a model because there is much information in the literature on the characteristics of drug-metabolizing enzymes in rat liver.

MATERIALS AND METHODS

WR 6026·2HCl AF, bottle number BK01845 (Starks Associates, Inc.), and ring-labeled (position 4 of quinoline structure) [^{14}C]WR 6026·2HCl (sp. act. 26.3 μCi/mg, Research Triangle Institute, Lot CT-3181-127-1) were used in all these studies. WR 225,742AC [8-(aminohexylamino)-6-methoxy lepidine] and WR 250,442AB* [8-(6-diethylaminohexylamino)-6-hydroxy-lepidine dihydrochloride] were obtained from the Walter Reed Army Institute of Research inventory. Other synthetic analogues of WR 6026·2HCl, obtained from the WRAIR inventory, which were utilized for mass spectral interpretation of the metabolites were: WR 6007 [8-(6-diethylaminohexylamino)-6-methoxy-quinoline, phosphate]; WR 242,784 [8-(6-diethylaminohexylamino)-2-hydroxy-6-methoxy-lepidine]; WR 29,595 [8-(6-diethylaminohexylamino)-lepidine, dioxalate]; WR 211,789 [6-methoxy-8-(6-ethylaminohexylamino)-lepidine, dihydrochloride, hemihydrate]; WR 225,742 [8-(6-aminohexylamino)-6-methoxyl-epidine]; WR 223,756 [6-methoxy-8-(5-diethylaminopentylamino)-lepidine, dihydrochloride]; and WR 223,658 [6-methoxy-8-(7-diethylaminoheptylamino)-lepidine, dihydrochloride]. Aroclor 1254 (polychlorinated biphenyls, PCBs) (Monsanto Co., Lot KD08-623) was provided by Dr. David Kupfer, and phenobarbital, sodium salt (PB) was obtained from Abbott Laboratories (Lot G858-0132). D-Glucose-6-phosphate, monosodium salt, glucose-6-phosphate dehydrogenase [EC 1.1.1.49] and NADP were purchased from the Sigma Chemical Co. (St Louis, MO) or from Calbiochem (San Diego, CA).

Animals and pretreatments. Male Sprague–Dawley rats, weighing 100–150 g, were obtained from the Walter Reed Colony. For all experiments animals were fasted for 16 hr before decapitation. In cytochrome P-450 induction experiments, PCBs were injected once i.p. in 0.2 ml corn oil at a dose of 100 mg/kg. Controls received the vehicle only. Animals were killed 7 days later, and incubations were performed with freshly prepared microsomes from individual control and PCB-treated animals. PB in water was injected i.p (75 mg/kg) in two divided doses daily for 4 days. Control animals received the vehicle 0.2 ml H_2O). Animals were killed 16 hr after the last dose, and the experiment was performed with freshly prepared microsomes.

Microsomal preparation. Liver microsomes were prepared by the method of Kupfer and Burstein [8]. The resulting microsomal pellet was resuspended in 1.15% KCl and centrifuged again at 105,000 *g*. The supernatant fraction was discarded, and the microsomes were resuspended in 1.15% KCl for use. In some initial experiments, the microsomal pellet was stored under a layer of 1.15% KCl at −70°; however, it was found that using frozen microsomes for incubation of WR 6026·2HCl dramatically decreased the rates of reaction. Thus, all results reported in this paper were obtained using freshly prepared microsomes. Unless stated otherwise, experiments were performed with microsomes which represented an equal mixture of tissue from six animals. This was accomplished by mixing an equal volume of 10,000 *g* supernatant fraction from each animal before the 105,000 *g* centrifugation. Protein concentrations were determined by the method of Lowry *et al* [9] as modified by Stauffer [10], using bovine serum albumin as a standard.

Microsomal incubation of WR 6026·2HCl. A final volume of 1 ml incubation medium contained; sodium phosphate buffer (pH 7.4, 50 mM), $MgCl_2$ (10 mM), EDTA (10 mM), KCl (45 mM), microsomal preparation (0.25 to 1.8 mg), [^{14}C]WR 6026·2HCl (0.5 to 2.0 μCi) and WR 6026·2HCl (0.02 to 0.75 mM). WR 6026·2HCl was added to incubation vials as a solution in isopropanol after which the isopropanol was evaporated using a stream of nitrogen. An aqueous solution containing sodium phosphate, KCl, $MgCl_2$, and EDTA was then added to the vial followed by microsomal suspension. The reaction was started by adding the NADPH-generating system consisting of glucose-6-phosphate (38.4 mM), NADP (1.3 mM), and glucose-6-phosphate dehydrogenase (3 I.U.) after a 3-min preincubation. The incubations were carried out by shaking in a Dubnoff incubator at 37°, in an atmosphere of air.

