Induction of Chloramphenicol Metabolism by Phenobarbital

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Received for publication 4 November 1971

The induction of hepatic microsomal enzymes leading to more rapid metabolism of antibiotics has been demonstrated in only a few prior studies. We have studied the induction of chloramphenicol metabolism in an isolated rat liver perfusion model and in intact dogs. Using these methods, we have demonstrated that the clearance of chloramphenicol from serum is markedly increased after treatment of animals with phenobarbital. This was demonstrated to be a function of increased clearance of the drug by the liver, accompanied but not paralleled by increases in liver weight and biliary excretion by the rat liver. In intact dogs, small doses of chloramphenicol were not sufficient to demonstrate this fact, whereas a large test dose of 100 mg/kg showed markedly enhanced clearance after 1 week of oral phenobarbital administration. Prolongation of phenobarbital treatment or increased dose did not further increase the clearance, and chloramphenicol could not be demonstrated to enhance its own clearance. Human data on induction of enzymes is to date inconclusive.

The induction by one drug of hepatic microsomal enzymes capable of inactivating or metabolizing another occurs frequently. The result can be as much as a 10- to 20-fold increase (2) in the rate at which such compounds may be removed from the circulation. Clinical alterations may be marked, as in the well-documented situation of increased clearance and decreased anticoagulant effectiveness ofbishydroxycoumarin after phenobarbital (11) treatment or in the development of tolerance to alcohol (15) or phenylbutazone by continued use (2). Conney (4), in a 1967 review, noted that more than 200 drugs or chemicals are known to stimulate the activity of drug-metabolizing enzymes in liver microsomes. These include sedatives, anesthetics, anticonvulsants, alcohol, tranquilizers, carcinogens, hypoglycemic agents, analgesics, steroid hormones, and insecticides. In several studies (1, 4, 6, 12), the more rapid serum clearance has been shown to be due to increases in activity or amount of directly measured hepatic microsomal enzymes, primarily of the smooth endoplasmic reticulum, which handle both substrates in an analogous fashion.

Aside from some controversial studies of the interaction of griseofulvin (14) with phenobarbital, there has been only one other demonstration of such enhancing effects on the metabolism of antimicrobial agents. In that in vitro study (13), metabolism of chloramphenicol by smooth and rough endoplasmic reticulum of rabbits was found to be increased seven- and fourfold, respectively, by prior phenobarbital administration. On the other hand, p-aminosalicylic acid has been shown to impede the hepatic metabolism of isoniazid (16), and chloramphenicol has been shown to inhibit the inactivation of a variety of drugs in man (3). In our studies, we wished to find out whether a commonly used inducing drug, phenobarbital, could enhance hepatic clearance of chloramphenicol in whole-organ systems and in intact animals. We also wished to learn whether postinduction differences might be of major magnitude, whether they would be dose-dependent, and finally whether these effects could be demonstrated in humans.

MATERIALS AND METHODS
Chloramphenicol was the antibiotic chosen because of the ease and accuracy with which it can be chemically measured in serum samples and because of its known enzymatic hydrolysis (7) and glucuronidation in the liver (10). The chloramphenicol assay utilizes the modification of the Kakeni (10) technique introduced by Hughes and Diamond (9), which measures only free (or active) chloramphenicol base. The antibiotic is extracted from serum with isopentylacetate, and in turn from this solvent with isonicotinic acid hydrazide and sodium hydroxide. Color development is proportional to concentration, and the reliability of the method is ±5%.
In initial liver perfusion studies, male Lee-Evans rats were used as donors for both blood and liver. They were maintained prior to testing on standard laboratory food in cages without insecticides. Ether anesthesia was used for laparotomy. An isolated rat liver preparation was made in a constant-temperature, oxygenating Lucite chamber with perfusion of the liver via portal vein and outflow from inferior vena cava (5). The livers were in line within 2 min of sacrifice, and bile production was used as a gauge of hepatic viability. Heparinized rat blood diluted to 50% with bicarbonate-buffered saline was used for perfusion, to which was added the test dose of 8 mg of chloramphenicol, giving an initial concentration of 100 to 130 μg/ml. Periodic samples of the perfusate and the entire biliary output were analyzed for chloramphenicol. Results of these experiments are summarized in Fig. 1. The curves are the average of six animals each and are significantly different at the P<0.001 level. Experimental animals had been pretreated for 3 days with daily intraperitoneal injection of 1.5 mg of phenobarbital. This dose did not produce sedation. The clearance of drug by livers of pretreated animals was more than four times as fast as in the control animals, as measured by the half-time of clearance. Although pretreated animals did have somewhat larger livers than controls (mean values of 12.3 versus 10.6 g), these differences were not statistically significant. Bile flow and biliary excretion of chloramphenicol were also slightly increased in the treated animals, but could account for only a small fraction of the total increased drug cleared by the liver. Mean increases were 18 and 25% for bile flow and total measured drug between untreated and treated animals. No attempt was made to extract and measure the activity of liver microsomes.

