Comparison of Shunt Fraction Estimation Using Transcolonic Iodine-123-Iodoamphetamine and Technetium-99m-Pertechnetate in a Group of Dogs with Experimentally-Induced Chronic Biliary Cirrhosis

Philip D. Koblik, William J. Hornof, Chi-Kwan Yen, Jan Komtebedde, Eugene Breznock, and Paul Fisher

Department of Radiological Sciences and Department of Surgery, School of Veterinary Medicine, University of California, Davis, California and the Department of Nuclear Medicine, Pacific Presbyterian Medical Center and Medical Research Institutes, San Francisco, California

Portosystemic shunt fraction estimation using transcolonic iodine-123-iodoamphetamine (IMP) has been previously validated relative to portal vein macroaggregated albumin injections using an experimental model of cirrhosis. Transcolonic technetium-99m-pertechnetate (TcO_4^-) has been proposed as an alternative tracer to IMP to study portal circulation in cirrhotic patients. We compared shunt fraction estimates from paired transcolonic IMP and TcO_4^- studies performed on a group of dogs before and after common bile duct ligation surgery. Pertechnetate overestimated shunt fraction in 6/7 postoperative studies relative to IMP. A good correlation between the two methods was demonstrated, however, the slope of the regression line was substantially less than 1.0 with TcO_4^- values reaching 100% at IMP shunt values of ~60%. This apparent inability to accurately assess high shunt flows may limit the quantitative aspects of TcO_4^- studies on patients with severe portosystemic shunting.

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In patients with progressive liver disease, onset of portosystemic shunting is an important milestone in the course of their disease. The ability to detect and quantify portosystemic shunts can affect the prognosis and treatment of these patients. In asymptomatic chronic hepatitis patients, the onset of cirrhosis is associated with a poor prognosis. Detection of portosystemic shunts using transcolonic radioisotope studies has been used to distinguish patients with and without cirrhosis and to identify patients with liver decompensation, and have been proposed as an alternative to liver biopsy as a method to identify those patients with poor prognosis in whom more aggressive therapy may be warranted (*1,2*). The magnitude of portosystemic shunting has been correlated with the incidence and severity of specific complications of cirrhosis such as hepatoencephalopathy and variceal bleeding (*3,4*). Finally, portosystemic shunt measurements may provide a quantitative assessment of pharmacologic intervention in patients with portal hypertension (*5*). In these clinical settings, serial studies are usually required, and the noninvasive nature of the transcolonic radioisotope studies is particularly attractive.

Portosystemic shunt fraction (SF) estimates using transcolonic iodine-123-iodoamphetamine (IMP) have been shown to correlate very well with estimates based on macroaggregated albumin (MAA) injections in experimental dogs with surgically created portocaval anastomoses or chronic cirrhosis (*6,7*). IMP has also been used to evaluate a group of human patients with cirrhosis and shown to correlate with the degree of liver decompensation (*2*). Because transcolonic technetium-99m (^{99m}Tc) pertechnetate (TcO_4^-) yields superior images of the portal system, results in lower patient dose, and is relatively inexpensive, it has been suggested as an alternative to IMP and has been used to study the portal circulation in human patients with a variety of liver diseases (*8–10*). Altered patterns of portal blood flow that occur with portosystemic shunting can be qualitatively assessed and in many cases the collateral channels themselves can be visualized.

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For reprints contact: P. D. Koblik, DVM, Dept. of Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA 95616.

Intravascular pertechnetate is freely diffusible into the extracellular fluid space of most tissues but is not actively extracted or bound by the liver, heart, or lung and, hence, acts as an inert tracer. Time-activity curves generated from liver (L) and heart (H) regions of interest (ROIs) show characteristic patterns in normal individuals and patients with hepatitis versus patients with cirrhosis and portal hypertension (*8–10*). Several methods to calculate shunt indices based on relative liver and heart activity have been proposed (*8–10*). In clinical studies, these indices have been shown to correlate with the degree of underlying hepatopathy and portal hypertension (*8–10*). It has been suggested that TcO_4^- shunt indices may provide quantitative information relative to the severity of portosystemic shunting (*9, 10*). Questions, however, have been raised regarding the validity of shunt fraction determinations using TcO_4^- as recirculation between target organs is unavoidable (*11,12*). The only published report comparing TcO_4^- shunt indices to any other established method of shunt fraction estimation was a letter to the editor in which Shiomi et al. noted a good correlation (r = 0.814) between shunt fraction determinations based on paired transcolonic IMP and TcO_4^- scans in 19 patients (*13*).