The reaction was terminated by the addition of 5 ml ethanol. This solution was then transferred to a capped extraction tube. The incubation vial was then washed with another 5 ml ethanol to remove any residual WR 6026 and metabolites, and this solution was then pooled with the first ethanolic solution. The solution was then centrifuged at 2000 rpm for 10 min to precipitate denatured protein. The supernatant fraction was transferred to another extraction tube, and the ethanol was evaporated overnight at room temperature under a stream of nitrogen. The dried residue containing WR 6026 and metabolites was dissolved in 2 ml of 0.1 M Tris buffer (pH 7.5). This solution was extracted with 3×4 ml of dichloroethane/isopropanol (3/1). The pooled organic extracts were evaporated to dryness at room temperature under a stream of nitrogen. The dry residue was then dissolved in methanol and subjected to high performance liquid chromatographic (HPLC) analysis. The overall recovery of radioactivity was $95 \pm 5\%$.

Detection and quantitation of WR 6026 *metabolites.* HPLC was utilized to assay the incubation extract for metabolites of WR 6026. Quantitation of metabolite formation was performed by utilizing previously constructed standard curves of peak area vs nmoles WR 6026 injected. Since the incubations were run with a known amount of radioactivity, equal aliquots of the solution containing incubation extract injected into the HPLC were taken and counted in a liquid scintillation spectrometer to determine the exact percentage of the sample injected. The total metabolism per sample could

* Unpublished synthesis, Dr. John Scovill, Walter Reed Army Institute of Research.

then be calculated by relating the nmoles metabolite observed on the HPLC to the percentage of the sample injected. To verify that the standard curve with WR 6026 did not change from experiment to experiment, the validity of the curve was checked by injecting known amounts of WR 6026 AF.

HPLC. Separation and quantitation of microsomal metabolites was performed on a Waters liquid chromatograph equipped with: models M-6000 and M-45 solvent delivery systems, model 440 dual channel UV detector, model 660 solvent programmer, Spectraphysics Minigrator, and a model U6K injector. Five micron Spherisorb cyano (4.6 × 250 mm or 10.0 × 250 mm) and 5 micron Spherisorb silica (4.6 × 250 mm) HPLC columns were purchased from the Thomson Instrument Co. (Newark, DE). Methanol and acetonitrile for HPLC and dichloroethane and isopropanol for extraction were purchased from Burdick & Jackson (Muskegon, MI).

Reverse phase chromatography was used to separate, quantitate and isolate all samples. Twenty percent acetonitrile (CH_3CN)/0.01 M ammonium phosphate (pH 3.0) at a flow rate of 1.5 ml/min was used as mobile phase for the 4.6 mm analytical cyano column. For semi-preparative isolation the same mobile phase was used at a flow rate of 5.0 ml/min with the 10 mm cyano column. Thirty percent CH_3CN/0.01 ammonium phosphate (pH 3.0) was used for further purification of some metabolites with the silica column. This system was run at 1.5 ml/min. Column elution was monitored by u.v. at 254 nm. In some cases the eluate was collected by use of a Gilson fraction collector and fractions were assayed for radioactivity in a Packard 460 scintillation counter using Aquasol (New England Nuclear) as the scintillation fluid. Comparison of the ratios of absorbance at 254 nm to radioactivity from WR 6026, metabolite 1 and metabolite 2, indicated that there were no statistically significant changes. These findings demonstrated that quantitation of metabolite formation by u.v. absorption at 254 nm is accurate.

Gas chromatography-mass spectrometry (GC-MS). Chemical ionization GC-MS was performed using a Finnigan 9500 gas chromatograph coupled to a Finnigan 3100 mass spectrometer equipped with an Incos data system and a Finnigan solid probe temperature programmer using methane as reagent gas. A 3 ft × 2 mm i.d. column packed with Supelco Sp2250 DB was used for GC runs at a flow rate of 25 ml/min using methane as both carrier and reagent gas. The metabolites were eluted from the GC column by using a linear temperature gradient from 120° to 270° at 12°/min. Injector temperature was 280°, and transfer line was at 260°. The mass spectrometer was operated at 70 eV and source temperature was either 100° or 180°. Computerized background subtraction was routinely used.

