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Evaluation of the Venous System of Wistar Rat Liver by Injection with Epoxy Resin

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SHORT COMMUNICATION

Abstract
The rat is the most widely used experimental model in surgical research due to several factors, including that it is easy to handle and inexpensive. It can be used in investigations related to liver regeneration, liver metastases, or transplant immunology. This study highlights the venous components of the hepatic circulation in the Wistar rat by intravascular injection of a polymer that allows the assessment of the distribution of vessels, regardless of their caliber. Five cadavers of 11-month-old male Wistar rats from the USAMV Cluj-Napoca biobase destined for incineration, were used to highlight the liver venous system. A dye mixture, consisting of epoxy resin-catalyst-blue acrylic dye in a 2:1:1 ratio, was injected. After 24 hours, biological tissues were macerated by immersion in 10% KOH solution for five days. The intrahepatic venous system is represented by the venous branches that continue into the liver lobes. Those vessels follow a parallel trajectory with the hepatic artery branches represented by the right and left ramifications. The right portal vein presents branches to the lateral and medial parts of the right lobe of the liver and the caudate lobe. The left branch of the portal vein has ramificationsfor the lateral and medial parts of the left lobe and the quadrate lobe. The technique of injecting the venous circulation of the liver, followed by tissue maceration, allowed the removal by anatomical dissection of all liver components that permits the identification of all components of the venous system, including the finest venous branches of the hepatic circulation.
Keywords: epoxy resin, venous system, Wistar rat

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INTRODUCTION
The rat is the most widely used experimental model in surgical research due to several factors, like the ease of handling and cheapness (Martins, 2003). It can be used in investigations related to liver regeneration, liver metastases, transplant immunology, or in other liver diseases. Moreover, all clinical liver surgery procedures can be performed on the rat. There are also some drawbacks to using the rat model in liver surgery, given its small size and insufficient information about the anatomy of liver vascularization (Martins and Neuhaus, 2007). Due to the great anatomical variability of the vasculature, knowledge of morphology is advantageous because it allows individualized dissection or specific ligation of vascular and biliary branches (Rodriguez et al., 1999). Knowing the diameter and length of the liver vessels is also important for the selection of catheters and perivascular devices (Martins and Neuhaus, 2007). Advances in experimental medicine have facilitated the treatment of diseases such as short bowel syndrome in neonates and adults, pancreatic dysfunctions, and end-stage liver diseases

through experimental surgical investigation. These particular diseases can only be treated by transplantation (Vdoviakova et al., 2016, Ma şi Guo, 2008, Majorová et al., 2004). Experimental transplants of digestive organs are performed using different methods and different combinations of donors and recipients, with the condition that the vascularization and lymphatic drainage are similar between the receiver and the donor (Vdoviakova et al., 2016, Li et al., 2000).

It is known that the vascularization can present anatomical variations, aspects that must be taken into account in experimental investigation and surgical practice (Vdoviakova et al., 2016). For the study of experimental transplantation, the laboratory rat appears to be the most suitable experimental animal, especially in liver and intestinal transplantation research (Lopes et al., 1998; Galvao et al., 2005). Having an organ structure similar in many aspects to that of humans, the rat is the most suitable species for anatomical, physiological and biochemical research of the digestive system.

MATERIALS AND METHODS

Five cadavers of rats (*Rattus norvegicus*), 11-month-old, males, belonging to the Wistar breed from the USAMV Cluj-Napoca biobase intended for incineration, were used to highlight the venous system of the liver. In order to perform the experiment, all five cadavers were opened on the abdominal white line, to highlight the large blood vessels in the abdominal cavity (i.e. descending abdominal aorta and caudal vena cava). After the identification of the vessels, the next step was the cannulation of the caudal vena cava with an 18 G cannula. Following, a heparinized saline lavage was performed, to remove any clots. Subsequently, a dye mixture, consisting of epoxy resin-catalyst-blue acrylic dye in a 2:1:1 ratio, was injected. The injection was performed at the level of the caudal vena cava. The cadavers were left at room temperature for 24 hours to allow polymerization to solidify the dye mixture. After 24 hours, the next step was represented by the maceration of the biological tissues by immersion in 10% KOH solution for five days.

Following the maceration stage, the pieces obtained were washed under running water, at which time any remaining undigested tissue was mechanically removed. The pieces obtained were photographed with a digital photo camera and processed using Adobe Photoshop software.

