POINT OF VIEW

Experimental models for hepatic encephalopathy

Daniel Díaz-Gómez, María Jover, José Antonio del-Campo, Antonio Galindo and
Manuel Romero-Gómez

UCM Digestive Diseases and CIBERehd. Hospital Universitario de Valme. Universidad de Sevilla. Sevilla, Spain

INTRODUCTION

Although there are many models for animal testing, the ideal model for chronic liver disease involving hepatic encephalopathy has not been described yet. Different problems associated with the models have led researchers to develop their own, sometimes unique experimental models, which makes difficult the comparison between the results of the conducted studies (1). Hepatic encephalopathy (HE) definition, nomenclature, diagnosis and quantification consensus were published in 2002 (2) where three types of HE were considered: type A, associated with acute liver failure, type B, associated with the existence of porto-systemic communication (by-pass) without intrinsic liver disease and type C, associated to liver cirrhosis. HE type C, in turn, is classified according to their form of presentation as spontaneous or episodic HE, in relation to precipitating factors, persistent HE is subdivided into mild (HE grade I), severe (HE II-IV) or treatment-dependent (early developed after abolition of treatment) and finally, the minimal HE, as the first manifestation of HE. HE type C is the most common form and from a clinical point of view, the episodic HE type because of liver cirrhosis decompensation is the most typical and relevant.

The ideal model of HE should reproduce most of the clinical features of this syndrome in humans, as occurring mainly in patients with chronic liver disease, being precipitated by defined factors, it should be reversible with the correction of precipitating factors and improved using rifaximin or ammonia lowering-drugs, being strongly associated with altered nitrogen metabolism and showing a wide spectrum of severity. Because most patients with HE also suffer from chronic liver disease, porto-systemic shunts, developed hyperammonemia and systemic inflammation it is highly desirable that animal models would include these facts (Fig. 1).

INDUCED CIRRHOSIS IN RATS BY CARBON TETRACHLORIDE

This model is reasonably easy to reproduce, although a significant number of animals die during CCl_4 treatment, especially if it is maintained until the onset of ascites. Multiple research groups have emphasized, moreover, that are usually detected little change in behavior despite very advanced and decompensated state of liver disease is achieved. Although the well-compensated cirrhosis model can only be useful to investigate the effects of hyperammonemia (HA) (3), which is usually moderate, it has been shown that there is not a severe reduction of enzyme activity of the urea cycle (4), which is commonly found in patients with cirrhosis. For this reason, Snodgrass (4) stated that the model was inappropriate for the study of chronic liver diseases. It might be interesting to reproduce an advanced stage of cirrhosis, but the presentation of severe ascites complicates the interpretation of results regarding the animals' behavior. Thus, currently, CCL_4 model was not utilized for HE research proposes any more.

BILE DUCT LIGATION

Obstruction of the main bile duct induces a secondary biliary cirrhosis, similar to that of humans, so it appears in ani-

mals a secondarily liver failure, developing jaundice, portal hypertension (5), porto-systemic shunt (6), bacterial translocation and immune dysfunction (7). Bile duct-ligated rats do not develop encephalopathy despite ammonia levels can be increased (8), although these animals have shown a loss of spontaneous motor activity (9) and memory deficits (10). Multiple attempts were carry out to find a model using a biliary ligation based technique –easy to perform– able to develop episodes of unambiguous hepatic encephalopathy. In this sense, a model was described in which encephalopathy was manifested after a portacaval anastomosis in cirrhotic rats by bile duct ligation, although this model was not widely distributed, probably because of the high mortality of the surgical shunt in cirrhotic animals (11). It has recently been proposed a model that combines bile duct ligation with rats fed with ammonia enriched diet, which have been found to increase the ammonia levels in the blood and in the brain (8) but the presence of behavioral changes in these animals, suggesting the development of encephalopathy, has not been found yet. Bile duct obstruction precludes testing biliary-secreted drugs. Nevertheless, BDL model is easy to perform and allow us to study ammonia metabolism in combination with an inflammatory environment. In preliminary studies, it has been shown that the reduction of ammonia with L-ornithine phenylacetate (OP) is effective in BDL model. Administration of OP results in increased conversion of glutamate to glutamine by stimulation of glutamine synthase activity in the muscle with the subsequent excretion of phenylacetylglutamine in the urine, a reaction in which one molecule of ammonia is removed. Also, OP resulted in normalization of glutaminase activity in the gut, indicating that OP effectively restricts the production of *in vivo* ammonia in a BDL model (9). These findings suggest developing approaches to target these enzymes to prevent ammonia release and HE is a valid therapeutic strategy in BDL model.

