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RENAL HOMOTRANSPLANTATION WITH VENOUS OUTFLOW OR INFUSION OF ANTIGEN INTO THE PORTAL VEIN OF DOGS OR PIGS

TRANSPLANTATION AT PORTAL SITE[1]

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SUMMARY

Kidneys were transplanted in mongrel dogs so that renal venous drainage was into the portal system of the hosts. Thirty-one recipients were not treated, 11 were given one dose of 3 mg of azathioprine per kg, and 11 were given 2 mg of azathioprine per day. Survival was not statistically increased compared with that in three comparable series in which renal venous drainage was into the vena cava, nor were the histopathological findings favorably altered in the "portal" kidneys. The injection of semisoluble antigen into the portal vein at the same time as renal transplantation at the caval site, had an effect no different from that if the antigen were given systemically during caval site transplantation. The conclusion that drainage of grafts into the portal vein was not beneficial was reached in 20 pigs evenly divided between the portal and vena caval sites, and in 12 pairs of dog to pig or pig to dog xenografts. Thus, none of these experiments has identified an advantage of antigen delivery into the portal as opposed to the systemic venous system.

There have been conflicting reports about whether or not rejection is reduced in homografts transplanted so that their venous drainage is into the portal circulation and then through the liver. Consequently, the question of portal site privilege, with or without azathioprine treatment, was examined again in mongrel dogs and in pigs using a kidney transplant model. In addition, the effect of donor semisoluble splenic antigen given via the portal versus systemic routes upon canine renal homograft survival was tested.

MATERIALS AND METHODS

Dog Homotransplantation

Randomly selected mongrel dogs that weighed 9 to 27 kg were operated upon with sodium pentobarbital anesthesia supplemented with phencyclidine hydrochloride and succinyl choline. Experiments were set up with weight-matched pairs. After bilateral nephrectomy, each dog received a kidney of its partner in the right subhepatic space (Fig. 1). The left kidney was usually used as the homograft, but if it had a multiple arterial supply, the donor right kidney was used instead. After removal, the kidneys were flushed free of blood with cold lactated Ringer's solution. The postoperative course was monitored by serial measures of blood urea nitrogen.

All animals that did not live a full 5 days were excluded from the final analyses, since the early deaths were almost always associated with technical accidents, acute respiratory complications, intussusception, massive infection, or hemorrhage. The number of experiments to obtain 53 completed portal site experi-

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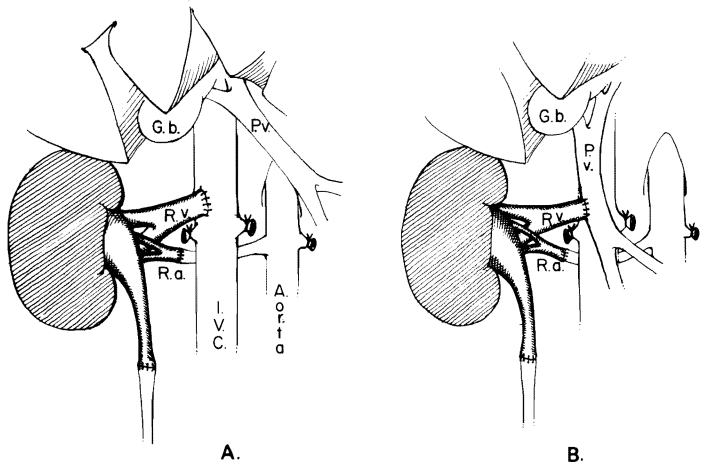

FIGURE 1. Experimental models. A, Left kidney of canine donor is placed in the recipient right renal fossa with renal venous drainage into the inferior vena cava. B, Same as A but with renal drainage into the portal vein.

ments was 80. The number of experiments to obtain 53 systemic site experiments was 84. The majority of discarded experiments in both portal and systemic outflow models was when azathioprine was given chronically.

For statistical analysis, credit for duration of survival of a given animal was limited to 40 days. The autopsy tissues were fixed in 10% formalin. Light microscopic criteria of rejection were used that have been described in past publications (*22*).

The experimental groups are outlined in Table 1. In groups 1, 3, and 5, the graft renal artery was anastomosed to the recipient renal artery, the renal vein was drained into the inferior vena cava, and end to end ureteroureterostomy was performed (Fig. 1A). In groups 2, 4, and 6, the same technique was used, except that the end of the renal vein was anastomosed to the side of the superior mesenteric or portal vein (Fig. 1B). When used, azathioprine was given i.v., and in groups 5 and 6 conversion was made to the oral route as soon as the dogs ate.

