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Homologous testis transplantation in dogs

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Abstract There is growing interest in the possible use of homologous testis transplantation for the treatment of anorchia and male infertility. In order to test the surgical and immunological feasibility of this therapy, three series of experimental studies of homologous testis transplantation were carried out in dogs. In the first pilot study, four beagles from the same litter were transplanted using microsurgical techniques for end-to-end anastomosis of the testicular vessels and the vas deferens. These dogs received cyclosporin A (CyA) for 3 months after transplantation. The longest functional graft survival in this series was 163 days, strongly suggesting that long-term survival of a homologously transplanted testis graft is possible. A second series of operations was performed on ten mongrel dogs. The same surgical technique was employed and the series was divided into three groups. Group 1 received CyA monotherapy, group 2 a combination of CyA and prednisolone, and group 3 received no immunosuppression. The average graft survival time in this series was 18 days, significantly less than the 71 days in the first series. The dogs in group 2, however, had graft survival times that were three times longer than those in the other two groups, suggesting that CyA in combination with prednisolone yields the best graft survival. In the third series, five littermates received a testis graft after castration. Immunosuppression was achieved by administration of CyA and prednisolone for 3 months. In three out of five animals, the graft survived until the immunosuppressive therapy was suspended. Histological biopsies of the graft 3 months after transplantation showed the same maturation of sperm cells as in the control testis of the same dog. The results of the last series suggest that long-term survival of homologously transplanted testis grafts in dogs is, indeed, possible with the aid of CyA and prednisolone.

Key words Testis transplantation, dog · Dog, testis transplantation

Introduction

As a result of important developments in immunosuppressive therapy and of greater knowledge of transplantation immunology during the last few decades [7], homologous transplantation of organs has become an established therapy for regaining vital physical functions. Since immunosuppressive therapy is no longer associated with severe morbidity, attention can be focused on regaining function by transplanting organs that are not life-sustaining.

Anorchia, the absence of both testes, due to congenital or acquired causes, and primary or secondary hypogonadism are not life-threatening states but cause specif-

ic problems. Androgen deficiency in men is associated with osteoporosis and impotence [14]. When treated, it can cause fluctuations in libido and, in addition, periods of depression, anger, and fatigue, due to the time intervals associated with the administration of artificial androgen [5]. Infertility and the inability to cause pregnancy is another problem that can be solved by techniques of assisted reproduction but, up until now, not by the patient himself. In view of the relatively low risk of immunosuppressive therapy, homologous testis transplantation could be considered an alternative solution to these problems.

The purpose of the present study was to investigate the problems related to the technique of homologous testis transplantation and to the immunosuppressive therapy and, in so doing, to develop a usable model for patients. Because of their cooperative characters and because of the similarity in vessel diameter between them and humans, dogs were chosen as subjects for our experiments.

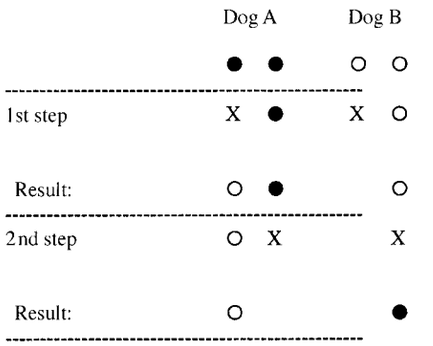

Fig. 1 Transplantation model, first operation. Dogs A and B are operated on at the same time by two surgeons. The first step is hemicastration. Dog A's right testicle is excised and kept for histological investigation; dog B's right testicle is transplanted into dog A (the recipient). Approximately 1 month later, the recipient becomes the donor. Both dogs' left testicle is excised. That of dog A is transplanted into dog B, and that of dog B is used to monitor histological pattern

Materials and methods

Three series of dogs were consecutively investigated. The first series initially consisted of six adult beagles from the same litter; however, two dogs could not be included in the study. There was one operative failure due to thrombosis of the vein, and one other dog was unfit for transplantation because of aggressive behavior. Thus, four animals remained for follow-up. The second series consisted of ten unrelated adult mongrels. For the third series, we again started with six adult beagle littermates, one of which could not be used as a recipient because of persisting wound infection after donor orchiectomy. Five animals thus received a graft and were included in this last series. The first series was considered to be a pilot study, the second was carried out to determine the effect of various types of immunosuppressive therapy, and the third was designed to further elaborate the data from the first two series.

