Absorption and Transport of Digitoxin in the Dog

By G. Charles Oliver, John Cooksey, Charles Witte, and Marlys Witte

ABSTRACT

Absorption of digitoxin was studied in 14 fasted anesthetized dogs after administration of 50 μg of 3H-digitoxin mixed with 1 mg of unlabeled digitoxin into either the duodenum or stomach. Radioactivity was measured in portal, hepatic, and central venous plasma, thoracic duct lymph, common duct bile and urine for 5 hours after digitoxin was given. Three dogs were given a single intravenous dose of 25 μg of 3H-digitoxin mixed with 0.5 mg of unlabeled digitoxin, and radioactivity was measured in central venous plasma and thoracic duct lymph. Digitoxin was absorbed rapidly and uniformly from the duodenum and entered the body entirely by way of the portal vein, reaching peak concentrations in portal blood in 1 to 15 minutes. Digitoxin was absorbed erratically from the stomach. No metabolites of digitoxin were observed in selected portal venous samples subjected to thin-layer chromatography. Absorption of orally administered digitoxin into the systemic bloodstream is retarded because of highly efficient hepatic extraction of the drug and subsequent transfer into bile.

KEY WORDS lymphatics thoracic duct lymph portal vein intestinal absorption entero-hepatic circulation

Despite widespread use and extensive research into the pharmacology of the cardiac glycosides, it has not been determined whether this group of drugs is absorbed by the mesenteric lymphatic or portal venous route (1, 2). To define the precise route of absorption of digitoxin, radioactive digitoxin was administered into the gastrointestinal tract of dogs and the time course of its appearance in portal, hepatic, and central venous plasma, thoracic duct lymph, common duct bile and urine was examined.

From the Department of Medicine (Cardiology Division), Washington University School of Medicine, St. Louis, Missouri 63110, and the Departments of Surgery and Medicine (Cardiology Section), University of Arizona College of Medicine, Tucson, Arizona 85719.

This study was supported by U. S. Public Health Service Grants HE 05332, HE 11233, HE 11034, HE 12179, and HE 13390 from the National Heart and Lung Institute, and a grant from the American Heart Association. Dr. Marlys Witte is an Established Investigator of the American Heart Association.

Received January 18, 1971. Accepted for publication July 1, 1971.

Methods

Seventeen fasted mongrel dogs weighing 10 to 25 kg were anesthetized using Myotal (0.5 mg/kg). Fourteen of the dogs were intubated and ventilated with a Harvard animal respirator. The thoracic duct was cannulated with polyethylene tubing in the right chest or left neck in 10 dogs. The spleen was removed and a cannula was inserted into the portal vein by way of the splenic vein. The tip of the cannula was carefully located adjacent to the liver so that portal venous blood was obtained above the entry of gastroduodenal venous tributaries. The right atrium or inferior vena cava and left hepatic vein (7 dogs) were cannulated through the femoral vein and right external jugular vein, respectively. Fifty μg of 3H-digitoxin (1.11 × 10⁷ dpm) and 1 mg unlabeled digitoxin (Crystodigin, Lilly) were instilled into the upper duodenum of seven dogs and into the stomach of seven others. The pylorus was ligated in two of the latter group. Heparinized samples of thoracic duct lymph and blood from the right atrium or inferior vena cava ("central venous blood"), portal, and hepatic veins were analyzed for total radioactivity at frequent intervals up to 5 hours. In two dogs receiving digitoxin by

*Supplied by New England Nuclear Corporation, Boston, Massachusetts.
Radioactivity (mean dpm/ml ± se) in portal (a) (5 dogs), hepatic (•) (5 dogs), and central venous plasma (•) (5 dogs), thoracic duct lymph (○) (4 dogs), common duct bile (○) (2 dogs), and urine (○) (2 dogs) after 50 μc (1.11 × 10⁸ dpm) ³H-digitoxin and 1 mg nonradioactive digitoxin were instilled into the duodenum at zero time. Note the very prompt appearance of radioactivity in portal venous blood in contrast to its slow appearance in lymph. Peak concentrations in urine and particularly in bile greatly exceed those in plasma and lymph.

intraduodenal instillation, bile and urine were collected simultaneously at intervals after cannulation of the common bile duct and catheterization of the urinary bladder.

Disappearance of radioactive digitoxin from central venous plasma and appearance in thoracic duct lymph were determined in three other dogs after intravenous administration of 25 μc (5.6 × 10⁷ dpm) with 0.5 mg of unlabeled digitoxin.

Blood samples were centrifuged and plasma and other fluid specimens were frozen until assayed. To 10 ml of Bray's solution (3) 0.1 ml of specimen was added, and radioactivity in each sample was determined by counting for 10 minutes in a 3-channel liquid scintillation spectrometer. Either internal or external standardization was performed to allow correction for quenching.

Chromatography was performed to ascertain whether radioactivity in portal venous blood was in the form of unchanged digitoxin or digitoxin metabolites. Selected samples of portal venous blood were extracted three times with 10 volumes of chloroform, and the chloroform extract subjected to thin-layer chromatography (Gelman glass fiber paper, type S.C., developing solvent cyclohexane:acetone 60:40). Digitoxin and digoxin were chromatographed simultaneously for reference. Radioactivity was located either with a radiochromatogram scanner or by cutting the chromatogram into 1 cm strips and counting each strip in a liquid scintillation counter.

Mark I liquid scintillation computer, Nuclear Chicago Corporation, Boston, Massachusetts.


