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Effect of the Level of Inbreeding upon Skin Graft Survival Times of in Rabbits

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Abstract

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The investigations were performed in December 2005- February 2006 with 15 male California rabbits. All animals had completer their growth and development (age of 8-12 months). They were divided into groups according to the degree of inbreeding as followed: outbred: Fx=0 - 4 rabbits (B&); inbred: Fx=0.25 - 3 rabbits (♂); Fx=0.375 - 4 rabbits (B ♂); Fx=0.500 - 4 rabbits (♂).

Prior to the operation, the rabbits were deprived from food and water for 12 hours. The general anaesthesia was done with ketamine hydrochloride (Ketaminol^{R}, Intervet Intenational B.V., Boxmeer - Holand) at 40 mg/kg w., i. m.; followed by xylazine hydrochloride (Xylazin^{R}, Alfasan, Woerden - Holland) at 5 mg/kg w., i. m 15 min later. The operation field behind the scapulas was shaved and prepped twice with iodininated alcohol. Rectangular skin flaps of 2x1 cm were cut from both sides of the dorsal line. After transfer of skin grafts from donors to recipients by the method of Bailey and Kohn (1965), they were fixed to wound margins with 4 sutures on each angle. Until transplantation, the grafts were put into a RPM medium. After the operation, the rabbits were administered antibiotic with a 72-hour effect.

The end result was determined in days on the basis of the time of graft persistence and rejection. The higher persistence of the graft was a criterion for a higher histocompatibility, i.e. a higher genetic similarity.

It was found out that in most cases, the increase in the degree of inbreeding resulted in statistically significant higher graft survival times and that at the same time, certain heterogeneity was present. In this case, it was more considerable as the achieved levels of inbreeding were not very high.

Key words: inbreeding, skin graft, rabbits, transplantation immunity

Introduction

The main histocompatibility complex (MHC) is a primary mechanism, controlling the specific immune response. It is discovered as a genetic system, whose products are responsible for tissue graft rejection among inbred murine lines. The genetic system, responsible for MHC antigens, is multilocus and highly polymorphic (multiallele), including more than 250 alleles in the population of some of genetic loci. MHC genes and their products are extensively studied with regard to their primary importance to transplantations (Janeway et al, 2001; Abbas and Lichtman, 2003).

Studies for homozygocity and histocompatibility control in inbred lines of laboratory mice and rats were performed by numerous authors (Bailey, 1966; Egorov and Blandova, 1972; Kohn and Melvold, 1974; Blandova et al., 1983; Moskalewski et al., 2002 et al.).

The studies upon skin transplantation as a test of homozygocity and histocompatibility (RL-A) in inbred rabbits are relatively few (Tissot and Cohen, 1976; Pennington et al., 1981; Pazdera et al., 1981; Hradecky et al., 1985; Contie, 1994; Sachs, 1994; Mezrich et al., 2003).

In all schedules and methods, one of criteria for homozygocity of inbred animals is the survival time of transplants within the studied inbred group in days or percentages (Petrov, 1978; Hud et al., 1985; Chardon et al., 1992).

The purpose of the present investigation was to determine the effect of the level of inbreeding upon survival times of skin grafts as a test of histocompatibility in reciprocal skin isotransplantation.

Material and Methods

The investigations were performed in December 2005- February 2006 with 15 male California rabbits, property of the Experimental Farm of the Trakia University – Stara Zagora. All animals had completer their growth and development (age of 8-12 months). They were divided into groups according to the level of inbreeding as followed: outbred: Fx=0 - 4 rabbits (♂); inbred: Fx=0.25 - 3 rabbits (♂); Fx=0.375 - 4 rabbits (♂); Fx=0.500 - 4 rabbits (♂).

Prior to the operation, the rabbits were deprived from food and water for 12 hours. The general anaesthesia was done with ketamine hydrochloride (Ketaminol^{R}, Intervet International B.V., Boxmeer - Holland) at 40 mg/kg w., i. m.; followed by xylazine hydrochloride (Xylazin^{R}, Alfasan, Woerden - Holland) at 5 mg/kg w., i. m 15 min later. The operation field behind the scapulas was shaved and prepped twice with iodininated alcohol. Rectangular skin flaps of 2x1 cm were cut from both sides of the dorsal line. After transfer of skin grafts from donors to recipients by the method of Bailey and Kohn (1965), they were fixed to wound margins with 4 sutures on each angle. Until transplantation, the grafts were put into a RPM medium. After the operation, the rabbits were administered antibiotic with 72-hour effect

The end result was determined in days on the basis of the time of graft persistence and rejection (Figure 2). The higher persistence of the graft was a criterion for a higher histocompatibility, i.e. a higher genetic similarity.