RESULTS AND DISCUSSIONS

From an anatomical point of view, the rat liver is systematized into 6 lobes: the left lateral lobe (*lobus hepatis sinister lateralis*), the left medial lobe (*lobus hepatis sinister medialis*), the right lateral lobe (*lobus hepatis dexter lateralis*), the right medial lobe (*lobus hepatis dexter medialis*), the quadrate lobe *(lobus quadratus*) and the caudate lobe (*lobus caudatus*) (Vdoviaková et al., 2016). The same morphological features were present also in the organs we harvested. The caudate lobe has 2 processes: papillary (*processus papillaris*) and caudate (*processus caudatus*), being located between the caudal vena cava (*v. cava caudalis*) and the left branch of the portal vein (*v. portae*). The following anatomical description represents the results of our study regarding the structure of the venous tree in Wistar rats.

The vascularization of the liver is double: nutritive - provided by the hepatic artery and its branches, and functional – provided by the portal vein. Functional vascularization consists of the extrahepatic and intrahepatic venous systems (Figure 1 A, B).

Hepatic veins

The right lateral lobe (*lobus hepatis dexter lateralis*) drains into the caudal vena cava (*vena cava caudalis*) in most cases via a single branch. The left lateral lobe was drained primarily by a large hepatic vein with 2 to 3 larger branches (Figure 1 A, B).

The left medial lobe (*lobus hepatis sinister medialis*) and right medial lobe (lobus hepatis dexter medialis) are each drained by one vein. The vein draining the left portion of the middle lobe (left median vein) enters the vena cava separately, before merging with the suprahepatic vena cava.

The left lobe and caudate lobe are drained by two large hepatic veins (left -*v. hepatica sinistra* and right -*v. hepatica dextra*) that open separately into the vena cava. The caudate process drains into the intrahepatic vena cava via multiple branches.

The extrahepatic venous system is represented by the portal circulation, which in turn consists of the portal vein (extrahepatic) and its tributaries (Vdoviaková et al., 2016). The intrahepatic venous system is represented by the venous branches that continue in the lobes of the liver. The main trunk of the portal vein is branched into right and left branches (Vdoviaková et al., 2016).

The right branch of the portal vein has ramifications to the lateral and medial parts of the right lobe of the liver and the caudate lobe. The dorsal branch of the right lateral lobe is located in the parenchyma of the dorsal part of

the right lateral lobe of the liver, between the lateral lobe and the caudate process of the liver. This branch has 2 terminal ramifications. The ventral branch of the right lateral lobe of the liver has 3 branches and along its course, it splits into collateral branches.

The branches of the caudate lobe are represented by 2 ramifications from the dorsal branch of the portal vein.

The branch of the right medial lobe is a tributary of the portal vein. The ventral branch of the right medial lobe (*lobus hepatis dexter medialis*) is found ventrally, deep in the liver tissue of the right medial lobe.

The left branch of the portal vein has ramifications for the lateral and medial sides of the left lobe and for the quadrate lobe (*lobus quadratus*). The left branch is considered the main tributary of the portal vein.

