IMPAIRED TRANSGENIC EFFICIENCY IN MICE AND RABBITS WITH HUMAN ERYTHROPOIETIN MAMMARY GLAND EXPRESSING TRANSGENES

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Introduction

The development of methods for the production of trangenic mammals has stimulated their use as bioreactors to produce large amounts of human proteins mainly in blood and milk (1, 2). We have previously reported the low level expression of active human erythropoietin (hEPO) in the mammary gland of lactating transgenic mice and rabbits with the hEPO cDNA (3). After, we generated transgenic mice and rabbits with the hEPO chromosomal gene under the 5' and 3' regulatory sequences of the rabbit whey acidic protein (rWAP) gene (4). The expression levels of hEPO were again very low. Here we report the comparative analysis showing that the frequency of generation of transgenic animals using hEPO containing transgenes was lower than the one obtained when other transgenes were employed. We hypothesize that this phenomena might be related to deleterious effects of the hEPO expression during the fetal period of transgenic animals.

Materials and Methods

Transgenic lines analyzed

Four lines of transgenic mice were studied which contained the following transgenes:

1. bovine aS1 casein > human tissue-type plasminogen activator (5).
2. rWAP > hEPO cDNA (3).
3. rWAP > hEPO genomic (4).
4. rWAP > IORT1 cDNA (chimeric anti CD6 antibody) (6).

Five transgenic rabbit lines were included in the analysis:

1. rWAP > hEPO cDNA synthetic variant (7).
2. rWAP > hEPO cDNA (3).
3. rWAP > hEPO genomic (4).
4. bovine aS1 casein > htPA (5).
5. mouse WAP > human growth hormone genomic (8).

Statistical analysis

Results obtained during the generation of transgenic lines with transgenes containing sequences derived from the hEPO were compared with the other transgenes. The analysis was done independently for mice and rabbits. Three indexes were calculated: A) number of transgenic founders out of total microinjected embryos, B) number of transgenic founders per live-born pups and C) number of transgenic founders out of transfered embryos. Besides, live-born pups and pregnancy rates were also studied for each group. A Student t-test for non paired samples with Levene's test for equality of variances was used (SPSS v5.01 for Windows, SPSS Inc., USA).

Results and Discussion

The results of statistical analysis appear in Table 1. In mice, we observed significant differences in the A and C indexes between the hEPO and the non-hEPO group. The other parameters tested also showed significant differences.

Because of the random integration of the transgenes into the host genome and the requirements of the WAP promoters to be properly regulated, it may be possible that the hEPO derived transgenes were incorrectly regulated during the embryonic period. If that was the case, then the embryos expressing high levels of hEPO might undergo reabsortion during the early stages of embryogenesis. That might be one of the possible explanations for the differences observed between the groups studied and for the fact that we only managed to obtain animals which expressed very low level of the protein in their milk.

Although the differences in rabbits were not significant, they showed the same general tendency.

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2. Carver AS, *et al.* Biotechnology 1993; 11:1263-1270.

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4. Manuscript in preparation.

5. Riego E, *et al.* Theriogenology 1993; 39:1173-1185.

6. Limonta JM, *et al.* Immunotechnology 1995;1:107-113.

7. Unpublished results.

8. Limonta JM, *et al.* Avances en Biotecnología Moderna 1992;1:19.

		Index A	Index B	Index C	Live pups	Transgenic pups	Pregnancy females
Mice	hEPO	0.089*	0.09	0.0164*	6.5714*	0.5714*	2.2857*
	non-hEPO	0.058*	0.20	0.1084*	18.125*	4.6250*	4.0000*
Rabbits	hEPO	0.022	0.0259	0.0029	4.5625	0.1875	1.7500
	non-hEPO	0.145	0.1884	0.0198	4.7368	0.7368	1.8947

*P < 0.05

Table 1. *Results of the statistical analysis.*