bind to the keratin in equal molar ratios as judged by comparing the amount of protein present (approximately 50 μg) to the amount of drug (approximately 1×10^4 cpm or 10 mmol) which was bound. It would be interesting to discover whether the drug binds primarily in the central core region or principally at the end domains of the keratin filaments. We also are interested in the details of the turnover rate of the drug within the cytoskeletal fraction since the drug binding could represent long term drug binding, i.e. that which is retained after the majority of the drug leaves the cell.

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Effect of chloramphenicol administration *in vivo* on cytochrome P-450-dependent monooxygenase activities in liver microsomes from uninduced male rats

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The cytochrome P-450-dependent monooxygenase system plays a key role in the detoxification and bioactivation of a wide variety of xenobiotics and endogenous compounds [1]. One important means of enhancing or attenuating the *in vivo* effects of compounds that are normally metabolized by cytochromes P-450 is through the use of chemical inhibitors. A common complication, however, is that many classical cytochrome P-450 inhibitors, including isosafrole, allylisopropylacetamide, metyrapone, and SKF-525A, that decrease monooxygenase activity within a few hours after *in vivo* administration, induce cytochromes P-450 after longer times, especially when given in multiple doses [2].

Our own efforts have focused on the inhibition of rat liver cytochromes P-450 by chloramphenicol and analogs. Chloramphenicol inactivates major forms of cytochrome P-450 induced by phenobarbital and pregnenolone-16α-carbonitrile, as well as several important cytochromes P-450 in uninduced rats [3, 4]. Chloramphenicol has also been shown to prevent the toxicity of such compounds as carbon tetrachloride, which require cytochrome P-450-dependent

bioactivation [5]. In our previous *in vivo* studies [3, 4], a single dose of chloramphenicol was administered, and the inhibition of cytochromes P-450 was examined in microsomes prepared from rats killed 1 hr later. To further explore the potential utility of chloramphenicol as a modulator of cytochrome P-450 function, we have now addressed two questions: (1) how long does enzyme inhibition persist after a single dose of chloramphenicol, and (2) do multiple doses of chloramphenicol cause enzyme induction? As substrates for assessing inhibition and/or induction, we have used *R*- and *S*-warfarin, androstenedione, and progesterone.

Experimental procedures

[4-^{14}C]Androstenedione and [4-^{14}C]progesterone were purchased from NEN Research Products (Boston, MA). Unlabeled steroids and steroid metabolites were purchased from the Sigma Chemical Co. (St. Louis, MO) or from Steraloids (Wilton, NH). Androstenedione metabolism was assayed as described previously [3]. A similar method was used to assay progesterone metabolism, except that 50 instead of 25 μg of microsomal protein was used per assay, and the separation of the progesterone metabolites was achieved by developing the thin-layer plates three times in benzene–ethyl acetate–acetone (10:1:1, by vol.). Metabolites were localized by autoradiography and identified by comparison with unlabeled standards. The R_f values of the standards were 16α-OH (0.10), 6β-OH (0.22), 21-OH (0.32), 2α-OH (0.40), and progesterone (0.69). The conversion of *R*- and *S*-warfarin to the corresponding 9,10-dehydro, 4′-OH, 6-OH, 7-OH, 8-OH, and 10-OH metabolites was assayed as described previously [6].

Adult male Sprague–Dawley rats (150–200 g) were used for all experiments, and two experimental protocols were followed. *Protocol 1:* twelve rats were injected i.p. with 300 mg/kg chloramphenicol in 0.5 ml of propylene glycol, and twelve animals received the vehicle only. After 1, 24, and 48 hr four rats per group were killed, and liver microsomes were prepared from each individual animal [7]. The results from the vehicle-treated rats were combined to form a single control group. Statistical analysis of the results of the monooxygenase activities was conducted using a one-way analysis of variance (ANOVA); subsequent comparisons between individual means was achieved using the Newman–Keuls' multiple-range test. Only those results that were statistically significant ($P < 0.05$) are commented on in the text. *Protocol 2:* four rats were injected i.p. with 300 mg/kg chloramphenicol once daily for 3 days, and four animals received vehicle only. Twenty-four hours after the last dose, the animals were killed and liver microsomes were prepared. Statistical analysis of the results of the monooxygenase assays was conducted using Student's *t*-test. Only those results that were statistically significant ($P < 0.05$) are commented on in the text.

