Usefulness of recombinant human prolactin for treatment of poor puerperal lactation in a rat model

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Recombinant human prolactin (r-hPRL) was produced by a line of murine C127 cells transfected with human PRL gene. To assess the biological efficacy of r-hPRL in vivo, we studied its influence on milk secretion using a rat model in which lactation was reduced by bromocriptine treatment. Puerperal rats were injected daily for 9 days after delivery with bromocriptine or bromocriptine plus r-hPRL and lactational performance was assessed by weighing the pups. The concentrations of rat and human PRL in rat serum were measured by specific radioimmunoassays and the mammary glands were examined on postpartum day 10. Daily injection of bromocriptine (0.1 mg/rat) significantly reduced the endogenous level of rat PRL and impaired the weight gain of the pups. Administration of r-hPRL increased the serum level of human PRL. Daily injections of r-hPRL (50 μg/rat, twice a day) restored lactational performance and significantly increased the weight of the pups. The detrimental effect of bromocriptine on the mammary glands, assessed by both weight and histological appearance, was reversed by administration of r-hPRL. These results demonstrate that r-hPRL is biologically active in vivo and replacement therapy of r-hPRL is effective in improving the lactational performance in bromocriptine-treated rats, and also that r-hPRL may be useful for the treatment of women with poor lactation.

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Breast feeding is known to be better than bottle feeding nutritionally, immunologically and in the formation of a mother–infant bond. Therefore, it is considered best for the development of babies (1–3) and there is increasing awareness of the need to treat women who have poor puerperal lactation (4, 5).

Prolactin (PRL) is synthesized by the lactotropes in the anterior pituitary and is well known to play a role in stimulating milk production. When puerperal women nurse their babies, PRL is released from the pituitary gland within 30 min from the start of suckling and strongly stimulates milk production (6). Furthermore, it plays another important role in the establishment and maintenance of breast feeding. The initiation of milk secretion in the early puerperal period is closely related to an increase in the serum PRL level induced by adequate suckling (7, 8). Puerperal women with poor PRL secretion thus have difficulty in breast feeding, and therefore there is a need for PRL in such women (4, 5).

Recently, recombinant human prolactin (r-hPRL) has been produced by a line of murine C127 cells transfected with human PRL gene (9). This should be useful for the treatment of women with poor lactation, so in the present study we evaluate the efficacy of r-hPRL for the treatment of poor puerperal lactation using a rat model.

Materials and methods

Animals

Female 9-week-old Wistar rats weighing 200–250 g were purchased from Japan Charles River Corporation (Kanagawa, Japan). They were housed in a controlled environment (25°C, 50% humidity), with lights on from 08.30 h to 20.30 h, and were provided with Purina rat chow and water ad libitum. They were paired with fertile males for mating and followed throughout pregnancy, delivery and early postpartum. The litter size was adjusted to 10 pups on the day of delivery.

Atrial cannulation

On the day of delivery the rats were anesthetized with a mixture of ketamine and xylazine (20:5 mg/kg ip), and a silastic tube (0.94 mm o.d., 0.51 mm i.d., Dow-Corning, Midland, MI) was inserted into the external jugular vein and sewn into position in the right atrium (10). The other end was exteriorized through the back of the neck. The tube was rinsed with heparinized saline (1 × 10⁴ U/l saline) and a steel pin was inserted into its open end. For collection of a blood sample, the intrathoracic cannula was rinsed and connected to a long.
polyethylene tube containing heparinized saline. This tube extended to outside the cage to permit rapid blood sampling without handling the lactating rats and the suckling pups.

Bromocriptine treatment

One milligram of bromocriptine (Sigma Chemical Co, St Louis, MO) was dissolved in 0.2 ml of 99.5% ethanol and diluted with 3.8 ml of distilled water. Injection of bromocriptine into puerperal rats at various doses showed that a dose of 0.1 mg/rat was appropriate because it impaired weight gain of the pups without causing mortality.

Recombinant human prolactin

Recombinant hPRL was expressed in a line of murine C127 cells transfected with hPRL gene (Genzyme Corporation, Framingham, MA). This was purified from conditioned media to >97% homogeneity by anion and cation exchange chromatographies. The protein concentration of the final preparation was 280 mg/l, as judged by amino acid analysis. The buffer used 25 mmol/l HEPES (pH 8.0), containing 150 mmol/l NaCl and 0.01% Tween 80. Murine C127 cells were found to produce both the 23- and 25-kD forms of r-hPRL, as shown by SDS-PAGE and immunoblotting. Lectin blotting, endoglycosidase digestion and monosaccharide analysis indicated that the 25-kD variant is glycosylated and the 23-kD variant is non-glycosylated (ratio 15 : 85, as judged by densitometry). The dilution curves of this PRL were parallel to those of pituitary-derived hPRL in several ELISA assays. The bioactivity in an Nb2 lymphoma cell proliferation assay was also similar to that of the pituitary-derived hormone (9).

