

Three Aspects Are Critical for Carrying Out Intraportal Islet Transplants Successfully in a Diabetes Mouse Model

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Intraportal islet transplant is an effective and common treatment for type 1 diabetes in clinical practice, though it is still not the most ideal method.¹ Early massive loss of islets after transplanting, resulting from inadequate oxygen tension and rejection, restrict the wider application of intraportal islet transplant in clinical practice.^{1,2} Scientists have been seeking to improve outcomes of intraportal islet transplant for many years.³⁻⁶ Mice, such as nonobese diabetic mice and NCR athymic nude (nu/nu) mice, are ideal tools for basic research because of the genomic and technologic advantages that the mice have.^{7,8}

Implanting islets into the liver via the portal vein successfully is the basis of intraportal islet transplant study. However, it is not easy work if the operator is not well experienced or does not have surgical training required. Our team has been engaged in studying intraportal islet transplant in the diabetes mouse model for 2 years. We find appropriate anesthesia, correct puncture, and hemostasis have a definite effect on completing the transplant and further research. During research, we have accumulated valuable experience that is critical for carrying out intraportal islet transplants successfully. The aim of the letter is to share our experience with those who are carrying out intraportal islet transplants but with poor results. We hope this article will help them to work better.

Appropriate anesthesia

Pay close attention to the animal anesthesia. Appropriate anesthesia is the basis of the entire work, as it ensures that the transplant goes on without the animal struggling, and the results of the study are reliable and the animal can survive after the operation. Inhaled anesthesia using isoflurane is strongly recommended. First, isoflurane gives the operator better control of the depth and time of anesthesia. Second, isoflurane is barely metabolized through the liver, so there is little hepatotoxicity.⁹ Third, the anesthetic effect would not be influenced, even though there is interference with the function of the mouse liver.^{10,11} To sum up, isoflurane is preferable when studying intraportal islet transplant. The common injection anesthetics, such as ketamine and pentobarbital sodium, should not be used. They cannot control the depth and time of anesthesia as well as isoflurane. They are also metabolized mainly by the liver and the kidney. When islet suspension is infused into the liver, liver function will be disordered. Metabolism of these anesthetics are interfered with, and eventually lead to death of the mouse.^{12,13} Meanwhile, the reliability of the study may be in doubt as the product of metabolism.

It is worth noting that the dosage of anesthetic is also important. The dosage may be influenced by mouse strain, sex, age, weight, the state of health and nutrition, even different batches of the same strain. Thus, the appropriate dose of each batch, in each strain, should be searched for before the study. Under anesthesia, the operator must pay close attention to the state of mouse to avoid excessive anesthesia, as an inexperienced operator is prone to give the mouse an overdose of anesthetic.

The depth and frequency of respiration, as well as mucous membrane color, are useful indicators of the anesthetic state; they should be observed during the entire procedure of anesthesia. Maintaining

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body temperature is also essential. Thermo-regulation of the mouse will be disrupted under anesthesia. The body's temperature will fall quickly and lead to hypothermia if the operator does not adopt the corresponding measure timely. Hypothermia may result in abnormal physiological states,¹⁴ intolerance of surgery, and further—death of the mouse. In our laboratory, we put a heated pad that can maintain a temperature of 25°C to 30°C between the anaesthetized mouse and operation table to avoid hypothermia during the surgery.

Correct method of puncture

Puncturing the portal vein is the core of the entire procedure. The correct puncture method can ensure that the entire bevel of needle is inserted into the portal vein lumen rather than transfixing it, and all the islets are infused into the liver successfully. The correct puncture point is important. The puncture point should be done easily and nearer to the liver on the premise that nerve and the other tissues are not injured. According to the anatomic structure of the mouse portal vein, the position between the first and second tributary vein, near the porta hepatic vein, is the ideal choice. The hepatic artery and nerve circumvolve the portal vein between the porta hepatic and the first tributary of portal vein, so if puncturing there—the hepatic artery and nerve might be injured. Furthermore, the liver also might be injured when the entire bevel is inserted into vein lumen at this position.

The inferior region of the second tributary of the portal vein is also unsuitable for puncture. The pancreas circumvolves the portal vein at this position, so it is hard to puncture the vein and may injury the pancreas. This position is far from the liver, and more liquid is required to force the islets into the liver. Excessive liquid may cause the mouse to have heart failure and die.¹⁴

Next, orientation of the bevel inserted into the portal vein is important to improve success of the puncture. We do not suggest that the bevel be inserted upwards. The portal vein is located in the middle and posterior region of the abdominal cavity, so the vein is easily transfixed when the bevel is inserted upwards, as the angle between the puncture needle and the vein is too big. If possible, a needle with short bevel is a better choice, as a short bevel reduces the risk of trauma to the endothelial wall of the vein.¹⁵

Hemostasis

Bleeding is one of the most-common complications of this surgery, and often leads to death. The portal vein is a major vessel in the abdomen, which collects blood from the small intestines, stomach, spleen, and pancreas. The puncture wound by a common 26-gauge or 27-gauge scalp vein needle is not small enough for the portal vein of mouse. Therefore, if the operator cannot stop the bleeding in a timely and effective manner, a large quantity of blood will flow from the puncture point. At the beginning, we try to stop bleeding by a cotton swab as some scientists do,^{16, 17} but we find that it is not a good choice, as it often causes bleeding again when we take the swab off.

We tested the gauze ball and cotton ball, but they still cause bleeding when taking them off. Finally, we chose an absorbable hemostatic gauze. The gauze can stop the bleeding, and decrease the risk of bleeding again as there is no need to take it off after use. The absorbable hemostatic gauze has antimicrobial function that can protect the puncture site from infection.¹⁸ It must be noted that the amount used of hemostatic gauze cannot be too little or too much. The bleeding cannot be stopped if too little, but the portal vein may be compressed if there is too much. In our laboratory, 5 layers of gauze are superimposed together, and then cut into many 0.3 cm × 0.3 cm pieces, one at a time.

Successful intraportal islet transplant in a mouse model demands that the operator knows the anatomic structure of mouse, and has a basic knowledge on anesthesiology, surgery, and pathophysiology. The 3 aspects mentioned above are important for the work. We hope our experiences help to raise the success rate of the operation.

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