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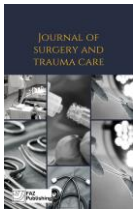

Spleen Autotransplantation in Rat

Adlina Mohammad[1,a], Affirul Chairil Ariffin[1,b], Fadlul Azim Fauzi Mansur[1,c] , Wan Omar Abdullah [1,d] Asral Wirda Ahmad Asnawi[1,e], Anuar Sani[1,f], Razrim Rahim[1,g*]

[1] Faculty of Medicine and Health Sciences,
Universiti Sains Islam Malaysia, MALAYSIA

*Corresponding Author

Email: [g]razrimrahim@usim.edu.my

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Abstract: This paper describes the surgical procedure for spleen autotransplantation in rats. Splenectomy is an important surgery protocol in research studies involving immunology and hematopoietic diseases. Spleen autotransplantation is an alternative method undertaken to preserve the splenic function as the absence of spleen (asplenia) is associated with lifelong risk for sepsis, especially by encapsulated bacteria. **Method:** Four six weeks old male Sprague-Dawley rats, with range of weight from 248-281 grams, underwent spleen autotransplantation via midline laparotomy incision. In this procedure the spleen was completely removed (total splenectomy); it involved removing the spleen and re-transplanted in greater omentum. A piece of the spleen weighing approximately 0.34 grams was transplanted inside the greater omentum and held in place with an absorbable suture knot. The muscle layer and skin incision were then closed using absorbable suture. The surgery was conducted under deep sedation and the rat was monitored post-operatively until it fully recovered. **Outcome:** The post-operative recovery of the rat was uneventful. Six-weeks after the surgery, the rat underwent a re-look laparotomy and the auto-transplanted spleen was examined. There was a three-fold increase in the size and weight of the spleen. **Conclusion:** This spleen autotransplantation model, which utilized an omental pouch, was not only successful in preserving the spleen, but also allowed for spleen regeneration. This model provides future platform for further research in auto-transplanted spleen.

Keywords: Splenectomy, spleen autotransplantation, surgery, rodent, immune system

1. Introduction

Splenectomy is a surgical procedure to remove the spleen. Splenectomy may be used to treat ruptured spleen mostly due to abdominal injury and in cases of splenomegaly due to blood disease such as thalassemia, sickle cell, and treat certain cancers, infection, and noncancerous cysts or tumors. Spleen is an important lymphatic organ that plays a major role in human immune system. Spleen is organized in regions called the red pulp and white pulp, which separated by interface called marginal zone (MZ).

The red pulp (RP) has a tight network of sinusoids that serves mostly to filter blood and recycle iron from the aging red blood cells. The white pulp (WP) has a large mass of lymphoid tissue, contains T cell and B cell zones that allow generation of antigen-specific immune responses that protect body against blood-borne bacteria, viral and fungal infections (Lewis et al., 2019). These splenic populations that initiate innate and adaptive immune response against pathogens. Marginal zone (MZ) contain dendritic cells and B cell (MZ B cell) capture and transport blood-borne antigen to WP for surveillance by T and B cells (Cerutti et al., 2013). Besides, the complex blood filtration and immunologic functions, spleen also act as a reservoir site of granulocytes and platelets. Therefore, if spleen does not work properly, it may lead to anaemia, bleeding or bruising and at high risk of infections. Therefore absence of spleen functions (asplenia) is associated with increase risk of infections. Patients who undergo splenectomy is at high risk of serious infections especially encapsulated organism such as *Streptococcus pneumoniae, Neisseria meningitidis, Escherichia coli, Haemophilus influenzae,* and *Staphylococcus.*

**Corresponding author: razrimrahim@usim.edu.my*

Spleen autotransplantation is a simple technical procedure aimed to compensate the absence of an intact spleen when its removal is inevitable. Clinical and experimental investigations have shown that spleen autotransplanted presents morphological regeneration and recover some functions of the spleen such as increase phagocytosis activity (Khorshidi et., 2017; Marques et al., 2012) and maintain normal immunological pattern of T-lymphocytes, B-lymphocytes, IgG and IgM levels (Resende et al., 2002) and lower inflammatory markers expression (Teixeira et al., 2008). Spleen autotransplantation is promising and widely investigated either in pre- or clinical researches.