In all three series, the investigative procedure went as follows. The operations were performed on pairs of dogs (A and B) and carried out in two steps with an interval of 1 month in between. In the first step, both dogs were unilaterally castrated, and the operation specimen from dog B was used as a graft in dog A. After 1 month, the former recipient became the donor and the former donor became the recipient, i.e. dog A's remaining testicle was removed and used as a graft in dog B. Thus, after completion of the second step, both dogs had one graft from the other and no remaining testicle of its own (Fig. 1). This two-step approach was taken for practical reasons. First and foremost, completing only one step of this model would have required too much narcosis time for the dogs and too much ischemia time for the grafts. Secondly, in this way, all of the dogs could be used for our experiments. As a result of this two-step approach, for 30 days after transplantation until hemicastration was performed, one half of the total number of dogs were producing testosterone from their own testis. The other half only had testosterone from the graft immediately after transplantation. The removed testicles were used as controls for histological investigations.

The operations were performed microsurgically by two surgeons at the same time. The anastomoses were made upon the stumps of the testicular arteries and veins and upon the vasa deferentia. If the veins of recipient and donor showed too large a discrepancy in diameter, the anastomoses were made with the lower superficial epigastric vessels [3]. The anastomoses of the arteries and veins were made in one circular end-to-end layer; the stumps of the vasa deferentia were anastomosed in two layers.

In order to prevent thrombosis of the graft after transplantation the animals in series I and II received 1000 IU subcutaneous heparin twice a day for 3 weeks. The animals in the third series received acetylsalicylic acid, 40 mg/day, orally for 3 weeks.

The period of immunosuppressive treatment was 3 months in all animals. In the first series, the animals received CyA, 15 mg/kg per day, by oral administration starting immediately after transplantation. The second series was subdivided into three groups: the first group ($n = 3$) received CyA 15 mg/kg per day, the second group ($n = 4$) received a combination of CyA, 15 mg/kg, and prednisolone, 10 mg/day, and the third group ($n = 3$) received no immunosuppressive medication. Immunosuppressive treatment in the third series was CyA, 15 mg/kg per day, and prednisolone, 10 mg/day.

Follow-up included physical examination of the grafts and measurement of serum testosterone levels twice a week with the aid of a radioimmunoassay [21]. During the physical examination, the size and consistency of the grafted testis were compared to those of non-grafted testes of other dogs. In addition, laboratory tests to monitor renal and hepatic function (creatinine, transaminases), leukocyte count, and CyA levels were performed twice a week. After 3 months, if testosterone could still be detected in the serum, a histological biopsy was taken from the graft via an open procedure.

The investigations were terminated 2–4 weeks after the serum testosterone fell into the castration range. The graft was removed from the animal after sacrifice and examined microscopically and microradiologically. The tissue was stained with hematoxylin-eosin and cut into 5- to 7-μ slices. Microradiographical examination was performed to determine the existence of functioning blood vessels in the specimen and to attempt to discriminate occlusion of the anastomoses from rejection. This was performed in 11 animals by infusion of the graft in vivo with a barium solution. Contact roentgen images were made on high-resolution plates with the aid of a microradiograph after fixation and cutting of the specimens.

In this study, the "Principles of laboratory animal care" (NIH publication nr 86–23, revised 1985) were followed.