In an attempt to investigate the accuracy of transcolonic TcO_4^- shunt index, we performed paired transcolonic TcO_4^- and IMP studies in a group of dogs before and 4 wk after chronic bile duct ligation, a proven method to induce cirrhosis, portal hypertension, and portosystemic shunting, and an accepted experimental model for human cirrhosis (*14*).

MATERIALS AND METHODS

An initial transcolonic TcO_4^- study and 30–34 hr later an initial IMP study were performed on a group of nine normal dogs ~1 wk before surgery. Common and cystic bile duct ligation was then performed using a modification of a previously described method (*14*). General anesthesia was induced with i.v. Suritol (Na-Thiamylal), and maintained following intubation with a mixture of oxygen and halothane. A midline laparotomy was performed. The cystic duct was ligated and the gallbladder was emptied by aspiration, eliminating the need to perform a cholecystectomy. The free portion of the common bile duct was isolated, double ligated using metal Versaclips, and sectioned between the clips. Postoperative care included daily administration of oral broad spectrum antibiotics and Vitamin K. Dogs were maintained on a special low-protein diet (K/D, Science Diet). Four weeks after surgery the same sequence of a transcolonic TcO_4^- study followed 30–34 hr later by an IMP study was repeated. As a part of a separate project, portal venous pressure recording, mesenteric angiography, mesenteric vein injection of ^{99m}Tc-MAA and liver biopsy were performed in a terminal experiment 6 wk after bile duct ligation to document alterations in liver structure and portal venous circulation (*7,15*). All protocols were approved by the UCD Animal Use Committee.

Transcolonic IMP Studies

Studies were performed without anesthesia or sedation. Dogs were restrained in a prone position over a LFOV gamma camera with a medium-energy parallel-hole collimator (Siemens Gammasonics, Sunnyvale, CA) interfaced to a dedicated nuclear medicine computer (Sophy GX, Sopha Medical, Inc., Columbus, MD). A flexible pediatric feeding catheter was introduced into the distal colon and 1 mCi of [^{123}I]IMP (SPECTamine, Medi-Physics, Inc., Paramus, NJ) was introduced into the colon using 10 ml of room air as a flush. A dynamic series of 10 1-min 64 × 64 images was recorded using a 20% window centered at 159 keV. Manual ROIs were created for the lungs and for the liver. Shunt fraction was calculated as the average background corrected L/(H+L) ratio for the 5th through 9th frames of the study. Background correction was based on the mean counts/pixel in one-pixel wide perimeter ring regions two pixels removed from each target organ.

Transcolonic TcO_4^- Studies

Studies were again performed without anesthesia or sedation. The dogs were positioned in right lateral recumbency over the gamma camera using a low-energy parallel-hole collimator. A 2-mCi/kg dose of TcO_4^- was administered in the same manner as described for the IMP studies. A dynamic series of 60 2-sec 64 × 64 images was recorded using a 20% window centered at 140 keV. Hand-drawn liver and heart ROIs were created and used to generate time-activity curves. The time of initial appearance of activity in each organ (T_L, T_H) was determined by visual inspection of the curves. The earliest arrival time for both organs, i.e., the shorter of the two arrival time values, was defined as T_0. Shunt indices were calculated using the following formula:

$$\text{SI-i} = \frac{\sum_{T_0}^{T_0+i} H}{\sum_{T_0}^{T_0+i} H + \sum_{T_0}^{T_0+i} L},$$

where i = 10 or 24 sec.

Resultant IMP shunt fractions and TcO_4^- shunt indices were compared by least-square's linear regression and paired-sample Student's t-test.