Pre-clinical animal testing provides reasonable evidence prior to early feasible testing in humans clinical trials to demonstrate that intervention, treatments and therapies are safe and effective. Rodent is most widely used in pre-clinical research specifically rats and mice. This is due to the 3Rs (Replacement, Reduction and Refinement) rules and a cost–benefit analysis. Rats or mice are most preferred for an experimental that need large sample sizes. Rats and mice have high population with litter size varying from eight to twelve pups per breeding and the breeding cost is lower than in other larger animals such as rabbits, dogs and non-human primates. Even though non-human primates are more than 90 percent genetically identical with human, but it is unethical to use them as experimental model particularly study that involves invasive procedure such as operation particularly that involve organ removal. Besides, genetic and physiological variations of laboratory rat and mice have been developed as inbred strains which have highly homogeneous genetic composition to increase the reproducibility of results and the statistical power of experiments (Barré-Sinoussi and Montagutelli, 2015). Hence, this paper will describe the surgical technique of spleen autotransplant in rat model.

Materials

Sterile surgical instruments: Metzenbaum scissor, adson tissue forceps, blunt forceps, mosquitoe hemostatic forceps, towel clamp forceps, needle driver, absorbable suture (Vestriyl Plus, Malaysia), scalpel handle and blade.

Surgical scrub solution: 0.5% chlorhexidine gluconate, 70% isopropyl alcohol and 12% povidone iodine.

Anaesthetic agents: Isoflurane, ketamine/xylazine, or Avertin (tribromoethanol).
Others; surgical gauze, surgical drape, small crepe bandage, and hair clipper.

Methods

This study was approved by the Ethics Committee on Animal Research Universiti Sains Islam Malaysia (USIM/AEC/APRV/2019(1)). Four healthy six week old male Sprague-Dawley rats supplied by Animal Resources Unit, Faculty of Veterinary Medicine, UPM Malaysia, that weighing 248-281 grams was housed in ventilated cage at room temperature with a natural day/night cycle. For immunological study, rats aged around four to eight weeks is recommended as it is considered adult and immunological matured (Jakson et al., 2017). The rats were acclimatized to the new environment for a week and had free to access to water and standard pellets diet (Altromin Germany). After the acclimatization period, the rats were prepared for surgical operation (Figure 1) by inducing anesthesia through intraperitoneal injection of Avertin (240mg/kg).

Surgical Operation Protocol

Refer to Figure 1. (1) Firstly, The surgical site was shaved three times the size of the proposed incision area. (2) The shaved area was disinfected using multiple surgical scrub solutions (0.5% chlorhexidine gluconate, 70% isopropyl alcohol and 12% povidone-iodine). Surgical scrubbing was performed in a circular pattern, beginning in the center and spiraling outward, or in a unidirectional pattern. The rat was moved to the surgical area with the surgical site facing upward to the surgeon, and draped using sterile disposable waterproof drape (3M Steri-drape). (3-4) A single incision was made in ventral midline abdominal along the linea alba penetrating the skin and muscle layer using a scalpel blade and scissor. (5-6) The spleen was identified and the blood vessels were transfixed ligated with absorbable suture to maintain haemostasis. (7) The spleen was removed and cut into pieces and wash with Hartman solution at four degrees. Maltos Filho et al., (2018) found that splenic tissue preserved in Ringer-lactate solution at four degrees maintains its functional and morphological structure that allows its recovery after transplanted on the greater omentum. (8-9) A single piece weighing approximately 0.34 grams was transplanted inside the greater omentum and held in place with an absorbable suture knot. The omental pouch and remaining part were place back into the abdominal cavity. (10-11)The muscle layer and skin incision were closed by continuous and subcuticular sutures using absorbable Vestriyl Plus 4/0 suture respectively (Worldwide Medivest, Malaysia). Absorbable subcuticular suture was reported to reduce surgical site infection (Bou et al., 2015) and less irritation to the rat. (12) Lastly, the wound was dressed with surgical gauze and small crepe bandage secured with surgical tape (10).