PORTOCAVAL SHUNT IN RATS

Many studies have been performed in rats with portacaval shunt (PCS) which have revealed some key knowledge about the hyperammonemia in liver diseases (12) (Fig. 2). Initially, doubts arose about the adequacy of the model to reproduce the symptoms in animals that could be equivalent to human HE, but according to Bengsston and co-workers (13-15) as well as other authors (16,17), changes in behavior have been demonstrated in this model, especially in the areas of spontaneous activity in response to a new environment and exploratory activity. Moreover, it was found that these changes were reversible by applying to the animal effective treatment for HE and, as mentioned before, the model can be relevant for studying the HE. Specifically, Conjeevaram and co-workers (18) demonstrated that by adding neomycin to drinking water to rats with PCS the motor activity was reduced after exposing them to a new and dark environment, while Coy and colleagues also did so using a more sophisticated technique
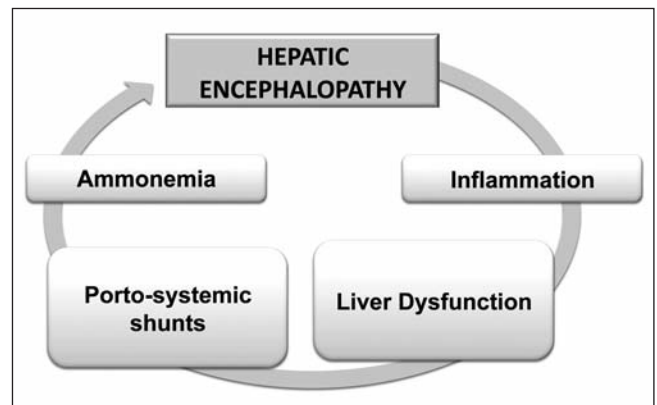

Fig. 1. Physiopathology of hepatic encephalopathy.

by measuring disturbances of the circadian motor activity (19). This group also demonstrated that operated rats shown the same improvement when they were subjected to low protein diet. It should be remembered, however, that the mere fact of subjecting rats to a PCS cannot guarantee the development of behavioral changes. Aspects such as the surgical technique employed or the size of the stoma of the microvascular anastomosis, which can have a significant influence on the shunt pressure gradient; or the diet provided, the time from the intervention until the study is performed in rats and even the age and weight of animals, may affect the ability of researchers to detect significant changes (20,21). Furthermore, it should be noted that hepatic neovascularization may occur spontaneously developing hepatopedal shunts, which are associated with recovery of weight and decrease in HAM and sub-clinical HE in these animals.

Lee and Fisher (22) in 1961 and Bismuth in 1962 (23) described a technique of direct portacaval anastomosis, reliable and reproducible, produced by vascular microsuture, which even today continues in force by virtue of its good results and reproducibility by different research groups. However, because of the technical difficulty that is associated, there have been several attempts to simplify it further. Funovics (24) in 1974 described a technique of anastomosis mediated by using a Teflon tube that was intended to avoid direct suturing and Jerkins (21) in 1988, one in which the junction between the portal and cava veins was performed using cyanoacrylate adhesive, with the intention to facilitate handling of microsurgical vessel and therefore, the technique itself. However, further studies comparing these alternative types of shunts described by Bismuth and Lee concluded that the pressure gradient between the portal and cava systems were lower after surgical shunts. This would suggest that the microsurgery anastomosis maintain a rate of long-term viability higher than the alternative shunts, which presented higher gradients because of their greater tendency to be in the postoperative period. This would explain the variability of results that had been previously described using these alternative surgical techniques (20).