RESULTS

One dog (#2198) developed signs of severe gastrointestinal distress (anorexia, vomiting, diarrhea, weight loss, dehydration) 3 wk after surgery and was terminated in accordance with the approved animal use protocol. Results of portal vein manometry, angiography, and MAA injection performed 6 wk after bile duct ligation confirmed the development of portal hypertension and portosystemic shunts in seven of the remaining eight dogs and were reported in previous publications (*7,15*). At the time of the terminal experiment dog #2197 had normal portal pressure (7 mmHg), had no evidence of shunts on angiography, had a shunt fraction of 0.04 (based on portal vein injection of ^{99m}Tc MAA), and had no evidence of cirrhosis on terminal liver

biopsy. At necropsy, a patent accessory bile duct was identified. Based on these findings the 4-wk post-surgery TcO_4^- (SI-10 = 0.06) and IMP (SF = 0.06) studies from this dog were excluded from further analysis.

Normal TcO_4^- studies (i.e., performed before surgery) were characterized by a rapid rise in liver activity a few seconds after delivery of the dose into the colon followed 8–10 sec later by a less rapid rise in heart activity (Fig. 1). IMP and SI-10 estimates of relative shunt flow were <0.05 in all normal studies (Table 1). Using the 24-sec time interval for TcO_4^- curve integration results in higher shunt index values (up to 10%) in the normal studies. In contrast, TcO_4^- studies performed on the same dogs 4 wk after surgical induction of biliary cirrhosis were characterized by a more rapid rise in heart activity, which occurred coincident with or prior to a markedly reduced rise in liver activity (Fig. 2). Relative to IMP, TcO_4^- shunt indices resulted in significant ($p < 0.05$) overestimation of shunt flow in 6/7 postoperative studies regardless of the time interval used for TcO_4^- curve integration (Table 1).

Although linear regression analysis indicated a good correlation ($R^2 \approx 0.90$) and relatively small residual error (<0.10) between IMP shunt fraction and TcO_4^- shunt indices, the slopes of the regression equations were considerably < 1.0.

DISCUSSION

Accurate, noninvasive estimation of TcO_4^- shunt flow has potential application in management of patients with cirrhosis and other liver conditions that develop portal hypertension. Techniques to directly quantitate shunt fraction using radioactive microspheres have been described but necessarily involve catheterization of the portal vein or spleen (*16–19*). Because of the efficient extraction and binding by amine receptors in the liver and lung, IMP shunt fraction calculations utilize the same theoretical approach as microspheres. The rate of colonic absorption and delivery of IMP to the liver and lung are relatively unimportant since observations are conducted over several minutes. Limited availability, high cost, and a relatively long half-life have limited its clinical use. Additionally, the potential exists that IMP receptor binding may be affected by administration of some drugs (*20*) or may be altered in patients with chronic active hepatitis (*13*). On the other hand, excellent correlations between IMP and portal vein MAA injections have been demonstrated in acute experimental studies that involved surgically created portosystemic shunts (*6*) and have been recently confirmed in a group of dogs under conditions of propranolol administration and experimentally induced liver cirrhosis (*7,15*).

TABLE 1
IMP and TcO_4^- Shunt Flow Estimates for a Group of Dogs Before and Four Weeks After Total Common Duct Ligation

Dog	Status*	IMP†	SI-10‡	SI-24§	DT (sec)¶
2161	Pre	0.03	0.02	0.03	10
2162	Pre	0.01	0.00	0.02	10
2164	Pre	0.02	0.00	0.02	10
2178	Pre	0.05	0.01	0.10	8
2180	Pre	0.00	0.00	0.04	10
2181	Pre	0.01	0.02	0.05	8
2196	Pre	0.02	0.00	0.07	8
2197	Pre	0.02	0.02	0.09	8
2198	Pre	0.00	0.01	0.10	8
2161	Post	0.44	0.78	0.76	−4
2162	Post	0.37	0.90	0.86	−6
2164	Post	0.58	0.61	0.71	2
2178	Post	0.63	0.99	0.93	−14
2180	Post	0.45	0.95	0.93	−4
2181	Post	0.57	0.92	0.85	−4
2196	Post	0.29	0.26	0.29	0

IMP = 0.03 + 0.55 X (SI-10) r = 0.94 s.e.e. = 0.09
IMP = 0.00 + 0.60 X (SI-24) r = 0.95 s.e.e. = 0.08

*Status: Pre = study performed before common bile duct ligation surgery and Post = study performed 4 wk after common bile duct ligation surgery.