Post-operative Care

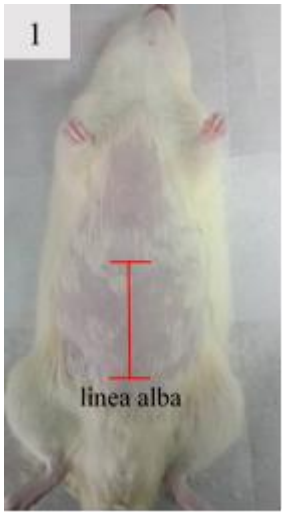
Post-operative care is important for rat's refinement, to prevent any complications and ensure the rat return to its normal state. Hypothermia is one of the common causes of death after surgery. Rodents such as rats and mice are prone to hypothermia during anaesthesia for surgery due to the inhalation of cold gases, disinfectant solution during surgical scrubbing and having body cavities exposed to the open air (Wenger, 2012). Therefore, during and after the operation, rat should be kept warm by placing it on a warming pad or under an infrared lamp. The rat is monitored every one hour until it fully recovers from the anaesthesia and in recumbency position before transferring into a housing cage. Then rat can be group together after at least 24-hour recovery post operatively. The use of analgesia to control post-operative pain management is an important component commonly neglected by surgeons. Hence, it is recommended to administer analgesia pre-operatively and post-operatively. The first seven days post-operative is a critical period where it is necessary for surgeons to assess the incision site daily to ensure any presence of unexpected signs of illness or infection. If the rat showed greater losses in weight and abnormal behaviour, an endpoint should be decided. Signs of infection include redness, swelling, discharge (purulent or serous), pain, or dehiscence (opening of the incision) (Pritchett-Corning et al., 2011). In general, animal loses a small amount of weight after surgery, but with proper analgesia and the provision of food, they will regain the weight quickly. Morbidity score is essential to assess and monitor the severity of disease in experimental model and to decide the euthanized point. In this study, Murine Sepsis Score (Appendix 1) was adapted from Shrum et al., (2014) to evaluate the rat's

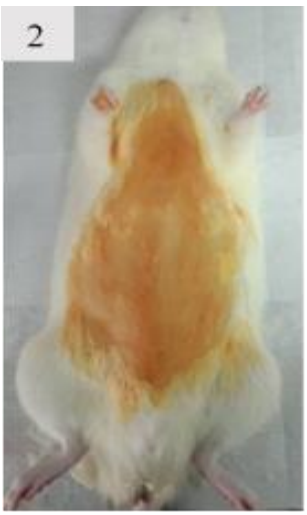
morbidity. Table 2 show the morbidity and mortality score of the experimental rats.