Electron impact mass spectrometry was also performed on a Finnigan 3100 mass spectrometer at 70 eV, and the solid probe was used for sample introduction for both synthetic compounds and isolated metabolites.

Structure identification by mass spectrometry. Incubation extracts were pooled, evaporated to dryness under a stream of nitrogen, and reconstituted in a small volume of methanol. Metabolites 1 and 2 were isolated by collecting fractions from the HPLC. The organic aqueous mixture containing the metabolites was then adjusted to pH 7.5 with 0.1 M Tris, pH 7.5, and extracted with 4 × 2 volumes of dichloroethane/isopropranol (3/1). The organic extract was evaporated to dryness under nitrogen. The dry residue was dissolved in a small volume of methanol, and a 10-μl aliquot of the solution was added to a solid probe capillary tube. The methanol was evaporated by heating the tube at approximately 40° in an oven, and the residue was subjected to MS. The sample was volatalized by running a linear temperature gradient from 30° to 300° at 60°/min. Mass spectra of authentic WR 6026 and other synthetic analogues were obtained in a similar manner.

For GC-MS, an aliquot of pooled extract was evaporated to dryness. Acetonitrile (100 μl) was added to the residue followed by *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (25 μl). The solution was heated to 60° for approximately 0.5 hr to form the trimethylsilyl derivatives. An aliquot of this mixture was then injected into the gas chromatograph.

RESULTS

Initial experiments focused on the development of appropriate enzymatic, extraction, and chromatographic conditions to monitor the microsomal metabolism of WR 6026·2HCl and to determine which peaks in a chromatogram were metabolites. This was accomplished by running control incubations in parallel with complete system incubations. Incubations, run in the absence of NADPH-generating system or in the presence of generating system and in the absence of microsomes, revealed that two peaks were present in the complete system incubations which were not present in the control incubations. Figure 2 illustrates HPLC chromatograms of incubation extracts from reactions both in the presence (Fig. 2B) and absence (Fig. 2A) of NADPH-generating system. A more polar major metabolite (#1) and an even more polar minor metabolite (#2) were formed in the presence of NADPH and microsomes. A chromatogram similar to that illustrated in Fig. 2A was also obtained with an extract from an incubation in which only microsomes were omitted from the complete system. Collection of fractions from the HPLC and assay for radioactivity in a liquid scintillation counter indicated that these compounds were derived from [^{14}C]WR 6026·2HCl.

To verify the enzymatic nature of the reactions, experiments were designed to gain information on the kinetic parameters of the reactions. A representative example of such an experiment is described in Table 1 for metabolite 1 formation. Increasing the WR 6026·2HCl concentration from 0.12 to 0.24 mM markedly increased the velocity of the reaction. However, a further increase in substrate concentration did not significantly enhance the rate of reaction. This observation, confirmed by other experiments, suggested that saturating substrate concentration was achieved around 0.24 mM WR 6026·2HCl. It is also apparent from Table 1 that the