† IMP = shunt fraction based on transcolonic IMP tracer study.

‡ SI-10 = shunt index based on transcolonic TcO_4^- tracer study using 10 curve integration interval.

§ SI-24 = shunt index based on transcolonic TcO_4^- tracer study using 24 curve integration interval.

¶ DT = time interval between appearance of activity in heart versus liver (T_H-T_L) for transcolonic TcO_4^- tracer studies.

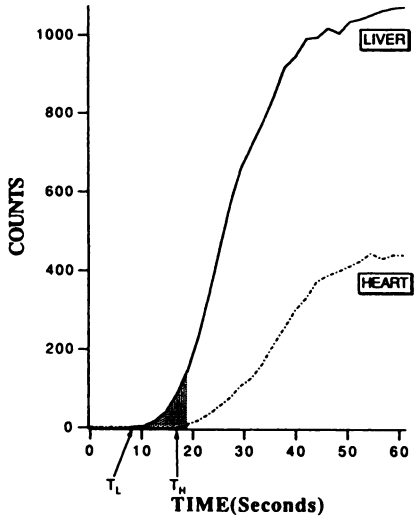

FIGURE 1
Example of liver and heart time-activity curves following transcolonic administration of TcO_4^- in a normal dog. Notice that arrival of tracer in the liver (T_L) occurs ~10 sec before arrival of tracer in the heart (T_H) and that liver activity rises at a much more rapid rate than heart activity. A ratio of heart/(heart + liver) activity integrated over a 10-sec interval beginning at the earliest time of arrival provides an estimate of portosystemic shunt flow. In this animal, shunt index = 0.01.

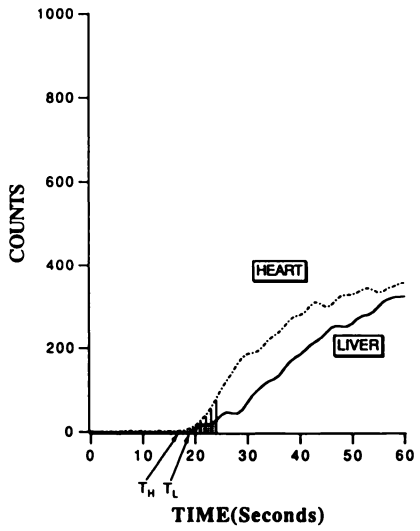

FIGURE 2
Example of liver and heart time-activity curves following transcolonic administration of TcO_4^- in a dog 4 wk after CBD ligation surgery. Notice that arrival of tracer in the liver (T_L) is delayed compared to normal studies and occurs a short time after arrival of tracer in the heart (T_H). The rate of increase of liver activity is also markedly reduced compared to normal studies. A ratio of heart/(heart + liver) activity integrated over a 10-sec interval beginning at the earliest time of arrival provides an estimate of portosystemic shunt flow. In this animal, shunt index = 0.61.

In contradistinction to the case of IMP where the shunt fraction is calculated on the basis of accumulation (presumably irreversible in relation to the observation time), in the case of TcO_4^- the calculation of shunt fraction is based on relative arrival times and integration over a time interval which could be longer than the residency time in the observed organs. In the absence of a shunt, the difference between the hepatic and cardiac arrival times is a function of the hepatic circulation time and the circulation time through the hepatic venous system. The arrival time in the heart of shunted blood is shorter, since the hepatic and hepatic venous circulation times are excluded and is influenced by the circulation times through the shunt pathways. Furthermore, appearance rate is modulated by the rate of TcO_4^- absorption from the colon. Although TcO_4^- represents a ubiquitous, inexpensive radioisotope ideally suited to clinical use and capable of providing scintiangiograms of the portal system, extraction of quantitative estimates of shunt flow using this tracer is difficult at best and has led to an empirical approach to calculation of shunt indices.