Protocol of experiments

When the litter was first noted, the mother rats were divided randomly into three groups. Group B (N = 13) received subcutaneous injections of bromocriptine (0.1 mg/rat) in the back once a day at 09.00 h from day 1 to day 9 postpartum. Group B + P (N = 10) was treated subcutaneously with bromocriptine in the same manner as group B and also with r-hPRL (50 µg/rat) twice a day (at 09.00 h and 17.00 h). Group C (N = 10) was treated with vehicle by the same schedule as group B + P.

Lactational performance was assessed by weighing the pups in the three groups daily from birth to postnatal day 10.

The weights of rats in groups B, B + P and C were 228.8 ± 15.3, 233.0 ± 16.8 and 234.9 ± 13.8 g (means ± sd), respectively, on the day of delivery; the values were not significantly different in the three groups. Consequently, the amounts of r-hPRL administered each time to rats in groups B, B + P and C were 0.219 ± 0.015, 0.216 ± 0.016 and 0.214 ± 0.013 µg/g rat (means ± sd), respectively; the values were not significantly different in the three groups.

Serum PRL assays

Blood samples (300 µl) were collected by cannulation and the blood was replaced by an equal volume of saline at 09.00, 11.00, 13.00, 17.00, 19.00 and 21.00 on postpartum day 1 and 09.00 on day 2 before the next injection. The blood was centrifuged (1000 g for 5 min) and the serum was stored at −40°C until assayed.

Endogenous rat PRL was determined with a double-antibody RIA kit supplied under the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Rat Pituitary Hormone Distribution Program. Rat PRL RP-3 was used as a reference. The inter- and intra-assay coefficients of variation (cv) of this assay were 6.9% and 5.5%, respectively.

Exogenous r-hPRL in rat serum was determined with an immunoradiometric assay kit (SPAC-S Prolactin Kit; Daiichi Radioisotope Laboratories, Tokyo, Japan). The inter- and intra-assay cv of this assay were 6.1% and 3.6%, respectively.

The cross-reactivity between these species, determined using these two PRL kits, was less than 1.0%.

Histological examination of rat mammary glands

The mammary glands were examined to study the effects of bromocriptine and r-hPRL on their development. On postpartum day 10, six pairs of mammary glands from rats in the three groups were removed 30 min after the beginning of suckling and were weighed immediately. The inguinal mammary gland on one side of each rat was fixed in 10% formalin, cut and embedded in paraffin. Sections of all the strips were stained with hematoxylin and eosin (H&E). Each specimen was examined histologically by light microscopy.

Statistical analyses

All results are presented as means ± sem. Statistical significances were assessed by two-way analysis of variance (ANOVA) for repeated measurements combined with Bonferroni’s post hoc test. Data on the weights of mammary glands were analyzed by one-way analysis of variance (ANOVA) by Bonferroni’s method. The Bonferroni critical significance level was obtained from tables of the t-distribution using a significance level of p/m, where ‘m’ is the number of comparisons of groups performed. In this study, a p value of 0.05 was replaced by 0.05/2 = 0.025 (11).
Results

Prolactin levels on bromocriptine treatment

The changes in the serum concentrations of rat PRL in the three groups on postpartum day 1 are shown in Fig. 1. Administration of bromocriptine resulted in a decrease in the serum concentrations of rat PRL, although the pups were allowed to suckle freely. The endogenous rat PRL levels in groups B and B + P decreased to less than 5 µg/l for more than 12 h after bromocriptine administration, and they were significantly lower than those of the normal control rats. Just before bromocriptine injection on the next day, however, the PRL level increased to that of control rats.

Human PRL levels after r-hPRL injection

The changes in concentrations of hPRL in rats on postpartum day 1 are shown in Fig. 2. Administration of r-hPRL rapidly increased the serum concentration of hPRL. After injection of r-hPRL (50 µg/rat) the concentration reached a peak of about 23 µg/l in 2 h, decreased to half this level after 4 h and had almost disappeared after 8 h. The concentrations of hPRL in groups B and C were below the level of sensitivity of the assay.

Changes in weight of pups after r-hPRL injection

Figure 3 shows the changes in mean weights of the pups in the three groups from postnatal days 0 to 10. The...
body weights of pups at birth were not significantly different in the three groups. Administration of bromocriptine resulted in a decreased weight gain of pups, the mean weights of pups in group B being significantly lower than those in group C from postnatal days 5 to 10. Treatment with r-hPRL counteracted the effect of bromocriptine: the mean weight of pups in group B + P was significantly more than that in group B from postnatal days 8 to 10 and was not significantly different from that in group C.

