Acta Medica Okayama

Volume 25, *Issue* 6 1971 *Article* 2

OCTOBER 1971

Improvement of the method of rats parabiosis with aortic anastomoses

C. H. Fang*

*Okayama University,

Improvement of the method of rats parabiosis with aortic anastomoses*

C. H. Fang

Abstract

This paper describes various aortic parabiotic procedures and discusses various problems concerning these procedures. The most satisfactory results, nearly 100 % of survival rate, can be achieved using longer sections of aortae of 2 to 5.month old rats. In these rats blood circulation between the parabionts has completely been established.

*PMID: 4264053 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **25**, 597—603 (1971)

IMPROVEMENT OF THE METHOD OF RATS PARABIOSIS WITH AORTIC ANASTOMOSES

C. H. FANG

Department of Pathology, Okayama University Medical School, Okayama, Japan
(Director: Prof. S. Seno)

Received for publication, Oct. 11, 1971

Parabiosis, the surgical union of the animals or grafting of one animal onto another has proved to be often an indispensable tool in research field of hematology, immunology and so on. As pointed out by KAWAI (1), parabiosis by skin and muscle anastomosis and celio-anastomosis of skin (2, 3, 4) does not give satisfactory results. By these methods, no one can expect the free exchange of the blood components or body fluid between two animals although capillary anastomosis may occur after the third postoperative day (5). And the average rates of the blood varied between 0.64 and 1.8 per cent of the blood volume per minute (6), and the time needed for complete mixing of cross-exchange of the labelled red cell ranges from 1 1/2 (7) to 24 (8) hours. All these data of the cellular constituents of the peripheral blood in irradiated parabionts by celio-anastomosis showed a great difference; *i. e.* red cell and white cell counts of the non-irradiated partner were usually 2 times greater than the irradiated one. But sealing the mouth of one rat of the parabionts 10 days after celio-anastomosis resulted in the death of the mouth sealed one by 5 to 6 days (1). Then, through these observations KAWAI tried to give complete blood exchange between parabionts by aortic anastomoses. Consequently, he has established the method of arterial parabiosis with "parallel anastomoses" by transplanted homologous thoracic aortae, in which abdominal aorta of one rat is transversely anastomosed with that of the other by using the aortae obtained from different rats of the same strain (Fig. 1).

When operation was accomplished, naturally peripheral blood showed the equal distribution of red cells and white cells in both animals, indicating the complete blood exchange between the parabionts. This method is a valuable tool in various fields, *e. g.*, hematology, immunology, etc. But the successful rate of the operation was very low because of the technical difficulties.

The author therefore tried to raise the successful rate, and finally,

succeeded in obtaining nearly 100 % of survival rate by improving the technique. Various technical problems of the operation concerning the original and the improved techniques will be described in this paper.

MATERIALS AND METHODS

1. *Animals*:

Seventy-six pairs of male Wistar rats of 4–5-month-old weighing 380 to 420 g were used. Parabiotic partners were of the same litter chosen from those of nearly the same body weight, less than 20 g in difference. In this experiment, all parabiosis was made between healthy rat and x-ray (1,000 r) whole body irradiated rat on the third day after irradiation.

2. *Procedures of parabiosis*:

(A) *Preparation for aortae transplantation*:

Some pairs were parabiosed in the same direction by cross-anastomoses of aortae. This way of parabiosis was unadaptable because of technical difficulties and early death of the parabionts. Therefore, the parabiosis in the opposite direction by parallel anastomoses of aortae with transplantation of the homologous aortae was adapted for experimental pairs.

The aorta for grafting was cut at the adequate portion as shown in Fig. 3. The canule was inserted into the aorta, the branches of the aorta were ligated with gut (No. 1) at the root, and were cut at the distal portion together with surrounding tissues. By this procedure, a pair of transplanting aortae was taken out. After taking out the aortae, the aortae were washed by injecting saline solution containing a small quantity of sodium heparin and agglutinated blood masses in aortae were removed. On this condition the removed aortae are ready to be transplanted. Up to this step it took about 20–30 minutes for one pair. The aortae were preserved in cold physical saline solution (Fig. 4). Simultaneously one pair of the rats for parabiosis was carried to a good anesthesized condition for operation. For anesthesia non-irradiated rat received the injection of Nembutal (50 mg/ml, Abott) 0.4–0.6 ml and atropin sulfate (0.5 mg/ml) 0.1 ml into gluteal muscle, the irradiated partner was injected less dose 0.3–0.5 ml of Nembutal. The anesthesized rats were disinfected by bathing with diluted osban solution.