Table 1 Pre- and postoperative serum testosterone levels and time of functional survival of the graft (*epig* lower epigastric artery and vein, *test* testicular artery and vein)

Series/ group	Dog number	Type of anastomosis	Ranges of serum testosterone level (nmol/l)				Functional survival days[a]
			With one own testis		With a transplant only		
			Mean	Range (min–max)	Mean	Range (min–max)	
I	505	test	4.6	1.6–13.6	< 0.3		< 42[b]
	506	epig	7.6	2.6–16.0	6.2	2.7–14.7	68
	509	epig	6.4	1.6–9.2	6.6		10
	510	test	5.3	1.0–9.9	4.4	0.3–19.0	153
II/1	522	epig	8.0	2.8–13.8	< 0.3		< 31[b]
	543	test	11.5	3.6–26.0	< 0.3		< 31[b]
	544	test	11.7	11.1–12.2	2.4	0.3–2.4	10
II/2	540	test	5.6	0.6–7.4	< 0.3		< 31[b]
	545	test	6.5	2.4–10.6	3.7	1.2–5.5	24
	552	test	5.5	0.6–10.4	5.5	2.3–7.7	24
	556	test	4.4	1.5–9.4	1.0		34
II/3	561	epig	7.6	1.3–13.8	5.6		10
	563	test	8.0	< 0.3–12.1	< 0.3		< 31[b]
	575	test	11.3	9.3–16.3	3.1		10
III	020	test	10.6	2.6–24.0	6.4	2.1–9.3	112
	021	test	14.9	13.9–15.8	< 0.3		2
	022	test	11.8	9.2–14.5	6.5	1.9–11.6	108
	023	test	6.3	2.6–13.4	6.0	3.0–9.0	105
	024	test	6.2	1.7–10.0	5.1	2.7–6.9	17

[a] Period that serum testosterone could be measured after transplantation

[b] No testosterone detected after orchiectomy of the remaining testicle after 1 month

Series I: Beagle littermates, immunosuppression by CyA
Series II,
group 1: Mongrel dogs, immunosuppression by CyA
group 2: Mongrel dogs, immunosuppression by CyA and prednisolone
group 3: Mongrel dogs, no immunosuppression
Series III: Beagle, littermates, immunosuppression by CyA and prednisolone

Results

To confirm our hypothesis that the testosterone measured in our described model actually originated from the graft and not from the adrenals, a trial was carried out to assess the endocrine function of the graft, as described by Garibyan et al. [2]. In two dogs in the first series, 500 IU of human chorionic gonadotropin (HCG) was administered 2 weeks after transplantation. This resulted in a rapid rise in the serum testosterone level, from 5.0 to 26.0 nmol/l and from 11.4 to 26.0 nmol/l, respectively. However, within 2 days after this rise, the testosterone level fell to castration level in both dogs. In one of them, testosterone could be detected again 1 week later, suggesting that the graft was still viable; in the other dog, this did not happen. Because of this observation, it was assumed that HCG was harmful to the graft's blood supply. Therefore, the tests were not performed in the other dogs.

In 18 out of 19 dogs, the transplantation of the testis graft was technically successful; in one dog the operation failed because of intraoperative graft thrombosis. The period in which testosterone was detected in the serum after the moment that all animals had lost their own testicles is shown in Table 1. Because half of the total number of dogs retained one of their own testicles during the 1st month (31 days), testosterone levels in these dogs up to 31 days would clearly not correlate with graft activity only. That is why, at 1 month after transplantation, no exact period of graft survival could be given for the five dogs in which no testosterone was detected after removal of their own testis.

The average time that testosterone could be measured was equated with graft survival time. In the first series, it was 71 days with a maximum of 163 days. In the second series, the average time was 18 days, significantly less than in the first series. In the group of dogs that received CyA in combination with prednisolone, however, testosterone seemed to circulate for a longer period in the serum. In the third series, three out of five grafts survived until immunosuppressive therapy was discontinued; after this, serum testosterone levels fell into the castration range within 1 week in two animals and within 3 weeks in one animal. The average graft survival period was 69 days in this series.