Circulation Research, Vol. XXIX, October 1971
Results

Figure 1 shows the radioactivity (mean dpm/ml ± se) in portal, hepatic, and central venous plasma, thoracic duct lymph, common duct bile and urine after intraduodenal instillation of 50 µc of ³H-digitoxin. Peak concentration of radioactivity in portal venous blood was reached in 1 to 15 minutes and in central venous blood only after 45 minutes. At no time was radioactivity in central venous blood or thoracic duct lymph greater than that of portal venous blood. After approximately 300 minutes, the concentrations of radioactivity in portal and central venous blood and lymph were nearly equal. Peak radioactivity in bile and urine greatly exceeded that of blood and lymph and was attained when blood and lymph concentrations were declining.

Radioactivity appeared in the first specimen of bile; peak concentration was attained at approximately 90 minutes and declined slowly thereafter. After low levels of radioactivity in urine for almost 60 minutes, there was a rapid rise and then a gradual increase up to 300 minutes.

Simultaneous samples of portal and hepatic venous blood showed a large portal vein–hepatic vein difference during the first 30 minutes after duodenal instillation of digitoxin, but the difference diminished thereafter and was negligible at 2 hours (Fig. 2).

In seven animals after intragastric instillation of digitoxin, absorption was delayed and erratic, particularly in the two in which the pylorus was ligated.

Radioactivity (mean dpm/ml ± se) in central venous plasma (•) and thoracic duct lymph (○) after intravenous administration of 25 µc (5.6 × 10⁷ dpm) of ³H-digitoxin and 0.5 mg nonradioactive digitoxin in three dogs. Note the similarity of the appearance curve in lymph to the curve obtained after intraduodenal administration and that values attained in lymph at equilibrium are approximately one-half those obtained after administration of twice the dose into the duodenum (Fig. 1).
Radiochromatograms of portal venous blood taken 1 and 4 hours after intraduodenal administration of 3H-digitoxin. A single radioactive peak is noted, corresponding to chromatographically pure digitoxin.

The results of intravenous injection of 25 µc of 3H-digitoxin in three dogs are shown in Figure 3. The appearance curve of radioactivity in thoracic duct lymph, however, was similar to that obtained after intraduodenal administration of digitoxin.

Extraction of selected samples of portal venous blood with chloroform removed 93% of the radioactivity from portal venous blood. Thin-layer chromatography of these chloroform extracts revealed only unchanged digitoxin. The results of radioactive scanning of the chromatograms of portal venous blood taken 1 and 4 hours following administration of tritiated digitoxin are shown in Figure 4.

Discussion
Digitoxin could be absorbed either into the mesenteric lymphatics or the portal vein. Nonpolar lipids (4–9) reach the venous circulation only after traversing mesenteric lymphatics and the thoracic duct, whereas amino acids and sugars are absorbed into the portal vein (4). The present study shows that digitoxin is absorbed into the portal vein rather than into the lymphatics. Cholesterol, a low molecular weight nonpolar lipid structurally akin to digitoxin is absorbed into the lymphatics (4), and its rate of entry into the blood stream is limited by the speed at which the thoracic duct drains into the systemic vein. Under normal circumstances, this transfer is relatively slow, and absorption curves for cholesterol show peak blood concentrations only several hours after presentation to the gut. Because digitoxin is extremely water insoluble (1 g/100 liters) and has solubility characteristics of a lipid, it is surprising to find not only that digitoxin reaches the blood stream entirely by way of the portal vein, but furthermore that absorption is extremely rapid, with peak concentrations in portal blood reached in less than 15 minutes. The rate of appearance in systemic venous blood, however, is retarded because of avid extraction across the liver and subsequent transfer into bile, which occurs despite tight binding of the drug to serum proteins. Delay in peak therapeutic effect of oral digitoxin may be explicable in part by slow entry into central venous blood, but other factors such as binding to serum proteins or to the myocardium itself probably also play a role, since the onset of action is delayed even after intravenous administration of digitoxin.

The exact mechanism responsible for rapid absorption of digitoxin from the gut into the portal vein cannot be defined precisely, but Greenberger et al. (10) concluded that digitoxin was absorbed from the gut of rats and guinea pigs by a nonsaturable (passive) transport mechanism. On the other hand, an active transport system such as described for the glycoside convallotoxin (11) may be involved.

Our data confirm earlier observations on the major role of the kidneys and biliary tract in the elimination of radioactivity in dogs given oral 3H-digitoxin. Katzung and Meyers (12) noted in dogs that diversion of the biliary stream from the intestine to a collecting vessel increased the rate at which digitoxin was
eliminated from the body. They concluded, as originally suggested by Okita et al. (13), that there is a significant entero-hepatic circulation of digitoxin. Furthermore, since at least 80% of bile radioactivity represents water-soluble, nonabsorbable metabolites of digitoxin, they suggested that prior to reabsorption by the gut, these metabolites are very likely transformed back into digitoxin. Our portal vein chromatographic studies are in agreement with this conclusion, since the only substance identified in portal venous blood during the period of the experiment was unchanged digitoxin.

In summary, digitoxin is rapidly absorbed from the upper gastrointestinal tract into the portal vein. Rapid removal by the liver with subsequent transfer into bile accounts for a delay in the appearance of digitoxin in central venous blood. The findings suggest further that patients with hepatobiliary or gastrointestinal dysfunction may have altered sensitivity to a given dose of this particular digitalis preparation.

Acknowledgment
The authors are grateful for the excellent technical help furnished by Mrs. Pamela Carter, Mrs. Clarina Tisdale, and Mrs. Adalene Boyd.

References
Absorption and Transport of Digitoxin in the Dog
G. Charles Oliver, John Cooksey, Charles Witte and Marlys Witte

Circ Res. 1971;29:419-423
doi: 10.1161/01.RES.29.4.419

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1971 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/29/4/419

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/