Two-cm incision in length was made along left postauricular line about one cm below costal margin. On the middle point of the line and at right angle another incision reaching to vertebral column was made (Fig. 2a). When the subcutaneous tissues and muscles were cut with scissors and abdominal aorta was exposed by tearing of fat tissues with fingers, the abdominal aorta and rt. renal artery were carefully separated from renal vein and surrounding connective tissues for the following operation of anastomosis. And the rt. renal artery was ligated at the distal portion with gut (No. 2), the ends of which were left about 5cm and tied together at the furthermost ends. Pulling up slightly the ends of the gut, rt. kidney was denuded from the surrounding tissues and capsule with fingers, the rt. ureter and rt. renal vein were ligated together with gut (No. 5), and the rt.

kidney was finally removed. The abdoimnal aorta of the partner rat was also exposed, and the rt. kidney was removed by the same procedures just described.

Two animals were placed side by side in opposite direction, and the abdominal opening weres conjugated on both sides sewing up together, skin to skin, and muscle to muscle, at the three points with gut (No. 7).

(B). *Transplantation of aortae* (Fig. 2b and 2c):

(1) *Dissected distal end of aorta of the hosts in each parabiont*:

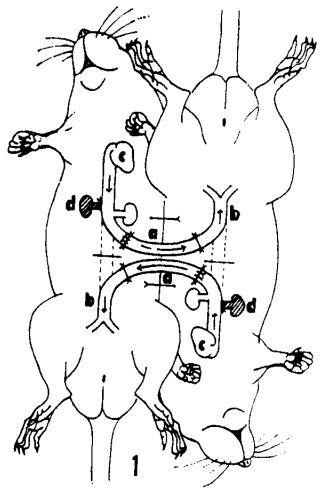
By using two small clips, the exposed abdominal aorta was clipped after separating carefully with tweezers from its surrounding tissues at two points just below the point of the bifurcation of abdominal aorta and ileolumbal artery and at the lower part of the abdominal aorta. Then the aorta was cut at the middle point as shown in Fig. 2b. The portion of the bifurcation of lt. and rt. common iliac artery was depressed with a forefinger to prevent bleeding, the clip of the distal end was taken away and the aorta prepared for transplantation with a steel canule inserted into the distal abdominal aorta. End-to-end anastomosis was performed with 8.0-nontraumatic filament nylon suture. The anastomosed portion was cemented with Alone Alpha (Sankyo Co.) in order to avoid thrombus formation due to infolding of the adventitial tissues into the inside of the anastomosed aortae (Fig. 2-c). Then the canule was removed, and the grafted aorta was perfused with saline containing a small quantity of sodium heparin. Immediately after these procedures the proximal end of transplanted aorta was clipped.

(2) *Proximal end of dissected aorta of the hosts* (Fig. 2b, 2c, 2d and 2e):

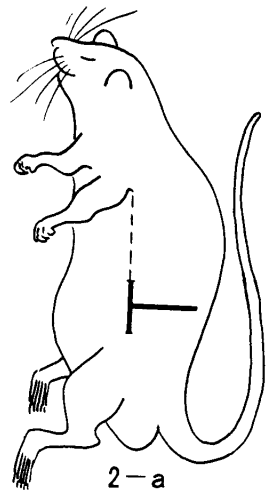
The abdominal aorta just above rt. renal artery was separated gently with smaller tweezers from abdominal vein and surrounding connective tissues. The portion was held with clip (c) to prevent bleeding. Then the clip (a) on the proximal end of dissected aorta was taken off (Fig. 2b). A steel canule inserted into the dissected proximal end of the host abdominal aorta and was pushed out through the wall of rt. renal artery nearby ligated portion. The clip at the end of the previously grafted aorta was moved to the distal side (clip (d)) and that canule was inserted into the grafted aorta of the partner (Fig. 2d). Both ends of the aortae were drawn close to meet. When the diameter of an aorta was larger than another, the diameter of the smaller one was extended by making a minor cut to adjust to the larger one. This will be described again in the section of discussion.

Anastomosis was made by 2-5 sutures followed by cementing with Alone Alpha. Immediately the canule was drawn out from rt. renal artery and about 0.5 ml of saline containing 0.1 ml of sodium heparin was injected into the renal artery and the clip (d) on the transplanted aorta was removed (Fig. 2e). When the anastomosis was not well made either leakage of injected saline or obstruction of flowing of the injected saline due to thrombus occulusion would be observed. Therefore, completely successful anastomosis is always required. After completion of the anastomosis, rt. renal artery was ligated with No. 2 silk at its origin.