1. Infrahepatic vena cava (left) and portal vein (right) exposure

2. Lateral clamp and junctions of the vena cava and portal vein

Numata Microsurgery 1983;4:243-4

3. Latero-lateral portocaval anastomosis with continuous suture

4. Ligation and section of the proximal portal vein, and final situation with a functioning end-lateral portocaval anastomosis
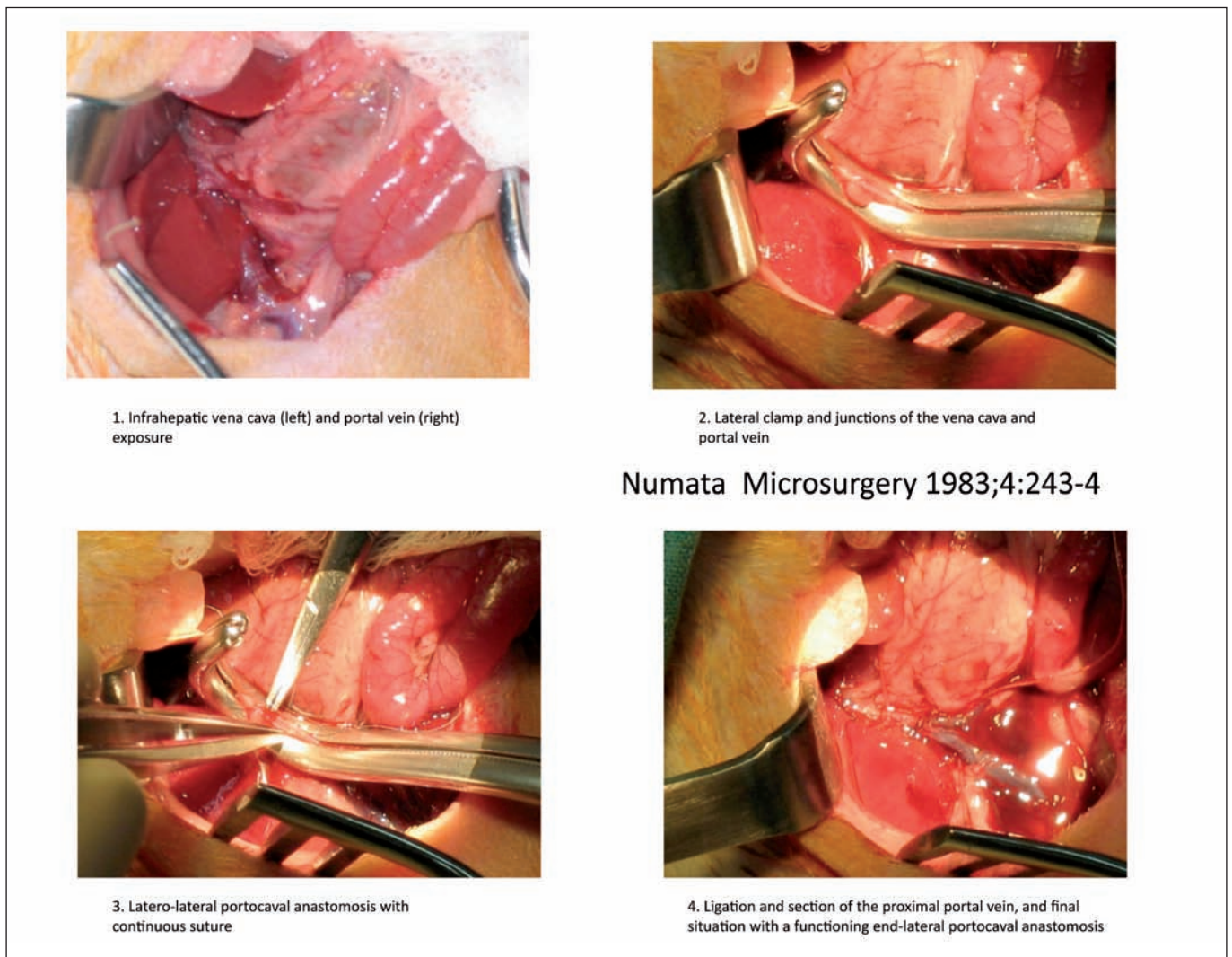
Fig. 2. Four steps of portacaval shunt in rats.