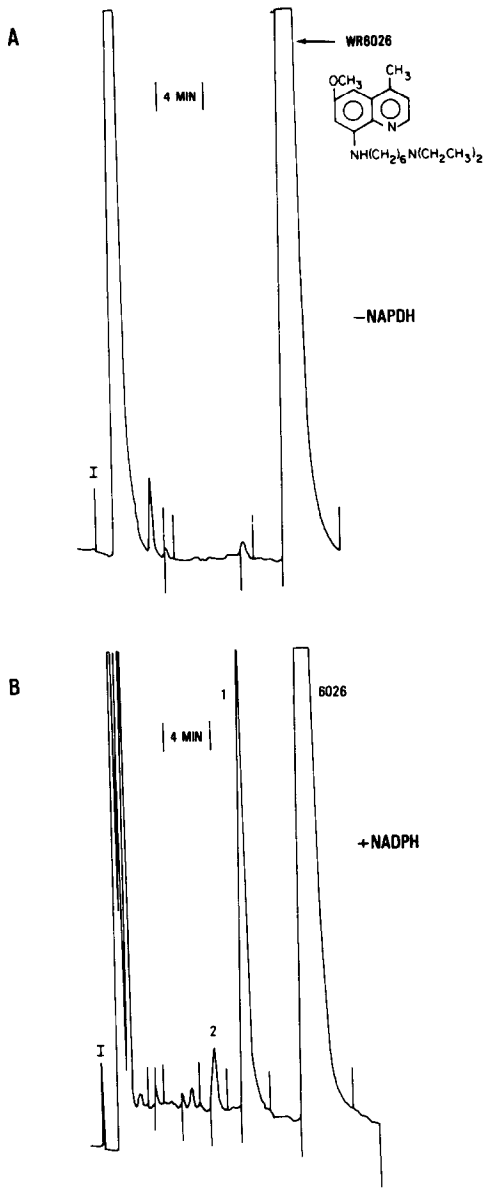


Fig. 2. HPLC chromatograms of rat microsomal extracts. Representative chromatograms of extracts from incubation of WR 6026·2HCl with rat liver microsomes. (A) Control incubation run in the absence of NADPH-generating system. (B) Complete system incubation extract. Metabolites are numbered 1 and 2 in order of increasing polarity. I: injection point. Conditions of HPLC: 4.6 × 250 mm 5 μm Spherisorb-cyano column; 20% CH_3CN/0.01 M ammonium phosphate (pH 3.0); flow rate, 1.5 ml/min; u.v. detection at 254 nm.

reaction was not linear from 30 to 60 min incubation time when the protein concentration was 1.6 mg/incubation.

Experiments characterizing the ranges of linearity of metabolite formation with increasing incubation time and increasing protein concentration are illustrated in Figs 3 and 4. Figure 3 shows the dependence of incubation time on the formation of metabolites 1 and 2. The formation of both metabolites was linearly dependent on incubation time with the rate

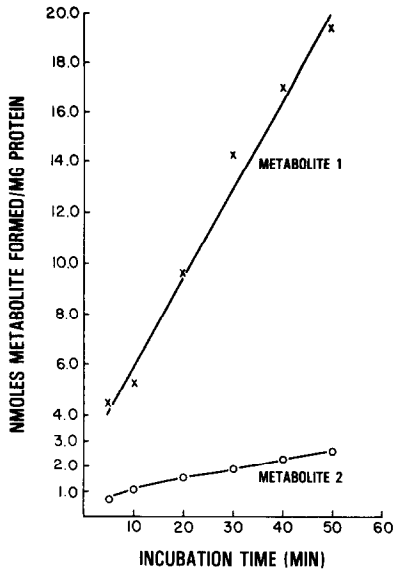


Fig. 3. Time course of formation of metabolites. Plot of formation of metabolites 1 and 2 with increasing incubation time. Values represent the mean of duplicate incubations with 1.0 mg microsomal protein/incubation. Microsomes were derived from a mixture of six animals. Conditions of HPLC were as in Fig. 2.

of metabolite 1 formation approximately nine times faster than that of metabolite 2 formation. Figure 4 illustrates the dependence of metabolite formation on microsomal protein concentration and shows that these two reactions were linearly dependent on microsomal protein added to the incubation medium.

The dependence of the formation of these two metabolites on substrate concentration, incubation time, and microsomal protein concentration proves that these reactions were enzymatic and that under

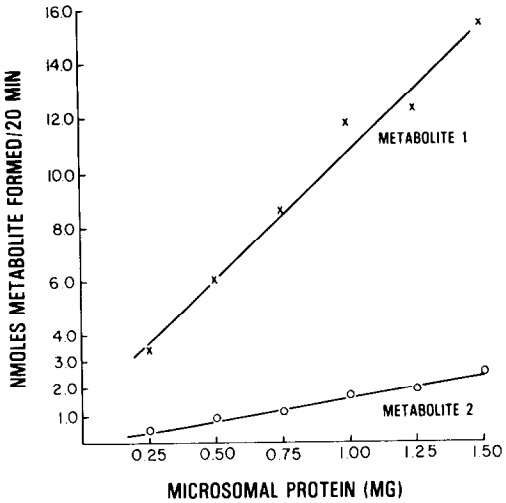


Fig. 4. Effects of increasing microsomal protein on formation of metabolites. Plot of linearity of metabolite formation with increasing microsomal protein. Values represent the mean of duplicate incubations run for 20 min. Microsomes were derived from a mixture of six animals. Conditions of HPLC were as in Fig. 2.

Table 1. Effects of WR 6026·2HCl concentration and incubation time on the formation of metabolite 1*

Incubation time (min)	6026 conc (mM)	(nmoles metabolite/mg protein)
60	0.12	15.4
60	0.24	22.3
60	0.48	24.5
30	0.24	13.8

* Values represent the mean of duplicate incubations. Each incubation contained 1.6 mg microsomal protein and represented a mixture of tissue from six animals.

the conditions utilized in these studies (i.e. 0.24 mM WR 6026·2HCl, 1.0 to 1.5 mg microsomal protein, and 10–30 min incubation time), the enzymes catalyzing these reactions were turning over at the observable V_{max}. These incubation conditions were utilized in subsequent experiments to examine the effects of inducers of cytochrome P-450 monooxygenase on the formation of these two metabolites.