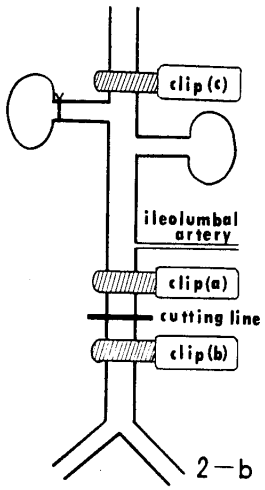
Immediately after completing all processes of aortic anastomoses the clips (d)



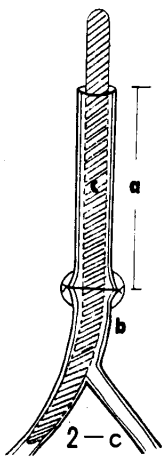
1



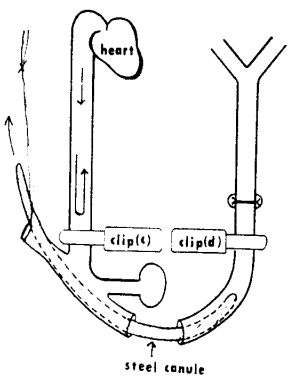
2−a



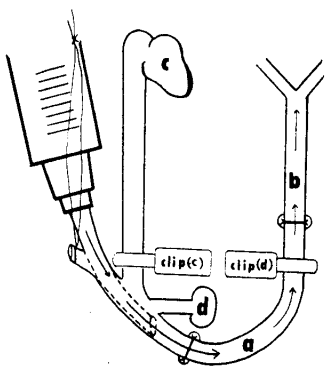
2−b



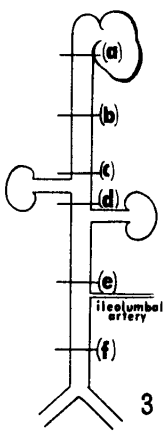
2−c



2−d



2−e



3

4

Fig. 1. Diagrammatic drawing of the parabiosis of rats with aortic anastomoses. a: transplanted aorta, b: abdominal aorta, c: heart, d: resected rt. kidney.

Fig. 2. Diagrammatic drawing of the process of operation

a): Site of incision for parabiosis. "T" indicates the site where the incision is made on lateral abdomen to open the peritoneal cavity.

b): Abdominal aorta is separated from the surrounding tissues and the abdominal aorta is clippd by 2 clipes at (a) and (b), and dissected on the middle line. Then the lower cut end is connected with the aorta for transplantation. Then the aorta is clipped at (c) and the clip (a) is removed. Next, rt. renal artery is ligated and the rt. kidney is cut off. The upper cut-end of the abdominal aorta is conjugated with the free end of the transplanted aorta of the partner. Finally the clip (c) is removed.

c): For the conjugation of aorta a steel canule, about 5 cm in length and 0.8 cm in diameter, is inserted into aorta, ligated with nylon fiber and cemented with Alone Alpha. a: transplanted aorta, b: abdominal aorta, c: steel canule.

d): Conjugation of the upper cut-end of aorta with the aorta for transplantation. From the upper cut-end of aorta the needle is inserted into rt. renal artery and the needle end is pushed out through renal artery. Another end of the needle is inserted into the free end of the transplanted aorta.

e): End-to-end anastomosis was done, then the needle is pulled out from the renal artery, and the conjugated area is washed with saline by using curved injection-syringe, and the root of the renal artery is closed by ligation. a: transplanted aorta, b: abdominal aorta of the partner, c: heart, d: It. kidney.

f): Diagrammatic drawing of the parabiosis of rats with aortic anastomoses. a: transplanted aorta, b: aorta, c: heart, d: resceted rt. kidney.

Fig. 3. Relation between the rat age and the useful part of the aorta for transplantation. (a)-(f); see text.

Fig. 4. Picture of two aortae for transplantation. Taken from healthy adult rats just before transplantation and preserved in cold saline.

Fig. 5. Picture of the parabiosed rats 3 days after operation.

on both transplanted aortae were removed first, followed by the removal of the clip. (c) on the aortae of both hosts (Fig. 2-e and Fig. 1). At the end of successful procedures the pulses of transplanted aortae were clearly observable. Fig. 5 shows the parabionts with aortic anastomoses on third-postoperative day.