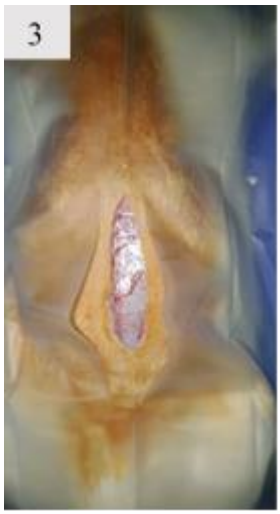
Result and Discussion

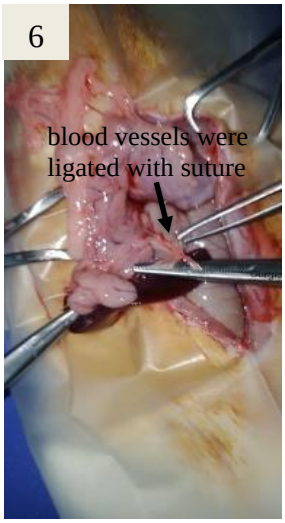
Spleen has complex functions including blood filtration and immunologic functions, in addition of hematopoietic and reservoir site of platelets, and granulocytes. Splenectomy after any trauma or diseases may lead to serious complications known as overwhelming post-splenectomy infection (OPSI), which may appear in the form of fulminant sepsis. Fulminant sepsis is commonly caused by encapsulated pathogens such as pneumococcus, *Haemophilus influenzae*, *Escherichia coli*, and meningococcus. Overwhelming post-splenectomy infection (OPSI) and fulminant sepsis ensuing splenectomy have been associated with significant morbidity with 50%-70% mortality rate (Standage & Goss, 1982). In recent years, vaccination and antibiotics against these bacteria is administered and provided for all post splenectomy patients. However, OPSI still develops in isolated cases and remain a lifelong problem in these patients. Yong et al., (2010) reported that splenectomized patients exposed to OPSI have the relative risk of death within 90 days post-splenectomy. Therefore, the intact splenic mass with good blood supply in an injured spleen should be preserved possibly by suturing or through bioplasts, tissue adhesives, meshes, spleen resection techniques. If these surgeries are not possible, splenectomy followed by autotransplantation is recommended.

Splenic preservation by autotransplantation has been pursued in both humans and animals and has been showed that implant tissue can act like the normal spleen. Greater omentum is the common spleen autotransplantation site because of the favorable revascularization, portal drainage, and contain inflammatory cells for omental bacterial clearance (Wilkosz et al., 2005; Miko et al., 2001). In this study, the rat survived until the tissue taking. The operation time was 30±5 minutes exclude the duration taken for analgesia. The recovery was uneventful with increasing body weight (Table 1) indicated the rat was healthy without any sign of infection with no mortality. The transplanted spleen showed regeneration of tissue (Figure 2) proving that spleen autotransplant to the greater omentum was successful. This finding was consistent with Marques et al., (2014) where the autotranplanted splenic tissues in rats showed regenerated mass approximately 54% at 24 weeks post-operative. Another study also proved that autotransplant able to preserve spleen functions, recover the blood deformability, increase splenic phagocytosis and reserved the structure and viability of transplanted spleen tissue (Khorshidi et al., 2017; Sajtos et al., 2012). It can be concluded that spleen preserving surgical techniques have a great significance in the clinical practice, in order to prevent possible complications originated from asplenia or hyposplenia. The implantation of splenic fragments on the greater omentum seems to be a safe and useful procedure for maintaining splenic function after total splenectomy. Future research is warranted to study the immunological function of the autotransplanted spleen and its effectiveness.