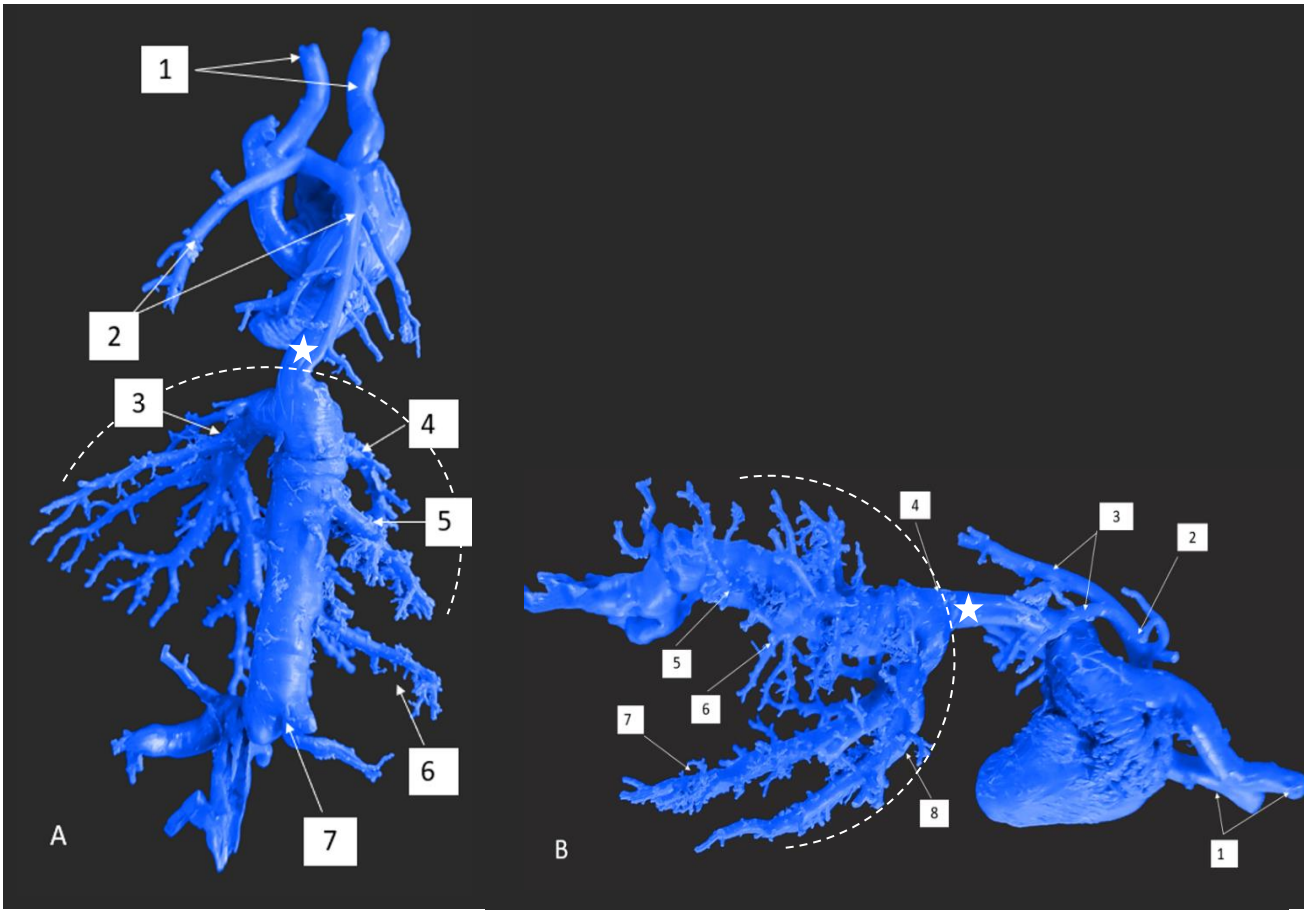

Figure 1. (A) 1. Left and right superior vena cava. 2. Pulmonary arteries. 3. Branches of the left medial lobe. 4. The branch of the right medial lobe. 5. Branches for the right posterior lobe. 6. Branches for the right posterior lobe. 7. Caudal vena cava. **(B)** 1. Left and right superior vena cava. 2. Pulmonary arterial trunk. 3. Pulmonary arteries. 4. Caudal vena cava. 5. Branches for the right posterior lobe. 6. Branches for the right posterior lobe. 7. The branch of the right medial lobe. 8. Branches of the left medial lobe. Marked area (☆) suggesting hepatic hilum

The dorsal branch of the left lateral lobe (*lobus hepatis sinister lateralis*) has several terminal ramifications that branch into the parenchyma of the dorsal portion that be longs to the left lateral lobe of the liver. From the ventral part of the left lateral lobe branch 3/4 of tributaries.

The branches of the left medial lobe are ramifications of the left branch of the portal vein). A single large dorsal branch drains blood from the dorsal portion of the hepatic left medial lobe. The other branches are smaller and drain blood from the remaining part of the left medial lobe.

The need to complete the information already existing in the specialized literature regarding the anatomical description of the vascularization of the venous system and its variations in the Wistar rat has also been stated by other anatomists who have conducted studies in this regard. The use of this species as a research model in experimental surgery is justified due to the reported multiple morphological similarities of the venous system of the Wistar rat with the human one (Vdoviaková et al., 2016; Martins and Neuhaus, 2007).

