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Correction of ornithine transcarbamylase (OTC) deficiency in spf-ash mice by introduction of rat OTC gene

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We introduced rat ornithine transcarbamylase (OTC) gene into OTC-deficient spf-ash mice by mating spf-ash heterozygotes with transgenic mice which carried recombinant DNA composed of 1.3 kb of the 5′ flanking region of the gene fused onto rat OTC cDNA. The liver OTC activity of hemizygous spf-ash mice which carried the transgene was about twice that of nontransgenic spf-ash mice, and the small intestinal OTC activity was 6 times higher; the values being 12% and 27% of the control levels, respectively. The transgenic spf-ash mice showed normal hair growth without sparse fur, nearly normalized urinary orotic acid excretion and normalized serum citrulline concentration.

Ornithine transcarbamylase; Transgenic animal; Enzyme deficiency; Citrulline; Orotic acid; Gene therapy

1. INTRODUCTION

Ornithine transcarbamylase (OTC) is a mitochondrial urea cycle enzyme located only in the liver and small intestine. Hepatic OTC plays an important role in ammonia detoxication and bicarbonate excretion [1] as a member of the urea cycle, whereas OTC in the small intestine is thought to supply citrulline mainly to the kidney for the synthesis of arginine [2]. It is not known whether small intestinal OTC plays any role in ammonia detoxication. Two types of OTC-deficient mice have been found, sparse-fur (spf) [3] and sparse-fur with abnormal skin and hair (spf-ash) [4], which had decreased OTC activity both in the liver and small intestine [5]. Their molecular and genetic defects have been analyzed [6–8]. As their names imply, they have delayed hair growth and, as in human OTC deficiency, they show hyperammonemia, orotic aciduria and low concentrations of serum citrulline and arginine [3,4,9,10]. Cavard et al. [11] produced a transgenic spf-ash mouse by introducing into the fertilized eggs of spf-ash mice a construct containing rat OTC cDNA linked to SV40 early promoter. They reported that the OTC activity in the liver and small intestine reached 80–90% of control values and that the phenotype and orotic acid excretion of the transgenic mouse was fully normalized. Murakami et al. [12] introduced recombinant DNA carrying 1.3 kb of the 5′ flanking region of the rat OTC gene fused to the rat OTC cDNA into fertilized mouse eggs and established transgenic mice which expressed rat OTC tissue-specifically. Hepatic expression, however, was low, probably because of the lack of the enhancer element [13]. This transgenic mouse in combination with OTC-deficient mice may be useful for studying the role of small intestinal OTC. We introduced rat OTC cDNA with its own promoter into spf-ash mice by mating spf-ash heterozygotes with the OTC-transgenic mice. Here we describe the increase in liver and small intestine OTC activities and the correction of the phenotypes of spf-ash mice. During the preparation of this manuscript, a paper by Jones et al. [14] has appeared that describes the correction of OTC deficiency in spf mice by introducing human OTC cDNA carrying mouse OTC promoter.

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2. MATERIALS AND METHODS

2.1. *Animals*

Heterozygous spf-ash mice (X^{+}/X), C57BL strain, were mated with transgenic mice (+/−) of the same (C57BL) strain, F4 of no. 94 described in [12]. We confirmed the presence of rat OTC gene in the genome by Southern blot analysis of the DNA extracted from the tail as described in [12] and confirmed the spf-ash gene by measuring OTC activity in the small intestine and liver. The mice were separated from their mothers at 21 days and maintained on a laboratory CE2 chow from Clea Japan Inc. Urine samples were collected in metabolic cage at 27 to 29 days of age. Serum samples were separated from blood specimens obtained by heart puncture.

2.2. *Assay methods*

For the assay of OTC activity, liver homogenate and extract of

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small intestine were prepared as described by Gushiken et al. [9] and Murakami et al. [12]. Activity was assayed by the method of Pierson et al. [15]. Urinary orotic acid and creatinine were measured according to the methods of Kesner et al. [14] and Bonnes and Taussky [17]. Serum amino acids were quantified by reverse-phase liquid chromatography after derivatization with o-phthalaldehyde according to the method of Godel et al. [18].