Results and discussion

In agreement with our previous results [4], the rate of formation of all six metabolites of both *R*- and *S*-warfarin was decreased in liver microsomes prepared 1 hr after administration of chloramphenicol, the residual activity in percent of control ranging from 0 to 70%. (The greatest extent of inhibition was observed in the case of *S*-10-OH warfarin, the formation of which was undetectable in the microsomes from the chloramphenicol-treated rats, compared to a rate of 0.06 ± 0.01 nmol/min/mg in the controls.) In contrast, the cytochrome P-450 content of the microsomes (0.68 ± 0.02 nmol/mg) was not different from that of the controls (0.76 ± 0.05). The residual steroid hydroxylase activities in percent of control were: progesterone 16α-OH (41%), progesterone 6β-OH (16%), progesterone 21-OH (44%), progesterone 2α-OH (54%), androstenedione 7α-OH (106%), androstenedione 6β-OH (23%), androstenedione 16β-OH (23%) and androstenedione 16α-OH (42%). These results confirm and extend our previous findings in which the inhibition of particular forms of cytochrome P-450 was inferred from decreases in the rate of formation of specific metabolites of warfarin and testosterone. Thus, based on decreases in warfarin *R*-6 and *S*-4′-hydroxylase activity and in testosterone 2α- and 16α-hydroxylase activity, we previously concluded that chloramphenicol inhibits cytochrome P-450 UT-A [4]. The inhibition noted in the present study of progesterone 2α- and 16α-hydroxylation and of androstenedione 16α-hydroxylation is totally consistent with this interpretation [8–11]. Likewise, we previously concluded that chloramphenicol inhibits cytochrome P-450 PB-C (warfarin *R*-7-hydroxylase), and this is consistent [11] with the decrease in progesterone 21-hydroxylase activity. The lack of inhibition by chloramphenicol of androstenedione 7α-hydroxylation is consistent with our previous results in which testosterone 7α-hydroxylase activity was monitored, and indicate that chloramphenicol does not inhibit cytochrome P-450 UT-F. Finally, the decreases noted here in androstenedione and progesterone 6β-hydroxylase activity [8–11] indicate that chloramphenicol inhibits a steroid-inducible cytochrome P-450 [12] that may be identical to P450PCN2 of Gonzalez *et al.* [13].

The time course of recovery of representative monooxygenase activities is illustrated in Fig. 1. Most of the activities, exemplified by *R*-warfarin 7-hydroxylase and androstenedione 6β-hydroxylase activity, exhibited a steady climb towards the control values, whereas four