Table 2 shows the effects of pretreatment of animals with cytochrome P-450 inducers. Both PB and PCB pretreatment of animals significantly stimulated the formation of metabolite 1, although PCBs were a more potent inducer of this activity. These results suggest that cytochrome P-450 catalyzed the reaction and, also, imply that both PB-inducible isozymes and other PCB-inducible isozymes can catalyze the reaction [11]. The increase in metabolite 2 formation after PCB treatment was statistically significant, whereas metabolite 2 formation was not significantly different in microsomes from PB-pretreated animals. The fact that PB pretreatment induced metabolite 1 formation but had no effect on metabolite 2 formation strongly suggests that different monooxygenases catalyze these two reactions.

Structural identification of metabolites. Figure 5 shows the electron impact (EI) and chemical ionization (CI) mass spectra of WR 6026·2HCl. Characteristic ions are at *m/z* 86, 158, 188, 201, and 269 with molecular ion at *m/z* 343 for the EI spectrum. The CI spectrum has ions at *m/z* 86, 201, and 269 with molecular ion at *m/z* 344 (M + 1) and CI adduct ion at *m/z* 372 (M + 29). Figure 5 also shows the assigned structures of the fragmentations in the spectrum. These assignments were made based on the EI mass spectra of several synthetic analogues of WR 6026 and are summarized in Table 3.

The base peak (*m/z* 86) of WR 6026 is diethylaminomethylene cation and is present in all compounds with a terminal diethylaminoalkyl moiety in the side chain (WR 6026, WR 6007, WR 242,784, WR 29,595, WR 250,442, WR 223,756, and WR 223,658), but is not present in WR 211,789 or WR 225,742 which have ethylaminoalkyl and aminoalkyl side chains respectively. The observation that the base peak of the desethyl derivative (WR 211,789) is 28 a.m.u. less (*m/z* 58; methylene aminoethyl cation) also supports this assignment (Table 3).

The ion at *m/z* 201 is in intact quinoline ring ion with cleavage alpha to the 8-amino group as indicated on Fig. 5. This ion was present in all compounds with identical substitutions as WR 6026 on the quinoline ring structure. Further support for this assignment is based on the fragmentations of WR 6007, WR 242,784, WR 29,595, and WR 250,442. With these compounds *m/z* 201 was not observed, but decreased by 14 a.m.u., increased by 16 a.m.u., decreased by 30 a.m.u. and decreased by 14 a.m.u. for these structures, respectively, in accordance with the substitutions in the quinoline ring structure (Table 3).

The presence of the ion at *m/z* 269 was dependent on both substitution in the quinoline ring structure and on the length of the alkyl side chain. Similar to the ion at *m/z* 201, *m/z* 269 is not present in WR 6007, WR 242,784, WR 29,595, and WR 250,442,

Table 2. Effects of inducers of monooxygenase on the metabolism of WR 6026 on rat liver microsomes*

Treatment	(nmoles/mg protein/20 min) Metabolite 1	Metabolite 2
Control (H_2O)	11.2 ± 1.1	1.4 ± 0.2
	$P \leq 0.005$	$P \geq 0.5$.
PB	18.3 ± 1.6	1.5 ± 0.2
Control (corn oil)	14.8 ± 0.6	1.3 ± 0.1
	$P \leq 0.001$	$P \leq 0.025$
Aroclor 1254	28.7 ± 1.3	1.7 ± 0.1

* Values represent mean ± S.E. of duplicate incubations with freshly prepared microsomes from each animal. There were six animals per group. Pretreatment of animals with PCBs or PB is described in Materials and Methods. Each incubation contained 0.24 mM WR 6026 and 1–2 mg microsomal protein. P = probability that treated values are identical to control values.