Numata published in 1983 a modification of the technique that greatly simplifies and makes it easier and more reproducible to minimize the operative time, a key issue in the viability of the animal after the intervention (25). This technique is based on a veno-venous microsurgical anastomosis side-to-side between the porta and cava veins of the rat, allowing a greater caliber of the shunt-side-end anastomosis for Lee, Fisher and Bismuth, with a theoretically lower rate of long-term stenosis. Moreover, this technique allows its use as side-to-side anastomosis, depending on how is left as following completion of the surgical shunt, or end-side, if it is tied and cut at the proximal side of the portal vein immediately after making anastomosis, enabling application to experimental studies based on different purposes (Fig. 3).

Since neither the psychometric tests nor clinical diagnosis for HE can be achieved in rats, we need to use alternative methods. Several authors have detailed some reflex tests and evaluating responses to stimuli (26), difficult to interpret, or computerized activity measurements (13,14) or circadian rhythms (19), which represent a forward step in this sense. According to Mullen (27), rats subjected to PCS have an uncertain response of the spontaneous motor activity, so even doing an adequate shunt; a large number of animals is required to demonstrate significant behavioral changes in operated animals compared to controls, despite the use of more sophisticated methods of measurement. Different systems have also been postulated to measure evoked potentials (28) or the analysis of encephalographic patterns (29), with low validation of data in HE animal models.

Another crucial aspect is the technique of the shunt. A wide sutured stoma, made with low vascular occlusion and an expert surgeon are the basis of a lasting time porto-systemic circuit and will be able to reproduce properly the effects of HE, compared to techniques in which the shunt
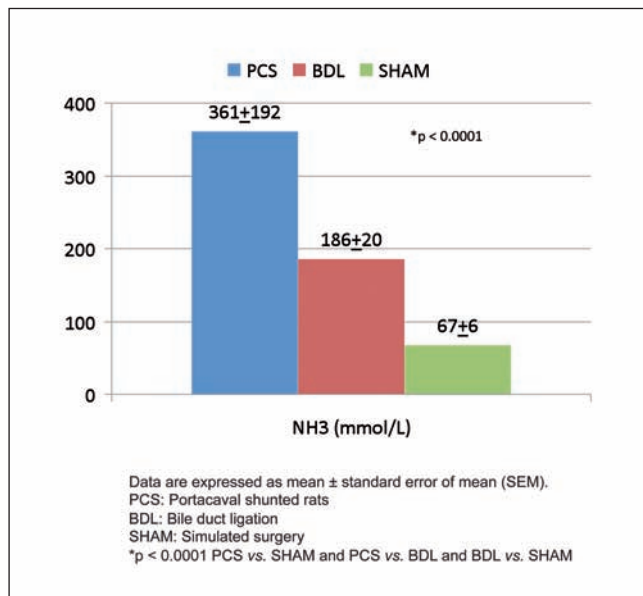
Fig. 3. Plasma ammonia levels in simulated surgery, portacaval shunted and bile duct ligation rats.

Table I. Ten leap to perform portacaval shunt

1. To use inhalation anesthesia
2. Wide dissection of porta and cava veins
3. Avoiding vascular injury
4. Correct lateral clamp of both veins
5. Clamp time shorter than 15 minutes
6. Stomas should be 5 mm at least
7. Braided suture 7/0 with cylindrical needles
8. Hemostasis after declamping by soft compression
9. Ligation and portal vein section
10. Isolation of animals in separate cages to avoid injuries

is mediated by a "button" or made with cyanoacrylate, associated with the reversibility of their effects (27). The complexity of studies to assess encephalopathy in rats in terms of their behavioral changes and lack of standardization, has led us to limit the validation of the model to weight loss and the study of biochemical changes that take place in the hepatic encephalopathy syndrome.