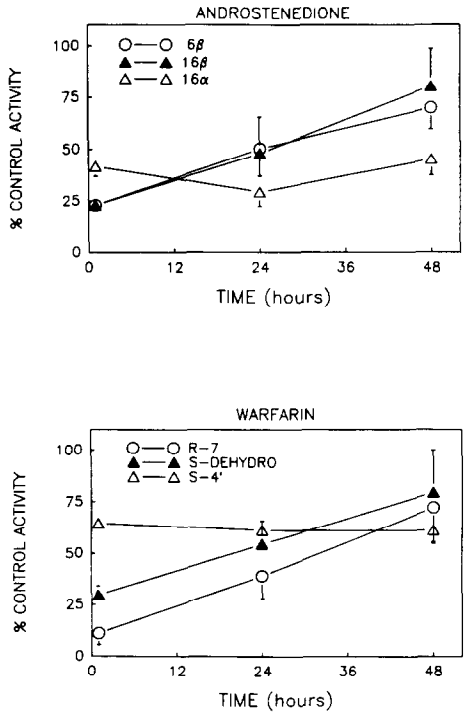

Fig. 1. Selected monooxygenase activities in liver microsomes harvested from rats 1, 24, or 48 hr after a single injection of 300 mg/kg chloramphenicol. Top: androstenedione hydroxylase activities; Bottom: warfarin hydroxylase activities. Details are given in Experimental Procedures. The 100% values expressed as nmol metabolite formed/min/mg protein ± the standard deviation (N = 4) were: androstenedione 6β-OH (2.04 ± 0.32), androstenedione 16β-OH (0.45 ± 0.05), androstenedione 16α-OH (1.62 ± 0.31), warfarin *R*-7-OH (0.18 ± 0.03), *S*-dehydrowarfarin (0.44 ± 0.09), and warfarin *S*-4′-OH (0.31 ± 0.07).

activities indicative of form UT-A (*S*-warfarin 4′-hydroxylase, androstenedione 16α-hydroxylase, and progesterone 2α- and 16α-hydroxylase) showed no evidence of recovery by 48 hr. At 24 hr, all of the activities with the exception of progesterone 6β-hydroxylase that showed inhibition after 1 hr were still lower than the controls. The time courses of recovery of most of the hydroxylase activities are consistent with the known half-lives (12–20 hr) of the relevant cytochromes P-450 in uninduced male rats [14]. This observation suggests that new enzyme synthesis is responsible for the reversal of inhibition. In this light, the lack of recovery of those activities attributed to UT-A is intriguing, since the half-life of this enzyme is similar to that of the other cytochromes P-450 that do recover after chloramphenicol treatment.

The results of the experiments in which multiple doses of chloramphenicol were administered are shown in Fig. 2. As can be seen, there was no evidence of any induction of monooxygenase activity; rather, with the exception of androstenedione 7α-hydroxylase, all the activities in the treated group were lower than the controls, the residual activities largely resembling those observed 24 hr after a single dose of chloramphenicol. There was also no evidence of induction of total cytochrome P-450 (0.58 ± 0.07 nmol/mg in the treated group compared to 0.75 ± 0.16 nmol/mg for the controls).

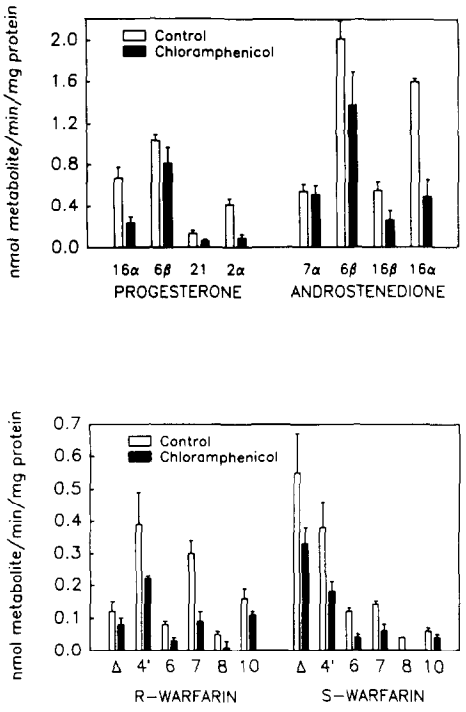

Fig. 2. Effect of chloramphenicol administration once daily for 3 days on monooxygenase activities in liver microsomes harvested from rats 24 hr after the last injection. Top: steroid hydroxylase activities; Bottom: *R*- and *S*-warfarin hydroxylase activities. The open triangle (△) indicates dehydrowarfarin. Details are given in Experimental Procedures. Values are means ± SD, N = 4.

In summary, the results of the present investigation indicate that chloramphenicol may have great utility as an inhibitor of cytochromes P-450 in uninduced rats. A single dose of chloramphenicol inhibited many of the major constitutive forms of cytochrome P-450, and the inhibition was still marked up to 24 hr. Moreover, there was no evidence that chloramphenicol induced any of the monooxygenase activities examined, suggesting that it may be feasible to maintain chronic monooxygenase inhibition by single daily doses of chloramphenicol. For all these reasons, chloramphenicol may have certain advantages compared to the more commonly used cytochrome P-450 inhibitors.

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