DISCUSSION

A parabiosis made by aortic anastomoses developed by KAWAI is a very useful tool in the studies of hematology, immunology and other various fields of experimental pathology. Nevertheless, the original KAWAI's method proves to be very difficult to duplicate by the author. He used thoracic aortae of 1 to 2-month old rats for transplantation since the diameter of the thoracic aortae was almost the same as the diameter of the host abdominal aortae.

The author recognized the difficulties in using thoracic aortae mainly due to its short length and often resulting in splitting apart on the walls of anastomosing aortae during the procedures. In addition to these problems, the diameter and the thickness of the walls of thoracic aortae are not always similar to those of the host abdominal aortae. The author, therefore, attempted to improve the original method in respect to obtaining enough length of transplanting aortae and adjusting these diameters to the host abdominal aortae, as previously mentioned. The adjustment of two aortae having different diameter was carried by making minor incision in adequate length on the smaller one. By this procedure, the aortae of various aged rats were anastomosed satisfactorily.

The most useful parts of the aortae for transplantation were determined depending on various ages of rats used and given as follows (Fig. 3):

One to 2-month old rats: The part between the upper end of thoracic aorta (a) and just above the aortae at the origin of rt. renal artery (c). In this case both resected ends of aorta have the same inner diameter of three to four-month old abdominal aorta, as already described by KAWAI.

Two to 3-month old rats: The part between the upper portion of the origin of rt. renal artery (d) and upper end of the thoracic aorta (a).

Three to 4-month old rats: The part between the upper portion of the origin of the lumbal artery (e) and the middle portion of thoracic aorta (b).

Four to 5-month old rats: The part between the lower portion of abdomial aorta (f) and the lower portion of thoracia aorta (c).

One to 2-month old rats supplied too short aortae, while other older rats supplied aortae long enough to be well transplanted without forming thrombus or splitting apart the anastomosed aortae. This is the key to be

accomplished in this operation.

KAWAI reported that the cross-anastomoses with polyethylene tubes were not suitable tool because of vascular obstruction in the anastomosed portions within 24 hours. The author tried it again, and obtained the same results. Further, the author tried the cross-anastomoses in the same direction of two animals. It became clear that it's not a suitable method becauce of various technical difficulties. In addition to this, in this instance, no symmetrical blood exchanges could be obtained and it means early death of parabiotic animals.

SUMMARY

This paper describes various aortic parabiotic procedures and discusses various problems concerning these procedures. The most satisfactory results, nearly 100 % of survival rate, can be achieved using longer sections of aortae of 2 to 5-month old rats. In these rats blood circulation between the parabionts has completely been established.

ACKNOWLEDGEMENT

The author is indebted much to Professor SATIMARU SENO for valuable advices throughout this work and painstaking proof-reading of the paper. Many thanks are also due to my wife for her encouragement and assistance and Dr. KAWAI for the demonstration of "aortic anastomoses" by his method.

REFERENCES

1. KAWAI, T.: Stem cell in peripheral blood: A study by parabiosis of irradiated and non-irradiated animals; I. New approach to parabiosis as a method for hematologic study. *Acta Med. Okayama* **23**, 257, 1969
2. BERT, P.: Greffe animale, Compt. rend. *Soc. de Biol.* **15**, 20, 1863
3. BERT, P.: Expérience et considération sur la greffe animale. *J. Anat., Paris.* **1**, 69, 1864
4. SAUERBRUCH, F. and HEYDE, M.: Ueber parabiose künstlich vereinigter Warmblüter. *Münch. med. Wschr.* **55**, 153, 1908
5. EICHWALD, E. J., LUSTGRAF, E. C. and STEINER, M.: Genetic factors in parabiosis. *J. Nat. Cancer Inst.* **23**, 1193, 1959
6. VAN DYKE, D. C., HUFF, R. L. and EVANS, H. M.: The efficiency of the vascular union in parabiosis. *Stanf. med. Bull.* **6**, 271, 1948
7. FLEMING, D. C., DALDWELL, L. and JACOBS, R.: Determination of rate of cross circulation in parabiotic rats with P^{32}-labeled erythrocytes. *Amer. J. Physiol.* **196**, 753, 1959
8. DOMÉNICO, A. D. and ANDREOTTI, H. T.: Mechanism of splenic protection against 》 Bartonella muris 》 in the parabiotic rat. *Amer. J. Physiol.* **197**, 795, 1959