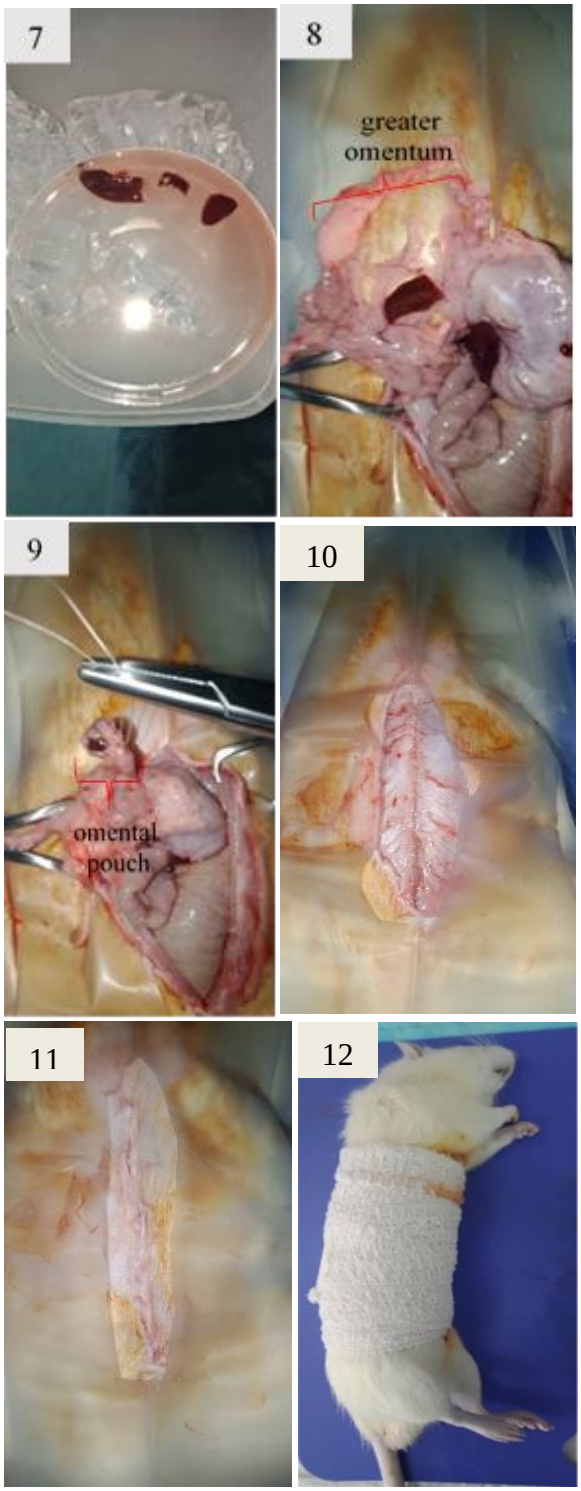


Fig.1 - The spleen autotransplantation procedure in male Sprague-Dawley rat

Table 1 - Post-operative body weight, initial and final transplanted spleen weight (n=4)

	Rat 1	Rat 2	Rat 3	Rat 4
Body weight (g)	280	267	281	248
Week 0	313	297	324	281
Week 1	329	309	335	313
Week 2	357	339	361	330
Week 3	391	367	378	364
Week 4	413	381	388	378
Week 5				
Weight of spleen (g)	0.35	0.37	0.34	0.34
Initial	1.25	1.38	1.18	1.24
Final				

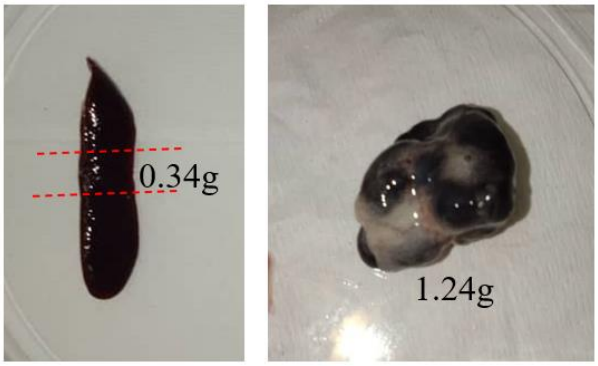


Fig. 2 - Regeneration of spleen tissue. Transplanted splenic tissue of Rat 4 (0.34g) showed 3-fold increase in size and weight (1.24g) after 6-week post-operation.

Table 2 - Morbidity score of rat after spleen autotransplantation (n=4).

		Rat 1	Rat 2	Rat 3	Rat 4
Appearance	Week 1	4	4	4	4
	Week 2	3	3	3	3
	Week 3	3	3	2	2
	Week 4	1	1	1	1
	Week 5	1	0	0	0
Level of consciousness	Week 1	1	1	1	1
	Week 2	0	0	0	0
	Week 3	0	0	0	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0
Activity	Week 1	1	1	1	1
	Week 2	0	1	0	0
	Week 3	0	0	0	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0
Response to stimulus	Week 1	0	0	0	0
	Week 2	0	0	0	0
	Week 3	0	0	0	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0
Eyes	Week 1	0	0	0	0
	Week 2	0	0	0	0
	Week 3	0	0	0	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0

Respiration rate	Week 1	2	2	2	2
	Week 2	1	1	1	1
	Week 3	0	0	1	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0
Respiration quality	Week 1	2	2	2	2
	Week 2	1	1	1	1
	Week 3	0	0	0	0
	Week 4	0	0	0	0
	Week 5	0	0	0	0

Conclusion

From the result, the operation was successfully conducted with zero mortality rate. The post-operative recovery of the rats was uneventful.

Acknowledgement

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Conflict of Interest

The authors declare that there is no conflict of interest.

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