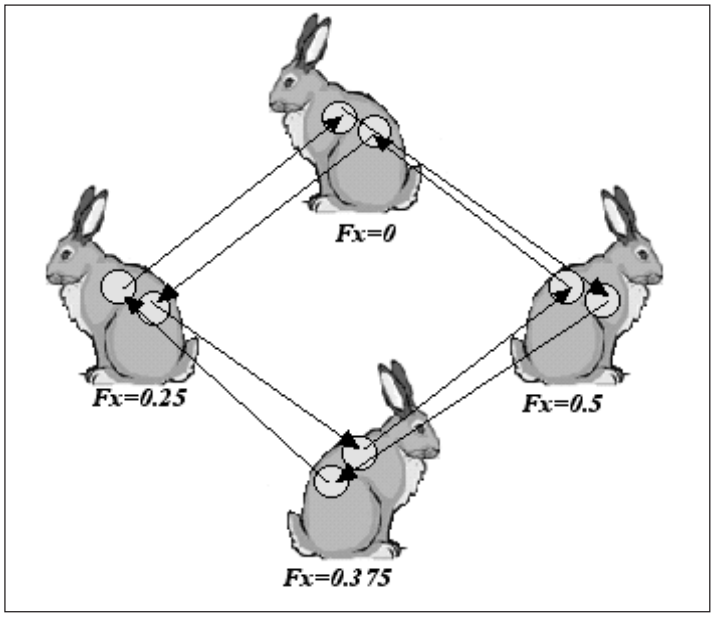

Fig. 1. Schedule of skin isotransplantation in rabbits

The exchange of skin transplants among male rabbits depending on the level of inbreeding was performed as shown on Figure 1. Thus, each recipient received grafts from two different donors on both sides of the dorsal line. The skin graft survival times in days were used as a criterion for histocompatibility and higher genetic similarity.

The data were statistically processed with the LSD test, using one-way fixed-effects ANOVA with the following additive model:

$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where

y_{ij} - graft survival times (days);

μ - general mean;

α_i - differential effect of the level of inbreeding;

ε_{ij} - random error

The analysis was done for transplantations on the left and the right sides independently as well as mean for both sides, by means of Statistica software package.

Results and Discussion

The multiple comparisons among groups were done by the LSD test.

The analysis of variance is presented in Table 1. The data show that the level of inbreeding had a statistically significant effect upon skin graft survival times on the left side, on the right side as well as on both sides.

Table 2 presents the mean values for each group, reflecting the skin graft survival times. It is evident that skin graft survival times increased with increasing of inbreeding levels. Therefore, the higher the inbreeding level, the higher the genetic similarity and the histocompatibility. It must

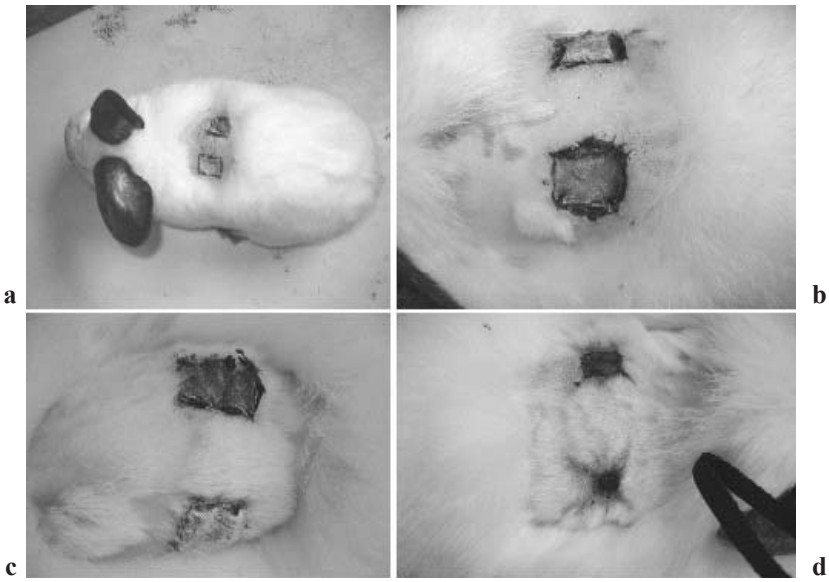

Fig. 2. a) Rabbits after reciprocal skin transplantation
b, c, d) Retainment and rejection of skin grafts

be emphasized that grafts on the left side had lower survival times

The differences between groups are statistically significant in most cases and are shown in Tables 3-5.