Dog Antigen Infusion and Renal Homotransplantation

Semisoluble antigen was prepared from the spleen of a mongrel donor according to the method of Brent and Kilshaw (*4*), as they modified it from the method of Medawar (*19*), and suspended in 400 ml of heparinized lactated Ringer's solution. The following day, the donor kidneys were removed at reoperation and one each was transplanted to the vena caval site (Fig. 1A) of two mongrel recipients. As the transplantation was being performed, one-half (200 ml) of the heparin containing semisoluble antigen suspension was infused into the jugular vein in control animals or into a mesenteric vein tributary in the test animals. Thus, each dog received the antigen equivalent of one-half a spleen. The mg wet spleen tissue per kg recipient was usually about 1,600 mg, but this varied considerably.

No immunosuppression was given. The same criteria were used to judge the results as described in the preceding section. To obtain 15 dogs with survival for at least a full 5 days (6 with systemic and 9 with portal antigen) 25 experiments were necessary.

TABLE 1. Experimental groups with canine renal transplantation

Series	No. of dogs	Renal venous drainage	Azathioprine day of operation (mg/kg)	Daily azathioprine postoperative (mg/kg)
1	32	Vena cava	None	None
2	31	Portal	None	None
3	11	Vena cava	3	None
4	11	Portal	3	None
5	10	Vena cava	3	2
6	11	Portal	3	2

Pig Homotransplantation

Landrace and large white pigs weighing 9 to 14 kg were used, with random donor recipient pairing. The conditions of anesthesia and care were essentially the same as with the dogs, except for appropriate diet adjustments. Survival for 5 full days was also requisite for inclusion in the final analyses. To obtain 10 complete portal site experiments, 13 transplantations were required. For 10 vena caval experiments, 15 transplantations were done. The most common causes of the 8 deaths before 5 days were thrombosis of the renal artery (4 examples) and pulmonary consolidation (2 examples).

Previously described surgical techniques were used (*17, 18*). These were similar to those shown for the dog (Fig. 1), with two common deviations. First, arterialization was usually by anastomosis of a Carrel patch, or an aortic graft to the recipient aorta. In portal site experiments, donor vena cava homografts were frequently used to bridge from the renal vein to the portal vein. Usually, one donor was used for two recipients; one of these kidneys was placed at the portal and the other at the vena caval sites. All grafts were cooled with the same infusion technique as was used for the dogs.

Heterotransplantation

Acute experiments were performed with the same techniques as above by transferring pig kidneys to dogs in three pairs of experiments and dog kidneys to pigs in nine pairs. With one member of a pair, venous outflow was directed by anastomosis into the vena cava and with the other member, the anastomosis was to the portal vein. The outcome was judged by the gross appearance of the heterografts at successive times, by evaluation of serial biopsies, and by the volume of urine output.

RESULTS

Dog Homotransplantation

No treatment. About one-fifth of the 32 dogs of group 1 with the systemic renal venous drainage survived for 2 weeks or longer. Two animals lived more than 1 month and one survived for 46 days. Similar results were obtained in the 31 animals of group 2 with renal venous drainage into the portal vein. Two of these dogs had survival of more than 1 month and one lived for 4 months (Fig. 2). The differences between groups 1 and 2 were not statistically significant.

There was essentially no difference in the histopathological findings in the homografts of groups 1 and 2 (Table 2).

One dose of azathioprine. A single dose of i.v. azathioprine slightly increased survival both in animals with systemic (group 3) and portal (group 4) venous drainage (Fig. 3), but this improvement was not statistically significant. There were no significant differences between groups 3 and 4. The only animal still alive at 40 days died after 44 days. The graft of that dog had systemic renal vein drainage.

Histopathologically, groups 3 and 4 were indistinguishable (Table 2).

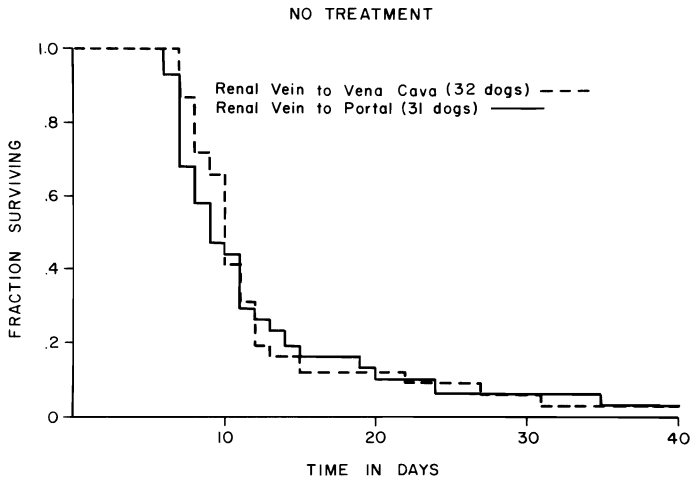

FIGURE 2. Portal versus systemic renal transplantation in untreated mongrel dogs.