The formula for shunt index calculation used in this project has been proposed as a valid method to estimate shunt flow provided that the time interval used for curve integration is short enough so as to minimize the effects of recirculation (*10,13*). The 24-sec time interval that has been recommended in humans represents the normal T_H-T_L time difference (*10*). Integration over a longer time interval following arrival of activity in the liver results in an increase in shunt index value in normal individuals (*12*). Since this study was conducted using dogs, we chose a 10-sec time interval for curve integration based on our observations of the more rapid normal circulation in this species (*21*). We included data using a 24-sec time interval to evaluate the effect of a longer time interval on shunt index. As anticipated, with the 10-sec time interval, TcO_4^- shunt index values in the normal (preoperative) studies were consistently near 0%, and higher normal shunt index values were obtained when the longer time interval was used.

We utilized right lateral images for the TcO_4^- studies because our previous experience performing this procedure in conscious dogs indicated that right lateral recumbency facilitated restraint, minimized patient motion artifacts, and provided adequate separation of liver and heart for ROI generation (*21*). We utilized ventral images for the IMP studies because they represent a good compromise in counting efficiency for and spatial separation of the target organs (lung versus liver). Since IMP studies involve the acquisition and analysis of a series of 1-min static images, they are much less affected by patient motion than the TcO_4^- studies.

Based on data from this study, TcO_4^- shunt indices are incapable of accurately predicting shunt flow in excess of 60% of total portal blood flow as both SI-10 and SI-24 values approached 100% at this level of shunting. Yet, microsphere and IMP studies conducted on human patients with decompensated liver cirrhosis (*2,17,18*) and IMP studies conducted on dogs with congenital and acquired TcO_4^- portosystemic shunts (*22*) indicate that shunt flow often far exceeds this value.

We feel that the failure of TcO_4^- shunt indices to maintain proportionality to shunt fraction with large shunts represents an inherent inability of this empirical method to appropriately model the complex kinetics of tracer uptake and distribution. Portal hypertension is known to have a variable effect on portal and hepatic circulation times (i.e., the length of time required for an inert tracer to pass through the portal venous system and liver, respectively) and on the rate of colonic absorption (*23–28*). In the absence of portosystemic collateral shunts, the predominant effort of cirrhosis on portal flow will be one of stagnation due to increased vascular resistance. Under such conditions portal and hepatic circulation times will be prolonged (*23–26*). Pertechnetate shunt index, however, will continue to approach 0% because all of the portal blood must pass through the liver before reaching the heart (Fig. 3A-B). If portosystemic collaterals exist, splanchnic blood flow may be hyperdynamic. Circulation time to the heart may be shortened via flow through the relatively low resistance collateral channels (*25–27*) and the ratio of heart and liver curve integrals becomes not only a