3. RESULTS AND DISCUSSION

We obtained four types of experimental male mice (X/Y, $-/-$) or control ($-$), (X'/Y, $-/-$) or ash ($-$), (X/Y, $+/-$) or control ($+$), and (X'/Y, $+/-$) or ash($+$). OTC activity in the liver of the ash($+$) mice was 1.9 times higher than that of ash($-$), the value being 12% of that of the control mice (Table I). On the other hand, OTC activity in the small intestine of spf($+$) mice was 6.3 times higher than that of ash($-$), reaching 27% of the control value. The low value in the liver is apparently due to the lack of OTC enhancer elements [12,13] in the present construct. No significant difference was observed between OTC activity in the liver and small intestine of control($+$) and control($-$) mice. In spite of lower than normal OTC activity in the liver and small intestine of ash($+$) mice, these mice showed no sparse-fur phenotype. Introduction of the rat OTC gene into spf-ash mice approximately doubled the serum citrulline concentration to a level comparable to the control value. These results suggest that small intestinal OTC activity (27% of the control) is enough to maintain serum citrulline at normal levels. There was no significant difference in the concentration of arginine among the different types of mice, probably because they were well fed. There was no increase in blood ammonia even in ash($-$) mice under the present conditions (data not shown).

The introduction of rat OTC gene into spf-ash mice resulted in a dramatic decrease in urinary orotic acid concentration (Table I). The level in ash($+$) mice was almost normal. Although a high concentration of orotic acid in the urine is a good indicator of OTC deficiency and is thought to be caused by deficiency of liver OTC, it is not known whether or to what extent deficiency of small intestinal OTC contributes to orotic aciduria. Recently Jones et al. [14] reported similar results with transgenic spf mice which carried human OTC gene with truncated mouse promoter; the mice showed no OTC deficiency phenotype, expressed a large amount of human OTC in the small intestine, but almost none in the liver. The difference in liver and small intestinal OTC expression between Jones et al. and the present study may be due to the OTC promoter used, mouse and rat, or to the insertion site of the transgenes. Their results described below are not easy to understand. Their spf mice had small intestinal OTC activity higher than control, and their transgenic spf mice had urinary orotic acid excretion reduced to the normal level in spite of the fact that there was no change in OTC activity in the liver where most of ammonia is formed and carbamylphosphate would accumulate. From our results, we cannot conclude whether the decrease in orotic acid excretion is due to increased OTC activity in the small intestine or in the liver of OTC-deficient mice.

In any case, it is noteworthy that the ash($+$) mice, having OTC activity about one-tenth and one-fourth of the controls in the liver and small intestine, respectively, showed almost no laboratory findings characteristic to OTC deficiency. Severe hyperammonemia was reported in human patients, with hepatic OTC activity at 30–50% of the controls [19]. This is probably due to species difference, or may support the observation that symptomatic manifestations break out in late-onset OTC deficiency following ingestion of a high protein diet or accelerated protein breakdown caused by injury or infection. It remains to be seen to what extent the ash($+$) mice are resistant to nitrogen load.

Table I

OTC activities in the liver and small intestine, concentrations of serum citrulline and arginine and urinary orotic acid excretion of control and spf-ash mice, and those carrying rat OTC gene