A review article has been recently published in relation to animal models for the study of HE (30) that stipulates that PCS in rats would be an appropriate model for the type B- EH, where there is a porto-systemic by-pass without liver failure. However, as noted by Gandhi (31), "the PCS is a model of liver atrophy for over 100 years", referring to the work of Hahn in the nineteenth century (32) and characterized in great detail in Bismuth's studies (33) during the 60s of last century. In his early work was described as "the appearance of the liver at laparotomy performed a few days later to the rats subjected previously to PCS is pale, and after sacrificing the animal and weigh it, there was a significant reduction in its size." This loss of liver size is not parallel to the reduction in weight of the rat, because the liver-weight / animal-weight ratio is also reduced (34). In the liver under the microscope, a secondary atrophy is observed that is not accompanied by changes in the composition in terms of the basic cellular components (water, lipids, carbohydrates or proteins) or the hepatic architecture. It seems to be related to hepatocyte atrophy, which begins very early after surgery and may last for several months during postoperative period (35). According to hepatocyte function studies, several changes have been demonstrated in these rats, as a decline in the production of bile salts (about 40%), a loss of conjugation of bilirubin, an alteration of the metabolism of biliary elimination of certain dyes,

such as indocyanine green –often used as a diagnostic method for assessing the hepatic functional reserve before hepatectomy in cirrhotic patients (36)–, alterations in platelet aggregation and hepatic drug metabolism (37).

Hemodynamic and hormonal factors may be involved in the genesis of this phenomenon. The PCS leads to a loss of blood flow through the liver, despite the significant increase, albeit not sufficient through the hepatic artery. The main arguments for this hypothesis derived from experimental observations where the atrophy is corrected almost completely after a portacaval transposition or modification of the portal vein (38). Moreover, the responsibility on the phenomenon of loss in substances passing through the liver with hepatotropic effect can be argued, mainly with pancreatic origin and that will affect liver regeneration. Among these, insulin and glucagon have been the most extensively studied (39). Bircher agrees "the highlights taking place in this model are the porto-systemic shunt and loss of functional liver parenchyma (40), implying that the model may be considered appropriate to represent alterations in HE type C" (which is associated with LC).

Neuron-anatomical lesions that appear in the encephalopathy of liver disease are similar to those found in patients with severe hyperammonemia by genetic defects of the enzymes of the urea cycle. These findings were first described by Von Hosslin and Alzheimer in 1912 (41) and in the case of liver disease, these lesions are usually reversible after correction of the increased level of ammonia. These cells show a very pale enlarged nucleus, sometimes deformed, with a prominent nucleolus and a marginal pattern of chromatin surrounded by a thin cytoplasmic layer, named as Alzheimer type II cells (42). It is not uncommon in these patients to find a spongiform degeneration of the deeper layers in the brain cortex and the basal glia (43). There are no studies on different experimental models of HE where the neuro-anatomical changes are described completely (44), although some authors have demonstrated the presence of characteristic Alzheimer cells type II after the completion of PCS in rats, considered as the most characteristic neuron-anatomical alteration of HE (45-48).

Pilbeam and co-workers published in 1983 (44) a study on rat brain subjected to PCS and sacrificed at different time points after surgery that questioned the presence of

Alzheimer type II astrocytes in the operated animals. They studied the brains of only 8 rats (2 animals with 2, 4, 16 and 30 weeks after surgery). They found that there were not significant differences in ammonia plasma values between operated and control animals (none of them the mean value of plasma AM was even double that of the control group). In a study performed by our group where 24 rats were used, 8 and 16 under PCS in two control groups of 8 each (control and sham), the values of ammonia plasma of operated rats were found 4 to 5-fold higher than control animals (49). Because of the details of the surgical technique, such as the extent of the stoma between the portal and cava veins, often have important results regarding the ability to reproduce the hyperammonemia in the liver (20,21), we thought that perhaps there were some problems in the surgical technique, leading to the low levels of plasma ammonia in rats reported in the study, responsible for abnormal brain histology.