Most probably, this was due to the better histocompatibility in the right circle from the rotation schedule of skin grafts. Also, it must be stated that within groups, there were considerable individual differences, as seen from the high standard errors of means (Sx). This indicates that practically, the increase in the theoretical genetic similarity through higher inbreeding levels does not guarantee for sure a higher percentage of real similarity with regard to the main histocompatibility complex (MHC) (Moskalewski et al., 2002). Hence, the effect of the progressive inbreeding in our experiment is essential for the increase in mean values of genetic similarity, evaluated through skin graft survival times, but the importance of genetic diversity, maintained by the genetic system, determining MHC, should not be ignored. Practically, it becomes clear that histocompatibility in rabbits is controlled by a considerable number of polymorphic loci. It is however accepted that RLA loci are the primary source of genetic heterogeneity. Their effect is the most important for the survival of skin grafts and isografts as a whole (Tissot and Cohen, 1976; Pazdera et al., 1981; Penington et al., 1981; Chardon et al., 1992). Our results, as well as the data of other investigators showed that there was no linear relationship between the level of inbreeding and survival times of recipient's grafts

Table 1

Effect of the degree of inbreeding upon the survival times of skin grafts

Traits	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Left side	2355.267*	3*	785.0889*	1035.667*	11*	94.15152*	8.33857*	0.0036*
Right side	2444.817*	3*	814.9389*	524.917*	11*	47.71970*	17.07762*	0.00019*
Mean of both sides	2377.871*	3*	792.6236*	431.229*	11*	39.20265*	20.21862*	0.000088*

* - statistically significant effect

Table 2

Mean survival times (in days) of skin grafts, depending in the degree of inbreeding

Level of inbreeding	Average survival times of grafts (days)					
	Left side		Right side		Mean of both sides	
	x±Sx	n	x±Sx	n	x±Sx	n
Fx=0 (outbred)	15.50±1.94	4	16.75±1.11	4	16.13±1.43	4
Fx=0.25	28.33±4.84	3	36.33±5.24	3	32.33±4.97	3
Fx=0.375	40.50±3.12	4	43.25±3.45	4	41.88±2.74	4
Fx=0.500	47.50±7.82	4	49.75±4.11	4	48.63±3.74	4

Table 3

Levels of statistical significance (p) of differences among groups with various levels of inbreeding with regard to left side skin graft rejection time, days

Level of inbreeding	Fx=0 (outbred}	Fx=0.25	Fx=0.375	Fx=0.500
	x±Sx	x±Sx	x±Sx	x±Sx
	15.50±1.94	28.33±4.84	40.50±3.12	47.50±7.82
Fx=0.25	0.111241			
Fx=0.375	0.003863 *	0.1289		
Fx=0.500	0.000689 *	0.025306 *	0.329532	

* - statistically significant differences

(Tissot and Cohen, 1976). That is why the results could not be used for prediction of histocompatibility with regard to the degree of inbreeding. Other authors (Mezrich et al., 2003) report that the increase of the level of inbreeding from F1 to F7 (at breeding of full sibs), the survival times of transplants (skin and tissues) in swine increased up to 10 times. The authors accentuated that for attainment of full of very high histocompatibility, the inbreeding should be used continuously as a method of breeding. Similar is the opinion of Hradecky et al. (1985).

Table 4

Levels of statistical significance (p) of differences among groups with various levels of inbreeding with regard to right side skin graft rejection time, days

Level of inbreeding	Fx=0 (outbred}	Fx=0.25	Fx=0.375	Fx=0.500
	x±Sx	x±Sx	x±Sx	x±Sx
	16.75±1.11	36.33±5.24	43.25±3.45	49.75±4.11
Fx=0.25	0.003431 *			
Fx=0.375	0.000209 *	0.216579		
Fx=0.500	0.000031 *	0.027336 *	0.210214	

* - statistically significant differences

Table 5

Levels of statistical significance (p) of differences among groups with various levels of inbreeding with regard to skin graft rejection time (days), average for both sides

Level of inbreeding	Fx=0 (outbred}	Fx=0.25	Fx=0.375	Fx=0.500
	x±Sx	x±Sx	x±Sx	x±Sx
	16.13±1.43	32.33±4.97	41.88±2.74	48.63±3.74
Fx=0.25	0.006041 *			
Fx=0.375	0.000117 *	0.071376		
Fx=0.500	0.000015 *	0.005858 *	0.155576	

* - statistically significant differences

With regard to inbred rabbits, Tissot and Cohen (1976) consider that the graft survival times over 60 days in 95% of cases was determined by 17 independent loci with confidence intervals from 11 to 28 loci.

Conclusion

It could be concluded, that the increase in the degree of inbreeding of rabbits in our experiment resulted in statistically significantly higher skin graft survival times in most cases and simultaneously, certain heterogeneity was preserved. In our trial it was higher, as the used inbred levels were not very high. The analysis of our data allowed us to assume that the intricate Philadelphia.

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