TABLE 2. Pathological findings in canine renal homografts[a]

	Groups					
	1	2	3	4	5	6
No. in group	32	31	11	11	10	11
Rejection						
Total	25	27	8	8	6	7
Cellular only	6	5	2	1	4	3
Cellular + humoral	19	22	6	7	2	4
Normal	4	3	1	1	3	3
Portal vein thrombosis; no rejection	0	1	0	0	0	1
Cortical necrosis; no rejection	1	0	1	2	0	0
Renal artery thrombosis; infarction	2	0	1	0	1	0

[a] The groups are defined in Table 1.

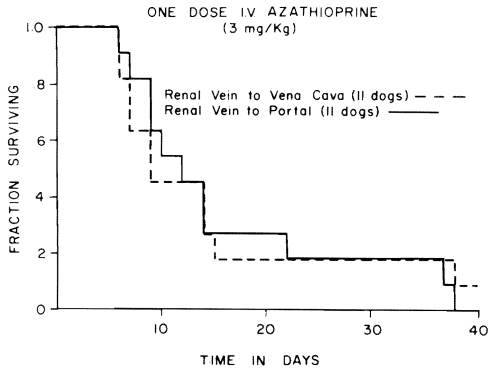

FIGURE 3. Portal versus systemic renal transplantation in dogs with one dose of 3 mg of azathioprine per kg on the day of operation.

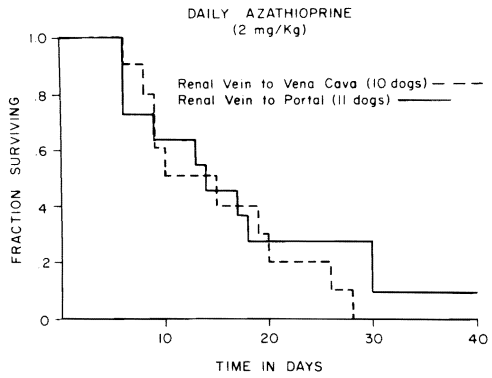

FIGURE 4. Portal versus systemic renal transplantation in dogs with chronic azathioprine treatment of low doses.

Chronic azathioprine. The survival of dogs in group 6 with portal venous drainage of the transplants was not significantly different from that of the group 5 animals with systemic drainage (Fig. 4), and both were slightly better than untreated controls. One of the portal site animals lived for 49 days.

Histopathologically, a protective effect of azathioprine was evident in groups 5 and 6 in that one-third of the homografts in each group was normal (Table 2).

Dog Antigen Infusion and Renal Transplantation

The administration of semisoluble antigen by the portal and vena caval routes at the same time as orthotopic renal transplantation did not significantly influence survival, which was 11.78 ± 6.26 (SD) days after the portal infusion and 12.67 ± 2.24 (SD) days after systemic infusion.

The histopathological findings were essentially the same in the two groups (Table 3).

Pig Homotransplantation

The survival was almost identical to transplantation to the inferior vena caval outflow site versus the portal site (Fig. 5). One animal in each group lived for about 1 month. The histopathological changes were almost the same in the two groups (Table 4).

Heterotransplantation

No gross differences in appearance or urine production were noted between transplantation at the portal and vena caval sites with either the pig to dog transplantation in which rejection occurred in a few min, or with the milder dog to pig transplantations in which rejection occurred after 30 min to 4 hr.

In the pig to dog renal xenografts, generalized and diffuse obstruction of all of the glomerular capillaries by aggregated platelets and

TABLE 3. Histopathological findings in orthotopically placed dog renal homografts after intraoperative infusion of semisoluble antigen via the portal vein and inferior vena caval routes

	Portal vein	Inferior vena cava
Total homografts	9	6
Rejection		
Total	7	6
Cellular only	1	1
Cellular + humoral	6	5
Normal	1	0
Renal artery thrombosis; infarction	1	0

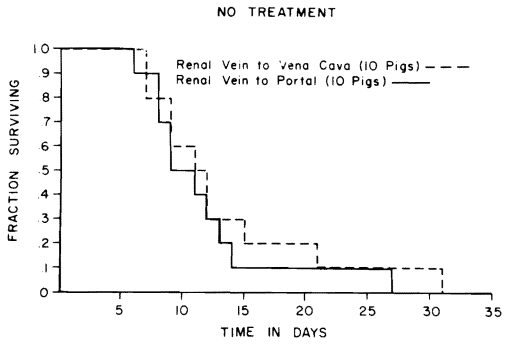

FIGURE 5. Portal versus systemic renal trans plantation in untreated pigs.