Postoperative serum testosterone levels fluctuated between castration level (< 0.3 nmol/l) and 26 nmol/l and showed large variations within the same animal. Average serum testosterone levels after castration and graft transplantation and before the decrease into the castration range were 10.2 nmol/l in the first series, 6.5 nmol/l in the second series, and 7.8 nmol/l in the

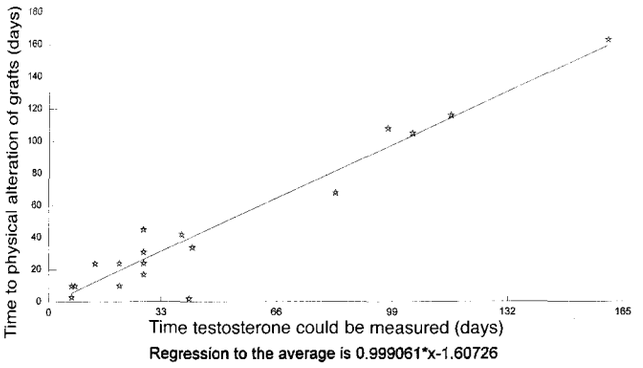

Fig. 2 Correlation between the time testosterone could be measured in the serum and time to physical alteration of grafts

third series. Preoperative testosterone values varied from 0.6 to 24.0 nmol/l, with an average of 7.6 nmol/l, which was almost the same as the total average postoperative level before reaching the castration range. In one dog in the third series, testosterone could only be detected until 2 days after transplantation. During transplantation, a 180° torsion of the vessels around each other persisted at the site of the anastomoses. Physical examination of the graft showed alterations as early as 41 days after transplantation. Stenosis of the vessels or early rejection could not be diagnosed because of severe degeneration at autopsy. The graft of another dog in this series survived for 17 days; after 28 days it gained volume and solidity for the first time. At autopsy, the vessels appeared to be open. This animal had probably experienced early rejection, although no lymphocytic infiltration could be detected.

The castration level appeared to be final in 14 out of 19 dogs when it was reached for the first time. In four animals, this level appeared once in the follow-up, but testosterone was measured again at various time intervals after this event, indicating that the graft was still viable at the time the testosterone had dropped to this low level.

The moment that alterations in the graft appeared at physical examination correlated highly with the time that the testosterone values fell into the castration range ($r = 0.96$; Fig. 2). The testosterone values above the castration range had no correlation with the findings at physical examination; only the castration range itself appeared to be relevant. The period between physical alteration in the graft and the moment that testosterone reached the castration range showed an average of 8.9 days (range 39 days). In nine cases the changes in the graft took place after the testosterone level had reached the castration range, and in five cases before this moment. The initial alterations were an increase in size and solidity. After an average of 8 days, the graft started to shrink and to gain solidity. Details are presented in Table 2.

In none of the animals were disturbances in renal function observed. There was a temporary mild elevation in transaminases in all animals in the first 2 weeks after transplantation. Two dogs in the second series that received prednisolone in addition to CyA had persistently elevated transaminases. CyA levels and ranges are presented in Table 3.

Table 2 Clinical follow-up of the grafts in relation to functional survival

Series	Dog number	Alteration	Alteration period (days)[a]	Functional survival (days)[b]	Difference (days)
I	505	Gain in solidity; shrinkage after 71 days	28	< 42	–
	506	Gain in solidity and size; shrinkage after 111 days	83	68	−15
	509	Gain in size; shrinkage after 76 days	21	10	−11
	510	Gain in solidity; shrinkage after 175 days	161	153	−8
II/1	522	Gain in solidity	28	< 31	–
	543	Gain in solidity	28	< 31	–
	544	Gain in solidity	8	10	+2
II/2	540	Gain in size	28	< 31	–
	545	Gain in size	28	24	−4
	552	Gain in size	14	24	+10
	556	Gain in size	42	34	−8
II/3	561	Gain in solidity	7	10	+3
	563	Gain in solidity	21	< 31	–
	575	Gain in solidity	7	10	+3
III	020	Gain in solidity	112	112	0
	021	Gain in solidity	41	2	−39
	022	Gain in solidity	98	108	+10
	023	Gain in solidity	105	105	0
	024	Abscess	28	17	−11