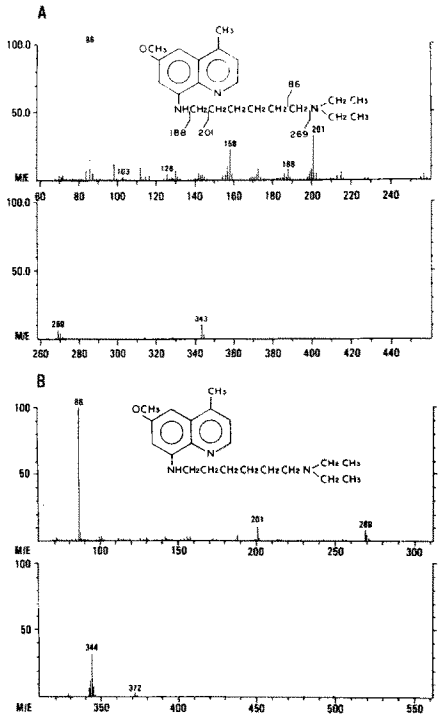


Fig. 5. EI and CI mass spectra of WR 6026·2HCl. Spectra were obtained at 70 eV with solid probe inlet. (A) EI spectrum. (B) CI spectrum: reagent gas, methane; source pressure 1 torr; source temperature, 180°.

but ions at *m/z* 255, 285, 239, and 255 were formed, respectively (Table 3). Also, in compounds with five carbon or seven carbon side chains (WR 223,756, WR 223,658), ions at *m/z* 255 and 283 were formed, which correspond to a 14 a.m.u. decrease or increase when compared to WR 6026 (Table 3). The ion at *m/z* 269 was also present in the spectrum of the desethyl derivative of WR 6026 (WR 211,789). These results prove that the ion at *m/z* 269 in the mass spectrum of WR 6026 arises from intact quinoline ring and side chain with cleavage of the terminal amino group.

The ions at *m/z* 158 and 188 arose from further fragmentation of the quinoline ring system and cleavage of the side chain at the 8-amino group respectively (Table 3). These assignments were made based upon the corresponding fragmentation ions of WR 6007, WR 242,784, WR 211,789, WR 225,742, WR 223,756, and WR 223,658. Compounds with identical substitution on the quinoline moiety had these ions in their spectra; however, alteration of quinoline substituents decreased or increased the masses of these ions in accordance with structural changes on the quinoline system.

Mass spectra of metabolites 1 and 2 were obtained in two ways. First these metabolites were separated and isolated by HPLC, and EI mass spectra were obtained for each isolated metabolite. Second, the microsomal extract was derivatized with BSTFA to form trimethylsilyl (TMS) derivatives. Figure 6A shows the EI mass spectrum of WR 211,789, the desethyl derivative of WR 6026, and Fig. 6B shows

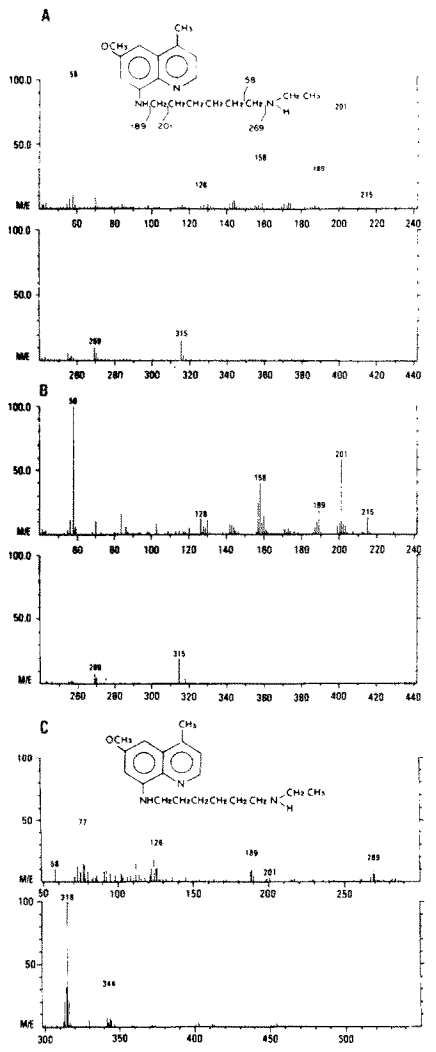
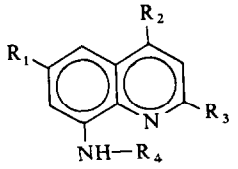


Fig. 6. Mass spectra of authentic WR 211,789 and isolated metabolite 1 from incubation of WR 6026 with rat liver microsomes. (A) EI spectrum of authentic WR 211,789 obtained with solid probe inlet. (B) EI spectrum of isolated metabolite 1 obtained with solid probe inlet. (C) CI mass spectrum of metabolite 1 obtained by silylation and GC-MS. CI conditions were as in Fig. 5.