TABLE 4. Histopathological findings in pig kidney homografts after transplantation to the portal vein or inferior vena caval sites

	Portal vein	Inferior vena cava
Total homografts	10	10
Rejection		
Total	10	10
Cellular	4	2
Cellular + humoral	6	8
Normal	0	0

clumped red blood cells was present 10 min after transplantation.

By contrast, most of the dog to pig renal xenografts appeared to be normal by light microscopy at this time, although electron micros copy did reveal early platelet aggregation. About 20 min later, however, there was obvious focal and segmental blockage of the glomerular capillaries by aggregated platelets and clumped red blood cells. These changes progressed and neutrophil polymorphonuclear leukocytes became prominent in the lesions, until by 2 hr after transplantation, all of the glomerular capillary loops were occluded. There were variations in the speed with which these events occurred in individual animals, but there were no significant histopathological differences between the grafts with venous outflow into the portal vein and those with systemic venous drainage.

DISCUSSION

Studies in the last 15 years have suggested or demonstrated that there is a reduced response to antigens that are introduced into the portal as compared with the systemic venous system (*2, 6, 7, 10, 14, 20, 24, 25*). Partial removal of the antigens by the liver and thus a diminished host reaction to their presence has been the most common explanation. Alternatively, the portal injection could have contributed to tolerance induction (*2, 8, 13, 16*). Pursuant to such reports, it was natural in a number of laboratories to test whether there was an immunological advantage for kidneys (*1, 11, 12, 15, 17, 18, 23*), hearts (*3*), pancreatic islets (*9, 26*), and parathyroid cells (*21*) transplanted into the portal circulation or liver.

The results have been variable. In rats, all reports have described a protective effect at the portal site but usually only with easy histocompatibility barriers (*3, 21, 23, 26*). Mazzoni et al. (*17, 18*) had positive experiments with pig kidneys but these findings were not confirmed by Hickman and Terblanche (*12*). Barker and Corriere (*1*), Fukada et al. (*11*), and May et al. (*15*) saw no amelioration of the rejection of primary canine kidney homografts that were drained into the portal vein. However, Fukada et al. (*11*) thought that sensitization directed to a second kidney from the same donor was thereby reduced.

The investigations herein reported failed to demonstrate any portal site privilege for primary transplants either in dogs or pigs. The canine results confirmed the negative findings of previous workers (*1, 11, 15*), and extended these conclusions to conditions in the dog of single-dose or low-dose chronic azathioprine treatment. Furthermore, concomitant delivery of semisoluble antigen into the portal vein had an effect no different from that of systemic antigen, and neither route produced a prolongation of survival of transplanted kidneys. The negative experiments were not different from the earlier ones by Calne et al. (*5*).

The results in pigs were different from those

observed several years ago by one of us (*17, 18*). Failure to reproduce the earlier work might be attributable to differences in the pigs in widely separated parts of the world, even though the breeds were the same. The pig as an experimental animal has been criticized as an unpredictable experimental model. Another possibility could have been the presence of inadvertent experimental bias in the earlier work, in which control experiments were carried out at a time different from the portal site experiments.

The importance of concurrent controls in dog and pig experiments is also illustrated in the studies herein reported. The number of long-term survivors in untreated mongrel dog recipients of portal site kidneys was so great that a therapeutic effect could have been inferred if historical control data had been accepted for comparison. Instead, it became clear that almost 10% of the untreated animals with homograft drainage through either the portal vein or vena cava had survived in excess of 1 month.

Under minimum treatment, these results were improved, both in terms of survival and histopathological criteria. Repeated doses of azathioprine that have been considered homeopathic by others had an effect. Thus, experiments that impute a zero effect of low-dose azathioprine alone upon survival contain a flaw in design, at least according to our data.

The better picture of control data or data from low-dose immunosuppression that is emerging is in part ascribable to the culling out of experiments in which the animals fail to live 5 full days. In untreated animals, the cause of early death was almost always for anesthetic or technical reasons. These factors are usually irrelevant or confusing in evaluating the antirejection effects of the drug or other system being tested. Thus, the propriety of removing the artifacts caused by the earlier deaths seems incontestable.

On the basis of these investigations, it seems unlikely that any specific benefit could be expected in patients from renal transplantation to the portal vein. The question that remains is whether there really is an amelioration of rejection in rats and mice. As already mentioned, any mitigating effects described to date have been small and seen only when there are minor histocompatibility barriers.

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