Having demonstrated the significance of hepatic clearance in the isolated liver preparation, unaccompanied by tissue protein binding or renal excretion, we next used intact dogs. Mongrel dogs weighing between 15 and 18 kg were anesthetized with intravenous pentobarbital, and immediately thereafter were given an intravenous test dose of 100 mg of chloramphenicol succinate/kg of body weight. Blood was sampled via an indwelling venous cannula for the 0.5 hr of the experiment, and urine was collected by catheter. As can be seen in Fig. 2, comparison of this control or initial clearance of chloramphenicol to that obtained in the same animals after 1 week of oral pentobarbital, at a nonhypnotic dose ranging from 60 to 240 mg per day, showed a markedly increased clearance of chloramphenicol. Although only two animals were tested at each dosage, a similar shortening of half-life was seen in all. It should be noted that in these studies every animal acts as his own control; that is, initial nonsimulated clearance was followed by phenobarbital and retest. Comparison of the plasma half-life was statistically significant between initial and 1-week posttreatment clearances at the 0.01 level by t test. However, no differences were apparent between large and small daily doses of phenobarbital. Also, a more prolonged period of phenobarbital administration, that is, 2 weeks versus 1 week, carried out in four of these same dogs showed no further increase in chloramphenicol clearance. Analysis of chloramphenicol in the urine showed that, although present in high concentration, the total quantities in urine were small and correlated poorly with rate of blood clearance.

As a control for possible effects of the pentobarbital anesthetic or the initial chloramphenicol test dose, as enzyme inducers, four dogs were retested after 1 week with no intervening administration of drugs. No change in the chloramphenicol clearance was noted. Thus, chloramphenicol or anesthetic did not induce increased clearance. As an additional control, when chloramphenicol was given at 2 or 4 g per day (two and four animals each) in the interval between initial and final clearance of chloramphenicol, no increase in clearance was seen. Thus, chloramphenicol alone could not be demonstrated to induce its own more rapid clearance. Finally, four dogs given phenobarbital pretreatment alone, without prior chloramphenicol test dose and accompanying anesthesia, gave a rapid clearance comparable to that of another four animals pretreated with chloramphenicol and given phenobarbital. That is, the induction effect occurred with phenobarbital alone, and did not need prior antibiotic or anesthetic usage.

With variation in chloramphenicol dosages, a
marked dose-dependent difference in clearance was seen. Comparison of the clearance of chloramphenicol before and after phenobarbital administration did not show any significant increase in clearance. This presumably demonstrates that mechanisms of the liver for clearing small doses of chloramphenicol are normally extremely rapid and cannot be significantly improved upon for detection by our methods.

Finally, we have studied too few humans to be sure of a comparable result. We elected not to administer chloramphenicol to human volunteers because of the known risks of this drug, but to depend on the use of patients receiving drugs for clinically indicated infections. In two patients studied, contradictory results were obtained, with one patient having slightly increased chloramphenicol clearance after receiving 60 mg of phenobarbital per day for 5 days and the other patient showing an unchanged clearance. The test dose of chloramphenicol in both cases was 1 g given intravenously, which is decidedly less than the test dose given to dogs.

**DISCUSSION**

These findings demonstrate a marked drug interaction between a sedative and an antibiotic. The mechanism for this enhanced removal is presumed to be, by our indirect testing, the induction of hepatic microsomal enzymes capable of increased glucuronidation or hydroxylation. An increase in biliary excretion of several drugs has been shown to result from phenobarbital in rats (11). However, the increases in biliary excretion seen in those studies were only approximately 50% more than control values, rather than the one- to fourfold increase seen here. In addition, in our liver preparation, only a modest increase (18%) in bile flow was noted. Others have shown that increased hepatic weight, as also demonstrated here, may be primarily due to an increase in this cellular subunit located in the smooth endoplasmic reticulum (4, 13). Other possible effects, such as changes in absorption, tissue protein binding, extracellular distribution, or excretion, are essentially ruled out by the intravenous route of administration or by the demonstration of an identical enhancement with an isolated liver perfusion model. Although congruence of hepatic and renal excretory function has been noted for other drugs (5), an increased renal clearance with stimulation could not be demonstrated here. However, our analytic technique measures only the free, biologically active chloramphenicol. The lack of an effect with small doses of chloramphenicol may be analogous to that seen with reticuloendothelial system (RES) function studies where the clearance of small doses merely reflects liver blood flow, and the stress of a large dose is needed to demonstrate RES stimulation. Indeed, some materials such as sulfonamides are known to be taken up and acetylated by the Kupffer cell, and not appreciably by hepatic parenchymal cells (8, 17). Although there is no direct means of translating dosage effects between animal species, it seems likely that the lack of demonstrable induction in humans may reflect this dose phenomenon. It is quite possible, however, that this variation in a very small sample merely reflects the extreme range in drug metabolism known to exist in genetically nonidentical humans (2).

Whether other antibiotics with hepato-biliary excretion may have similar enhancement of their blood clearance by prior or concomitant drug administration awaits to be seen. Indeed, these drugs may induce higher levels of hepatic drug-metabolizing enzymes, and hence clearance may be increased during prolonged use. This induction of self-metabolizing enzymes seems common (1, 2, 15), and the lack of such an effect in our studies with chloramphenicol is unusual. Possibly the known ability of chloramphenicol to inhibit microsomal drug metabolic pathways (3) may impede its own enzymatic destruction.
LITERATURE CITED