Mice	OTC activity (U/mg protein)		Serum amino acid (nmol/ml)		Urinary orotic acid (nmol/mg cr)
	Liver	Small intestine	Cit	Arg	
Control($-$)	1.09 ± 0.42	0.258 ± 0.065	76 ± 16	196 ± 50	477 ± 303
(n)	(6)	(6)	(4)	(4)	(6)
Control($+$)	0.928 ± 0.256	0.229 ± 0.079	67 ± 6	176 ± 48	634 ± 255
(n)	(9)	(9)	(4)	(4)	(6)
Ash($-$)	0.067 ± 0.014 [b]	0.011 ± 0.004 [b]	36 ± 16[a]	165 ± 50	7850 ± 4280[b]
(n)	(6)	(6)	(4)	(4)	(6)
Ash($+$)	0.126 ± 0.027[b,c]	0.069 ± 0.019[b,d]	63 ± 11[c]	188 ± 21	907 ± 141[a,c]
(n)	(4)	(4)	(4)	(4)	(4)

Values significantly different from those of control($-$) - [a]$P<0.05$ and [b]$P<0.01$; and values of ash($+$) significantly different from those of ash($-$) - [c]$P<0.05$ and [d]$P<0.01$. 'Control' and 'ash' denote control and spf-ash mice; ($-$) and ($+$), absence and presence of rat OTC gene. cr, creatinine; (n), number of animals examined.

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REFERENCES

[1] Haussinger, D., Meijer, A.J., Gerok, W. and Sies, H. (1988) In: pH Homeostasis (Haussinger, D. ed) pp. 337-377.
[2] Windmueller, H.G. and Spaeth, A.E. (1981) Am. J. Physiol. 241, E473-E480.
[3] DeMars, R., LeVan, S.L., Trend, B.L. and Russell, L.B. (1976) Proc. Natl. Acad. Sci. USA 73, 1693-1697.
[4] Doolittle, D.P., Hulbert, L.L. and Cordy, C. (1974) J. Hered. 65, 194.
[5] Malo, C., Qureshi, I.A. and Letarte, J. (1986) Am. J. Physiol. 250, G177-184.
[6] Briand, P., Francois, B., Rabier, D. and Cathelineau, L. (1982) Biochim. Biophys. Acta 704, 100-106.
[7] Veres, G., Gibbs, R.A., Scherer, S.E. and Caskey, C.T. (1987) Science 237, 415-417.
[8] Hodges, P.E. and Rosenberg, L.E. (1989) Proc. Natl. Acad. Sci. USA 86, 4142-4146.
[9] Gushiken, T., Yoshimura, N. and Saheki, T. (1985) Biochem. Int. 11, 637-643.
[10] Inoue, I., Gushiken, T., Kobayashi, K. and Saheki, T. (1987) Biochem. Med. Metab. Biol. 38, 378-386.
[11] Cavard, C., Grimber, G., Dubois, N., Chasse, J.-F., Bennoun, M., Minet-Thuriaux, M., Kamoun, P. and Briand, P. (1988) Nucleic Acids Res. 16, 2099-2110.
[12] Murakami, T., Takiguchi, M., Inomata, T., Yamamura, K. and Mori, M. (1989) Dev. Genet. 10, 393-401.
[13] Murakami, T., Nishiyori, A., Takiguchi, M. and Mori, M. (1990) Mol. Cell. Biol. 10, 1180-1191.
[14] Jones, S.N., Grompe, M., Munir, M.I., Veres, G., Craigen, W.J. and Caskey, C.T. (1990) J. Biol. Chem. 265, 14684-14690.
[15] Pierson, D.L., Cox, S.L. and Gilbert, B.E. (1977) J. Biol. Chem. 252, 6464-6469.
[16] Kesner, L., Aronson, F.L., Silverman, M. and Chan, P.C. (1975) Clin. Chem. 21, 353-355.
[17] Bonsnes, R.W. and Taussky, H.H. (1945) J. Biol. Chem. 158, 581-591.
[18] Godel, H., Graser, T., Foldi, P., Pfaender, P. and Furst, P. (1984) J. Chromatogr. 297, 49-61.
[19] Shih, V.E. (1976) In: The Urea Cycle (Grisolia, S., Baguena, R. and Mayor, F. eds) pp. 367-414, Wiley, New York.