the EI mass spectrum of the isolated rat metabolite 1. The isolated metabolite 1 spectrum is identical to that of the authentic compound, proving that this metabolite is the desethyl derivative of WR 6026. Figure 6C shows the CI spectrum of the metabolite 1 peak after silylation with BSTFA and GC-MS. A pseudomolecular ion ($M^+ + 1$) at *m/z* 316 and C_2H_5 chemical ionization adduct ion at *m/z 344* ($M^+ + 29$) were present. Other significant ions in the spectrum were present at *m/z* 77, 126, 189, and 269. This spectrum further supports the EI data, indicating that this metabolite was a desethyl derivative of WR 6026. It was surprising that neither the 8-amino nor terminal secondary amines were silylated by BSTFA. Other studies with some of the authentic compounds

Table 3. Electron impact mass spectra of synthetic analogues of WR 6026*



Compound	R_1	R_2	R_3	R_4	Major fragments *m/z* (relative intensity)	Molecular ion *m/z* (relative intensity)
WR 6026	OCH_3	CH_3	H	$(CH_2)_6N(CH_2CH_3)_2$	86 (100), 158 (23), 188 (18), 201 (35), 269 (8)	343 (12)
WR 6007	OCH_3	H	H	$(CH_2)_6N(CH_2CH_3)_2$	86 (100), 98 (10), 112 (10), 144 (22), 174 (3), 187 (38), 255 (2)	329 (7)
WR 242,784	OCH_3	CH_3	OH	$(CH_2)_6N(CH_2CH_3)_2$	86 (100), 98 (12), 112 (12), 174 (8), 204 (2), 217 (16), 285 (4)	359 (7)
WR 29,595	H	CH_3	H	$(CH_2)_6N(CH_2CH_3)_2$	86 (100), 143 (24), 159 (9), 171 (27), 239 (5)	313 (5)
WR 250,442	OH	CH_3	H	$(CH_2)_6N(CH_2CH_3)_2$	86 (100), 112 (10), 158 (6), 187 (16), 255 (5)	329 (8)
WR 211,789	OCH_3	CH_3	H	$(CH_2)_6NHCH_2CH_3$	58 (100), 126 (16), 158 (36), 189 (27), 201 (76), 269 (11)	315 (16)
WR 225,742	OCH_3	CH_3	H	$(CH_2)_6NH_2$	130 (28), 143 (24), 158 (58), 189 (52), 201 (100), 257 (5)	287 (56)
WR 223,756	OCH_3	CH_3	H	$(CH_2)_5N(CH_2CH_3)_2$	86 (100), 112 (15), 143 (11), 158 (28), 188 (14), 201 (36), 215 (11), 255 (15)	329 (10)
WR 223,658	OCH_3	CH_3	H	$(CH_2)_7N(CH_2CH_3)_2$	86 (100), 144 (14), 158 (31), 172 (17), 188 (7), 189 (5), 201 (45), 215 (9), 283 (5)	357 (10)

* Chemical names of compounds are listed in Materials and Methods. Solid probe inlet with temperature programming was used to obtain all spectra.

shown in Table 3 also indicated that WR 211,789 was not silylated by BSTFA, nor was WR 6026. WR 225,742, a primary amine, was monosilylated with BSTFA, and the hydroxyl derivatives, WR 242,784 and WR 250,442, were also monosilylated.

The EI mass spectrum of isolated metabolite 2 is shown in Fig. 7A, and the CI mass spectrum of the silylated metabolite is shown in Fig. 7B. The base peak in the EI spectrum is at *m/z* 86, with other prominent ions at *m/z* 217, 285, and 359. The mass spectrum of this compound indicates that metabolite 2 was a hydroxylated derivative of WR 6026. The ions at *m/z* 217 and 285 indicate that the hydroxylation occurred at some position on the quinoline ring system since ions at *m/z* 201 and 269 in the WR 6026 spectrum were not present but had increased by 16 a.m.u. The base peak at *m/z* 86 proves that the diethylaminoalkyl side chain of this metabolite was identical to that of WR 6026.

The CI mass spectrum of the silylated derivative is shown in Fig. 7B. The pseudomolecular at *m/z* 432 ($M^+ + 1$) and CI adduct ion at *m/z* 460 ($M^+ + 29$) are consistent with a hydroxylated monosilylated derivative of WR 6026 since the secondary amines of WR 6026 and analogues were not silylated by BSTFA.