**Changes of the mammary glands**

The weights of the mammary glands in groups B, B + P and C were 14.9 ± 0.9, 19.8 ± 1.0 and 20.8 ± 1.1 g/rat respectively, on postpartum day 10, their weights in group B being significantly less than those in groups B + P and C (p < 0.01). The weight of the mammary glands correlated well with the weight of the pups on postpartum day 10 (r = 0.786, p < 0.001).

The histological appearances of the mammary glands were also examined by light microscopy. The glands in group C showed well-developed delineation of interlobular connective tissue, a well-defined lobuloalveolar structure and alveoli with a wide lumen and thin, dilated walls. Group B showed a decrease in density and a nodular–glandular structure, resulting from a relative reduction of alveoli and an increase in fat stroma and connective tissue. The histological changes caused by bromocriptine were reversed by simultaneous administration of r-hPRL (group B + P), resulting in a similar appearance to that in group C.

**Discussion**

To treat women with poor puerperal lactation, it is effective to increase the serum PRL level. Administration of exogenous PRL seems, logically, to be the best method to elevate the PRL level. However, extraction of PRL from human pituitary on a commercial scale is not possible owing to a shortage of material and the potential biohazard of Creutzfeldt–Jakob disease (12). Alternatively, several drugs (e.g. sulpiride, TRH, metoclopramide) that stimulate PRL secretion are used to increase endogenous PRL and to augment lactation (13–16). We use sulpiride for women with poor lactation because it stimulates PRL release from the pituitary by blocking dopamine receptors and increases milk secretion from the mammary glands (13, 14).

Recently, many recombinant products are available for clinical use (e.g. GH, FSH, TSH) (17–19). Usually, such recombinant proteins have more advantages than its extracts. They can be supplied stably in invariable quality and adequate quantity at low price and free from infectious virus. Recombinant hPRL has also been produced by genetic technology (9). In the present study, we demonstrate the efficacy of r-hPRL for treatment of poor puerperal lactation using a rat model. Therefore, our results suggest that r-hPRL is useful for the treatment of breast-feeding women with poor lactation.

In this study we used a rat model. The human and rat PRL genes have 73% nucleotide and 63% amino acid sequence homologies. Moreover, the structures of human and rat PRL, which both have three disulide bonds, are similar (20, 21). The structure of the PRL receptor has been identified recently by screening cDNA libraries (22, 23), and the extracellular domains of the human and rat PRL receptors have been shown to have high structural homology (24). The long form of the PRL receptor, which is involved in milk protein gene transcription, is present in the mammary glands of both humans and rats (25). Moreover, PRL can easily bind to the PRL receptors of other species because the species specificity of its ligand-binding domains is low. The decreased weight gain of rat pups caused by bromocriptine treatment was reported to be reversed partially by simultaneous administration of ovine PRL during the treatment period (26). These reports suggest that the action of r-hPRL in this study was caused by its direct binding to the receptors in the mammary glands of rats.

In our rat model bromocriptine was used to decrease the serum PRL level and lactation. We found that injection of 0.1 mg bromocriptine/rat effectively decreased the serum PRL level (Fig. 1). The increase of PRL during suckling was prevented for several hours by bromocriptine, and daily treatment with bromocriptine impaired lactational performance and decreased the weight gain of the pups (Fig. 3). After subcutaneous injection of r-hPRL (50 μg/rat) the serum hPRL level promptly increased to a maximum of 23 μg/l in 2 h then decreased to less than half this level after about 4 h and to almost zero after 8 h (Fig. 2). Data obtained by more frequent sampling in other rats (not shown in this paper) showed a similar change.

Twice-daily injections of r-hPRL (50 μg/rat) completely restored the weight gain of the pups and the weight of the mammary glands to that of normal controls. We also preliminarily examined the effect of a half-dose of r-hPRL (25 μg/rat, twice a day: data not shown). This dose of r-hPRL only partially restored the weight gain of the pups and the weight of the mammary glands in the bromocriptine-treated rats compared to the normal controls. These findings indicate that r-hPRL improved the milk yield of rats with poor lactation and that its effect was dose dependent.

Histological examination of the mammary glands showed that PRL was important for the development of the mammary glands in the puerperal period. We also observed that the histological changes of the mammary glands caused by bromocriptine were reversed by r-hPRL.

In conclusion, our results clearly demonstrate that r-hPRL is biologically active in vivo and replacement
therapy of r-hPRL was effective in improving the lactational performance in a rat model. Our results strongly indicate the value of studies on the effect of r-hPRL on lactational performance in women with poor lactation.

Acknowledgments. We are indebted to the NIDDK (Baltimore, MD) for generous supply of a rat PRL kit. We are also grateful to Dr Susan M Richards and Dr Paul T Gelpe of the Genzyme Corporation (Framingham, MA) for supplying the recombinant human PRL. This work was partly supported by a grant from the Japanese Ministry of Health and Welfare (Tokyo, Japan).

References