[a] The period between transplantation and physical alteration in the graft, related to its physical state before transplantation
[b] The period that testosterone was detected in the serum after transplantation (and orchiectomy)

Table 3 Cyclosporin A (CyA) levels in serum

Series	Dog number	Serum cyclosporin level (μg/l)			
		With one own testis		With a transplant only	
		Mean ± SD	Range	Mean ± SD	Range
I	505	83.6 ± 56.8	45–232	79.0 ± 30.9	41–126
	506	< 10	–	140.4 ± 66.4	37–230
	509	< 10	–	140.3 ± 206.0	54–950
	510	222.8 ± 177.6	82–517	86.3 ± 56.1	41–216
II/1	522	73.6 ± 26.7	23–115	92.7 ± 70.9	10–250
	543	81.4 ± 36.4	30–142	70.4 ± 25.8	33–103
	544	–		172.0 ± 150.3	33–545
II/2	540	219.1 ± 125.8	69 – 465	176.3 ± 110.3	38–370
	545	–		141.3 ± 122.4	24–498
	552	–		106.3 ± 49.6	24–187
	556	192.4 ± 170.4	41–555	171.5 ± 95.5	23–330
III	020	377.8 ± 78.3	251–457	373.6 ± 126.3	183–594
	021	–		341.0 ± 146.5	195–647
	022	–		333.1 ± 110.4	238–732
	023	529.1 ± 217.3	152–928	657.9 ± 230.5	312–1300
	024	491.8 ± 328.3	219–1120	318.7 ± 128.4	182–601
Series I:		Beagle littermates, immunosuppression by CyA			
Series II, group 1:		Mongrel dogs, immunosuppression by CyA			
Series II, group 2:		Mongrel dogs, immunosuppression by CyA and prednisolone			
Series III:		Beagle littermates, immunosuppression by CyA and prednisolone			

Reference value (in humans) 100–200 μg/l

According to the protocol, at 3 months after transplantation, histological biopsies of the grafts were taken in two dogs from the first series and in three from the third series. In the other animals the testosterone levels fell into the castration range and the grafts deteriorated within 3 months. In one specimen from the first series, histological examination showed progressive degeneration of the tubules, whereas the stromal Leydig cells appeared to be identical in number and shape to those of the control testis of the same animal. In the other specimen from the first series, the tubules as well as the Leydig cells appeared to be the same as in the tissue of the control testis of this animal, showing normal maturation of spermatic cells and spermatocytes in the lumina of the tubules. In this specimen, no signs of degeneration, fibrosis, or lymphocytic infiltration were noticed. The material of three dogs in the third series showed tubules with the same maturation of spermatic cells and the same count of spermatocytes in the lumen as in the control testis of these animals. This was also true for the stromal Leydig cells. Histological examination of the final specimens always showed various stages of degeneration.

Microangiography of the grafts ($n = 11$) at the end of the experiment showed normal blood vessels in the peripheric zone and absence of these in the central zone of the graft in 7 out of 11 cases, indicating that vascularity was at least partly preserved. In 4 of 11 specimens there was no vascularity left at all because of severe degeneration. In these cases, no discrimination between occlusion of the graft vessels and rejection could be made.

Discussion

The first group to describe experimental homologous testis transplantations in dogs was that of Attaran [1], who performed the procedure using caval and aortic patches. Since the development of microsurgical techniques, it has become possible to perform the procedure reliably on the testicular vessels [17, 18], as confirmed in this study (1 operative failure out of 15).

To date, homologous testis transplantations in humans have been performed by Kirpatowskij [8, 9] and by the groups of Wang [23, 24] and Zhao [25]. Apart from these authors, few experiments have been performed to develop a model that can be used in patients, with the aid of modern microsurgical techniques and immunosuppressive agents. Cyclosporin A, probably the most potent single immunosuppressive agent known at present, showed no distinct advantage in our material, except when it was combined with prednisolone.