These spectra prove that WR 6026 was hydroxylated on the quinoline ring system, but they give no information on the exact position of hydroxylation. However, other indirect evidence suggests that this metabolite was hydroxylated on the methyl group at C-4. First, this metabolite had an ultraviolet absorption spectrum identical to WR 6026 and metabolite 1. If aromatic hydroxylation had occurred, one would expect a shift in the absorbance maximum to a longer wavelength. (The absorbance maximum for WR 6026, metabolite 1 and metabolite 2 was 265 and 270 nm for WR 242,784, the 2-hydroxyl synthetic analogue of WR 6026.) Furthermore, metabolite 2 was very stable, in contrast to 5- or 7-hydroxylated quinoline derivatives which are easily oxidized and rapidly degrade in solution. Also, metabolite 2 did not co-chromatograph with WR 242,784, the 2-hydroxyl analogue, on HPLC. Second, this metabolite had chromatographic mobility identical to the major metabolite formed from incubation of WR 6026 with hamster liver microsomes.* The hamster metabolite has been shown to be the hydroxymethyl metabolite by mass spectrometric and proton NMR studies and, also, to have u.v. absorption maxima identical to WR 6026 and both rat microsomal metabolites.*

* A. D. Theoharides, A. J. Lin, H. Chung and H. Velazquez, unpublished results.

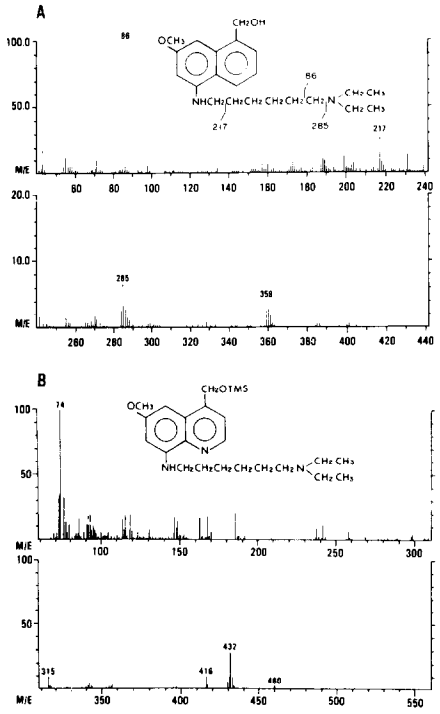


Fig. 7. EI and CI mass spectra of isolated metabolite 2. (A) EI spectrum obtained with solid probe inlet. (B) CI spectrum of silylated derivative and GC-MS. CI conditions were as in Fig. 5.

DISCUSSION

The results of this study prove that the 8-aminoquinoline WR 6026·2HCl was enzymatically deethylated or hydroxylated by rat liver microsomes. The rate of the deethylase pathway was approximately nine times faster than that of the hydroxylase. Studies with inducers of cytochrome P-450 monooxygenase suggest that both reactions were catalyzed by cytochrome P-450 monooxygenase. The observation that PB pretreatment of animals significantly stimulated the formation of metabolite 1 but had no effect on the rate of metabolite 2 formation indicates that these reactions were catalyzed by different cytochrome P-450 isozymes.

Although the mass spectrum of metabolite 2 gives no information as to which position of the molecule is hydroxylated, this metabolite had both a chromatographic mobility by HPLC and an ultraviolet absorption spectrum that were identical to those of the major hydroxylated metabolite formed from incubation of WR 6026·2HCl with hamster liver microsomes [12]. The hamster metabolite was shown to be hydroxylated on the methyl group at position 4 of the quinoline ring by proton NMR studies,* i.e. [4-hydroxymethyl-6-methoxy-8-(6-diethylaminohexylamino]quinoline. Although the quantities of the rat metabolite 2 were insufficient to allow further structural characterization of this compound by NMR, the HPLC chromatographic mobilities of this compound strongly suggest that the rat hydroxylated metabolite 2 was identical to the hydroxymethyl compound formed in hamster liver microsomes.

Studies on the metabolism of WR 6026·HCl in hamster liver microsomes have also shown that N-deethylation is a major enzymatic reaction; however, the rate of 4-hydroxylation is much higher in this species than in the rat [12].

The studies in the rat reported in this paper were performed to develop appropriate analytical and enzymatic methodologies to monitor the microsomal metabolism of this drug. Since the rat is the classical animal used in drug metabolism studies, we felt that it was important to initially study the metabolism of WR 6026·2HCl in this species. We are now using these techniques to study the metabolism of this drug by hepatic microsomes from the hamster, the laboratory host for leishmania species in which the pronounced activity of this drug has been demonstrated.

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