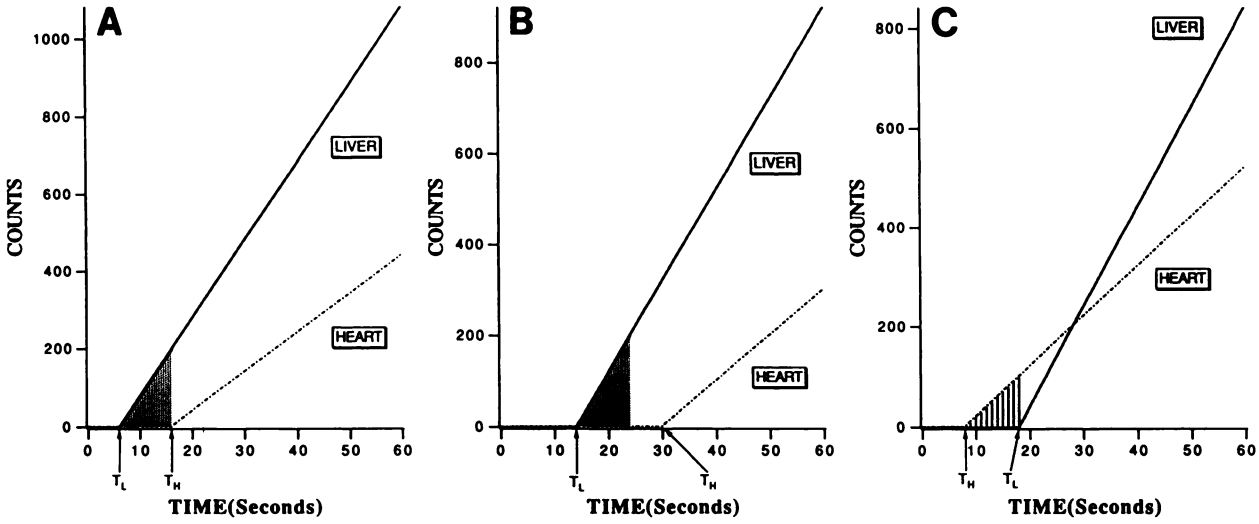

FIGURE 3

(A) Hypothetical liver and heart time-activity curves that could be expected to occur in a normal individual following transcolonic administration of TcO_4^-. After a short delay, tracer first arrives in the liver (T_L) and after several seconds arrives in the heart (T_H). By choosing a time interval that corresponds to the hepatic circulation time for curve integration, shunt index values will be forced toward 0.00. (B) Hypothetical liver and heart time-activity curves that could be expected to occur in an individual with prolonged portal vein and hepatic circulation times subsequent to portal hypertension and cirrhosis following transcolonic administration of TcO_4^-. The time delay before arrival of tracer in the liver and the hepatic circulation times are prolonged, however, in the absence of portosystemic shunts, shunt index values will remain near 0.00. (C) Hypothetical liver and heart time-activity curves that could be expected to occur in an individual with portosystemic shunts subsequent to portal hypertension and cirrhosis following transcolonic administration of TcO_4^-. The shunt channels provide an alternative pathway for tracer to reach the heart and in this example result in the heart arrival time (T_H) to occur considerably earlier than the arrival time in the liver (T_L). Although the rate of increase in activity in the heart and liver have not been altered relative to the other examples, shunt index in this case will primarily reflect the change in circulation times and will approach 1.00.

function of blood flow but also a function of circulation times through these alternate pathways. In those cases where the circulation time through the collateral shunt channels is considerably shorter than through the portal vein and liver, shunt index is more likely to reflect the early arrival of activity in the heart rather than relative blood flow (Fig. 3C). This hypothesis is supported by the observation that the two postoperative TcO_4^- studies with closest approximation to IMP shunt fraction also had the least shortening of T_H relative to T_L (Table 1).

Another explanation for the disparity of IMP and TcO_4^- results is that they represent some systematic error introduced by observer intervention during ROI creation or determination of T_0. In our experience, the variation in shunt index when different observers process the same study is too small to account for the large discrepancy (≈0.30) in postoperative IMP and TcO_4^- values. The similarity of the SI-10 and SI-24 results further indicates that the shunt index is relatively insensitive to the portion of heart and liver curves used for its calculation.

A means to noninvasively monitor portosystemic shunt flow would be useful in staging and managing patients with progressive liver disease. Radioisotopic tracers that directly enter the portal blood stream via colonic absorption can be used to detect the presence of portosystemic shunts and offer the potential for shunt quantitation. Pertechnetate has a number of advantages relative to other transcolonic tracers and TcO_4^- shunt indices have been shown to correlate well with other physiologic and clinical parameters which reflect the severity of hepatopathy and portal hypertension. However, based on the results of this study, TcO_4^- shunt index calculations using currently accepted methods appear to have limitations in measuring relative shunt flow in an experimental model of chronic cirrhosis and portal hypertension. The extent to which this flaw will limit its clinical utility in human patients is, at present, an open issue.

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