PORTOCAVAL SHUNT WITH ADDITIONAL HANDLING

The idea of reproducing the symptoms of a more severe encephalopathy in rats (and occasionally in dogs) has been the origin of the design of different manipulations in the operated animals. A maneuver with potential utility is the administration of ammonium resins to rats with PCS, which apparently causes a reversible encephalopathy. Ammonium managed by other means (for example i.v.) clearly produces coma total, while still is not determined what this means in human. Potentially, this coma is associated with induced brain edema, even though there is no evidence on this issue. Furthermore, there is no data demonstrating the reversibility of the coma. Normal rats which were fed with ammonium acetate developed hyperammonemia with a similar degree to rats with PCS, and yet they were resistant to the time-point administration of additional ammonia, but the rats with PCS were, as mentioned above, in the opposite situation (50).

The superposition of an acute toxic hepatitis with dimethyl-nitrosamide in dogs with PCD has been shown to induce a severe HE (51). Alternative ways to induce HE on animals with PCS included blood extraction or the intake of high amounts of protein in the diet, which was effective in dogs, but did not in rats. Partial hepatectomy has also been used in some cases in PCS rats, or PCS to rats with cirrhosis induced by tetrachloride carbon, but changes in animal behavior were not reported.

Different works have been recently published using a complete biliary ligation in rats with previous PCS, which induced an acute or subacute cholestasis (11). This model has not been fully characterized and has the added difficulty of inducing a severe weight loss, probably caused by anorexia or malabsorption.

In summary, the ideal HE animal model remains elusive. Portocaval shunted rat remains as the gold standard method for HE in animals. Easier to perform models like bile duct ligation could be useful as complimentary model. The usefulness of adding methods require further studies. Portacaval shunt in CCL_4-induced cirrhotic rats could be intriguing but extremely difficult with higher rat mortality. Hyperammonia-based diet or thiacetamide-induced liver failure could also be taken in mind when developing combination models. Portacaval shunt is a good model for minimal hepatic encephalopathy and could be useful for the development of new therapeutic agents.

REFERENCES

1. Mullen KD, McCullough AJ. Problems with animal models of chronic liver disease: suggestions for improvement in standardization. Hepatology 1989;9(3):500-3.
2. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy -definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002;35(3):716-21.
3. Bengtsson F, Bugge M, Vagianos C, Jeppsson B, Nobin A. Brain serotonin metabolism and behavior in rats with carbon tetrachloride-induced liver cirrhosis. Res Exp Med (Berl) 1987;187(6):429-38.
4. Snodgrass PJ. Urea cycle enzyme activities are normal and inducible by a high-protein diet in CCl4 cirrhosis of rats. Hepatology 1989;9 (3):373-9.
5. Kountouras J, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rat. Br J Exp Pathol 1984;65(3):305-11.
6. Dunn CW, Horton JW, Megison SM, Vuitch MF. Contribution of portal systemic shunt to Kupffer cell dysfunction in obstructive jaundice. J Surg Res 1991;50(3):234-9.
7. Greve JW, Gouma DJ, Soeters PB, Buurman WA. Suppression of cellular immunity in obstructive jaundice is caused by endotoxins: a study with germ-free rats. Gastroenterology 1990;98(2):478-85.
8. Rodrigo R, Jover R, Candela A, Compan A, Saez-Valero J, Erceg S, et al. Bile duct ligation plus hyperammonemia in rats reproduces the alterations in the modulation of soluble guanylate cyclase by nitric oxide in brain of cirrhotic patients. Neuroscience 2005;130(2):435-43.
9. Huang LT, Hsieh CS, Chou MH, Chuang JH, Liou CW, Tiao MM, et al. Obstructive jaundice in rats: cause of spatial memory deficits with recovery after biliary decompression. World J Surg 2004;28(3):283-7.
10. Jover-Cobos M, Noiret L, Habtesion A, Balasubramaniyan V, Sharifi Y, Romero-Gómez M, et al. The mechanism behind synergistic action of L-Ornithine and Phenylacetate to reduce ammonia in Cirrhotic rats. Hepatology 2010;52(Supl.);893A.
11. Maddison JE, Dodd PR, Morrison M, Johnston GA, Farrell GC. Plasma GABA, GABA-like activity and the brain GABA-benzodiazepine receptor complex in rats with chronic hepatic encephalopathy. Hepatology1987;7(4):621-8.
12. Cooper AJ, Lai JC. Cerebral ammonia metabolism in normal and hyperammonemic rats. Neurochem Pathol 1987;6(1-2):67-95.
13. Bengtsson F, Nobin A, Falck B, Gage FH, Jeppsson B. Portacaval shunt in the rat: selective alterations in behavior and brain serotonin. Pharmacol Biochem Behav 1986;24(6):1611-6.
14. Bengtsson F, Nobin A, Falck B, Gage FH, Jeppsson B. Effect of oral branched chain amino acids on behavior and brain serotonin metabolism in portacaval shunted rats. World J Surg 1988;12(2):246-54.
15. Bengtsson F, Bugge M, Brun A, Falck B, Henriksson KG, Nobin A. The impact of time after portacaval shunt in the rat on behavior, brain serotonin, and brain and muscle histology. J Neurol Sci 1988;83(1):109-22.
16. Tricklebank MD, Smart JL, Bloxam DL, Curzon G. Effects of chronic experimental liver dysfunction and L-tryptophan on behaviour in the rat. Pharmacol Biochem Behav 1978;9(2):181-9.
17. Herz R, Sautter V, Robert F, Bircher J. The Eck fistula rat: definition of an experimental model. Eur J Clin Invest 1972;2(6):390-7.
18. Conjeevaram HS, Nagle A, Katz A, Kaminsky-Russ K, McCullough AJ, Mullen KD. Reversal of behavioral changes in rats subjected to