Our study used the technique of injecting the venous system with epoxy resin to present accurate knowledge of liver morphology and vascularization in the rat, which is a very important aspect when experiments are to be performed using this laboratory animal model. The results obtained cannot be exactly extrapolated to humans, but

there are currently studies, in which the rat liver has been compared to the human liver and have shown that the lobes of the rat liver correspond to the divisions of the human liver (Martins and Neuhaus, 2007; Vdoviaková et al., 2016).

Most authors consider the rat liver is divided into four lobes: the left lateral lobe, the medial lobe (left and right), the right lobe, and the caudate lobe (the caudate process or Spiegel lobe which in turn is divided into two portions-anterior/inferior and posterior/superior) (Vdoviaková et al., 2016, Martins and Neuhaus, 2007 described and named the lobes of the rat liver: middle lobe, left and right lateral lobe, right lobe, and caudate lobe or Spiegel lobe, which is divided into two portions, anterior and posterior. Madrahimov et al., 2006 state that the rat liver consists of 4 lobes, and Lorente et al. divided the rat liver into two parts: upper and lower liver, and 6 sectors: sector 1 (caudate process), sector 2 (caudate lobe), sector 3 (lower right lobe), sector 4 (right portion of the medial right lobe), sector 5 (central and left portion of medial right lobe plus medial left lobe) and sector 6 (lateral left lobe) (Martins and Neuhaus, 2007). Couinaud, 1994 reported that the human liver is divided into 8 segments.

Three hepatic veins divide the liver into four sectors (right lateral sector, right paramedian sector, left paramedian sector, and left lateral sector) and in turn, each sector receives a portal pedicle, which bifurcates and supplies each lobe (I-VIII). Later, the same author classified the paracaval portion of the caudate lobe as an independent segment (Martins and Neuhaus, 2007).

Human and swine anatomy are very similar; therefore, the pig is currently the most frequently studied potential source of organs and stem cells to be used in human medicine (Cooper et al., 2002; Cooper, 2003). Some studies have shown that the functional morphology of the rat liver differs from that of the human liver, but is similar to that of the pig (Lorente et al., 1995; Zanchet and Montero, 2002). The morphological relationship between the human and rat liver is still relatively undefined. However, among laboratory animals, the morphology of the rat is very similar to that of the human and morphofunctional, the rat liver resembles the pig liver but differs from the human one.

There is no direct communication between the portal system and the venous system of the rat liver, but each of the 6 sectors receives a portal branch and has its venous tributary (Lorente et al., 1995). However, at the microscopic level, interdigitations may occur at this level (Gershbein and Elias, 1954). In terms of branches, in the individuals studied, the splenic vein consists of 3 venous ramifications, namely the left gastric vein (*v. gastrica sinistra*), the right gastroepiploic vein (*v. gastroepiploica dextra*) or left gastroepiploic vein (*v. gastroepiploica sinistra*), and the cranial pancreaticoduodenal vein (*v. pancreaticoduodenalis cranialis*), which is in agreement with the existing data in the literature at the time. The cranial pancreaticoduodenal vein has been identified as an independent branch of the portal vein in our study, but in other studies, it has been tributary to the splenic vein (*v. lienalis*) (Vdoviaková et al., 2016).

The hepatic veins, specifically the veins draining the lower lobe and the upper lobe correspond to the right hepatic vein and the lower right hepatic vein in humans, with the right hepatic vein representing a variation of normal anatomy. Withal, the lower lobe and upper lobe were drained by a single vein in the individuals under study, but instances were reported in which these lobes were drained by the same branches mentioned above, each drained by two veins (Martins and Neuhaus, 2007). In addition, the vein draining the left median vein may enter the vena cava separately, or join with the left hepatic vein to form a common trunk before uniting with the suprahepatic vena cava (Martins and Neuhaus, 2007; Lorente et al., 1995; Gershbein and Elias, 1954). In the present study, the veins draining the left lobe and caudate lobe are drained by two large hepatic veins that open separately into the vena cava, but in some cases, they can be joined by a common trunk.

CONCLUSIONS

The technique of injecting the hepatic venous circulation, followed by tissue maceration, allowed the removal of all hepatic components and facilitates a clear delineation of all components of the venous system, from the largest to the finest branches. The technique utilized by intravascular injection of a polymer is a promising method in the assessment of the hepatic venous system, highlighting details regarding the venous branches, their caliber, and distribution.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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