In this model of one testicular graft in an animal with no testicles of its own, the period of functional survival of the graft was considered to be the period during which testosterone was detectable in the serum. The correlation in time interval between physical alterations in the graft and the period of measurable testosterone levels supports this theory, as well as the rapid rise in testosterone levels after endocrine stimulation of the graft. However, in our experiments, this endocrine stimulation led, after the initial rise, to a fall in testosterone levels to the castration range. This was probably due to vasoconstriction in the graft or in the testicular vessels. A re-

duced blood flow in the testis by precapillary vasoconstriction after administration of HCG has been described in rats [22], but to our knowledge not in dogs. Because of these observations and the probability of losing animals to follow-up as a result of this test, we did not perform it on the other dogs.

When testosterone fell into the castration range, it generally appeared to be final, with the exception of some incidental measurements below 0.3 nmol/l during the time of graft function. This further confirmed the theory we previously described.

A maximum period of detectable serum testosterone in one of the dogs from the first series of 163 days, without tissue typing before transplantation, strongly suggests the possibility of long-term survival of testis homografts in dogs. The results of the second series suggest that CyA in combination with prednisolone gives the best results for graft survival. The outcome of a far lower median graft survival time compared to the first series was probably due to a mismatch of the animals. The results of the third series strongly suggest that long-term survival of testis homografts in dogs is possible, given the fact that three out of five grafts functioned until immunosuppressive therapy was discontinued. This outcome, however, is partly in contrast with the observation in the first series in which one graft preserved endocrine function 2 months after the immunosuppression was suspended (153 days after transplantation). The probable cause of this phenomenon was the degree of serological and histological matching of the animals, which could not be predicted in the three series. A much larger group would be necessary to form a pool in which the most compatible animals could be found and brought together. A suitable species for this would be the rat, in which the technique and outcome of testis transplantation have been described by Lee et al. [11] and Goldstein et al. [4].

In view of the fact that there was only 1 operative failure out of 19 transplantations, we feel that the technique we used, previously described by Silber [17], would alos be valid in humans. The immunosuppressive therapy of combined CyA and prednisolone is promising and has a possible clinical application. With regard to kidney transplantation [15], it is possible that, after careful HLA-matching of patients, immunosuppressive therapy will, in time, become less crucial for testis graft survival. Long-term use of prednisolone would, however, have to be avoided because of its well-known side effects. CyA has side effects also, especially nephrotoxicity [20], but in general these are of less importance [13] and pose no direct threat to fertility in humans [6].

The immune privilege of the testis, i.e., the protection within the testis of homologously transplanted tissue against rejection [12], is obviously of no importance in homologous transplantation of the entire organ. The results of the third series of this study show a clear dependence of the graft on immunosuppressive therapy to survive.

What happens with the genetic constitution of the reproductive cells of a testis graft in the long term after transplantation is as yet unknown. Local chimerism, i.e., coexistence of donor and recipient cells in homografts, has been demonstrated for various organs [16, 19]. Infiltration of genetic material from the host, however, involves leukocytic cells, and to date the graft's parenchymatous cells have shown no evidence of genetic alterations. Neither Wang [23] nor Zhan [24] discuss the genetic constitution of the offspring of their patients with a testis homograft. It would, therefore, be necessary to examine offspring of homologously transplanted animals before performing the operations on humans. In our material, three out of five dogs were seemingly fertile during the time their grafts functioned. Although possible [10], collecting semen in dogs is a complicated and time-consuming procedure and, due to the design of our investigation, these semen collections were not performed. Histological investigation of the differentiation of spermatogenic epithelium of a testis, however, also gives reliable information on its potency to function in terms of fertility [2].

If homologous testis transplantation were to be done in humans to restore their hormonal status, the advantage of a physiological testosterone level would need to be carefully weighed against the disadvantage of possibly life-long immunosuppressive therapy. It is the opinion of the authors that the "pros" could outweigh the "cons" for carefully selected patients. If homologous testis transplantation were to be applied to infertile patients, serious issues regarding the ethical aspects of the procedure, compared to those related to assisted reproduction, would certainly have to be discussed.

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