portacaval shunt with oral neomycin therapy. Hepatology 1994;19 (5):1245-50.

19. Coy DL, Mehta R, Zee P, Salchli F, Turek FW, Blei AT. Portal-systemic shunting and the disruption of circadian locomotor activity in the rat. Gastroenterology 1992;103(1):222-8.

20. Coy DL, Srivastava A, Gottstein J, Butterworth RF, Blei AT. Postoperative course after portacaval anastomosis in rats is determined by the portacaval pressure gradient. Am J Physiol 1991;261(6 Pt 1):G1072-G1078.

21. Jerkins AA, Steele RD. Diet composition and surgical technique influence the postoperative recovery of portacaval shunted rats. Hepatology 1988;8(4):855-60.

22. Lee SH, Fisher B. Portacaval shunt in the rat. Surgery 1961;50:668-72.

23. Bismuth H, Benhamou JP, Lataste J. Experimental portacaval anastomosis in the normal rat. Technic and preliminary results. Presse Med 1963;71:1859-61.

24. Funovics JM, Cummings MG, Shuman L, James JH, Fischer JE. An improved nonsuture method for portacaval anastomosis in the rat. Surgery 1975;77(5):661-4.

25. Numata M. A modified technique to make a portacaval shunt in rats. Microsurgery 1983;4(4):243-4.

26. Rigotti P, Jonung T, James JH, Edwards LL, Peters JC, Fischer JE. Infusion of branched-chain amino acids and ammonium salts in rats with portacaval shunts. Arch Surg 1985;120(11):1290-5.

27. Mullen KD, Birgisson S, Gacad RC, Conjeevaram H. Animals models of hepatic encephalopathy and hyperammoniemia. In: Felipo V, Grisolía S, editors. Hepatic encephalopathy, hyperammoniemia and ammonia toxicity. New York: Plennum Press; 1994. p. 1-10.

28. Jones DB, Mullen KD, Roessle M, Maynard T, Jones EA. Hepatic encephalopathy. Application of visual evoked responses to test hypotheses of its pathogenesis in rats. J Hepatol 1987;4(1):118-26.

29. Bosman DK, van den Buijs CA, de Haan JG, Maas MA, Chamuleau RA. The effects of benzodiazepine-receptor antagonists and partial inverse agonists on acute hepatic encephalopathy in the rat. Gastroenterology 1991;101(3):772-81.

30. Jover R, Madaria E, Felipo V, Rodrigo R, Candela A, Compan A. Animal models in the study of episodic hepatic encephalopathy in cirrhosis. Metab Brain Dis 2005;20(4):399-408.

31. Gandhi CR, Murase N, Subbotin VM, Uemura T, Nalesnik M, Demetris AJ, et al. Portacaval shunt causes apoptosis and liver atrophy in rats despite increases in endogenous levels of major hepatic growth factors. J Hepatol 2002;37(3):340-8.

32. Hahn M, Massen O, Nenecki M, Pavlov J. Die Eck sche fistel zwischen der unteren Hohlvene und der Pfortader und ihre Folgen fur den Organismus. Arch Exp Pathol Pharm 1893;32:161-210.

33. Oudea P, Bismuth H. Experiemental portacaval anastomosis in normal rats. Study of hepatic lessions by means of electron microscope. Pathol Biol (Paris) 1965;13:288-96.

34. Terlunen E, Altenahr E, Becker K, Ossenberg FW. Liver atrophy following portacaval shunt in normal rats –a morphometric study–. Res Exp Med (Berl) 1977;170(2):133-42.

35. Bioulac P, Dubuisson L, Saric J, Despuyoos L, Bedin C, Magne F, et al. Chronic biliary drainage corrects liver atrophy induced by portacaval shunt in the rat. Gastroenterology 1981;80(5 pt 1):1023-30.

36. Miyagawa S, Makuuchi M, Kawasaki S, Kakazu T. Criteria for safe hepatic resection. Am J Surg 1995;69(6):589-94.

37. Castaing D, Bismuth H. Portacaval anastomosis in the rat: 20 years of experimental models. Gastroenterol Clin Biol 1984;8(5):469-79.

38. Castaing D, Beaubernard C, Ariogul O, Gigou M, Franco D, Bismuth H. Liver atrophy and encephalopathy after portacaval shunt in the rat. Eur Surg Res 1982;14(3):192-202.

39. Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, et al. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973;137(2):179-99.

40. Bircher J. The rat with portacaval shunt: an animal model with chronic hepatic failure. Pharmacol Ther [B] 1979;5(1-3):219-22.

41. Von Hosseln C, Alzheimer A. Ein Beitrag zur klinik und pathologischen anatomie der Westphal- Strumpellschem pseudosklerose. Z Neurol Psychiat 1912;8:183-209.

42. Butterworth RF, Giguere JF, Michaud J, Lavoie J, Layrargues GP. Ammonia: key factor in the pathogenesis of hepatic encephalopathy. Neurochem Pathol 1987;6(1-2):1-12.

43. Adams RD, Foley JM. The neurological disorder associated with liver disease. Res Publ Assoc Nerv Ment Dis 1953;32:198.

44. Pilbeam CM, Anderson RM, Bhathal PS. The brain in experimental portal-systemic encephalopathy. I. Morphological changes in three animal models. J Pathol 1983;140(4):331-45.

45. Cavanagh JB, Kyu MH. Colchicine-like effect on astrocytes after portacaval shunt in rats. Lancet 1969;2(7621):620-2.

46. Cavanagh JB, Kyu MH. On the mechanism of type I Alzheimer abnormality in the nuclei of astrocytes. An essay in quantitative histology. J Neurol Sci 1971;12(3):241-61.

47. Cavanagh JB, Lewis PD, Blakemore WF, Kyu MH. Changes in the cerebellar cortex in rats after portacaval anastomosis. J Neurol Sci 1972;15(1):13-26.

48. Kyu MH, Cavanagh JB. Some effects of porto-caval anastomosis in the male rat. Br J Exp Pathol 1970;51(2):217-27.

49. Romero-Gomez M, Jover M, Diaz-Gomez D, de Teran LC, Rodrigo R, Camacho I, et al. Phosphate-activated glutaminase activity is enhanced in brain, intestine and kidneys of rats following portacaval anastomosis. World J Gastroenterol 2006;12(15):2406-2411.

50. Azorin I, Minana MD, Felipo V, Grisolia S. A simple animal model of hyperammonemia. Hepatology 1989;10(3):311-4.

51. Zeneroli ML, Baraldi M, Ventura E, Vezzelli C, Tofanetti O, Germini M, et al. Alterations of GABA-A and dopamine D-2 brain receptors in dogs with portal-systemic encephalopathy. Life Sci 1991;48(1):37-50.