Influence of Antisense IGFBP-2 Oligo Deoxynucleotide Administration on Tissue IGFBP-2 Gene Expression in Chicks

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ABSTRACT : We have examined the influence of antisense oligo deoxynucleotide (ODN) of IGFBP-2 on tissue IGFBP-2 gene expression in chicks. Antisense IGFBP-2 ODN was directly injected into the liver or cerebroventricle. Control birds were injected with vehicle. The hepatic IGFBP-2 gene expression was decreased to approximately 30% of the control at 2 h after injection of antisense ODN. In the brain of chickens injected with antisense ODN, IGFBP-2 mRNA level did not change after 2 h of injection and decreased to approximately 60% of the control after 6 h of injection. These results showed that the expression of IGFBP-2 gene in the liver and brain was successfully suppressed by administrating antisense ODN and that hepatic IGFBP-2 gene expression was quickly suppressed by antisense ODN compared with the brain. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1781-1784)*

Key Words : Chickens, Antisense, IGFBP-2, Liver, Brain

INTRODUCTION

The chicken insulin-like growth factor-I (IGF-I) has been characterized and shown to be 70 amino acid polypeptides (Ballard et al., 1990). Some findings pointed out an important role for IGF-I in the control of growth and metabolism in chickens, as in mammals. In most circumstances, IGF-I makes a complex with specific high-affinity IGF-binding proteins (IGFBPs). So far, six different IGFBPs have been found in mammals and their genes have also been cloned and identified (Shimasaki and Ling, 1991). IGFBPs are thought to prolong the half-life of circulating IGFs (Ketelslegers et al., 1996) and also act as modulators of IGF activity (Elgin et al., 1987; Rutanen et al., 1988). Several species of IGFBPs have been found in blood of avian species (Armstrong et al., 1989; Schoen et al., 1992). Recently, Schoen et al. (1995) have cloned and characterized a cDNA of chicken IGFBP-2, and revealed that it codes for a mature 275 amino acid protein. Thereafter, in the chicken, we revealed that hepatic IGFBP-2 gene expression was increased by fasting and decreased by refeeding (Nagao et al., 2001). It has also become apparent that IGFBP-2 inhibits physiological activities of IGF-I and suppresses cell proliferation (Corkins et al., 1995). These results suggest that IGFBP-2 is increased under malnutritional conditions and is one of various factors arresting animal growth. Recently, it has been reported that the administration of antisense oligo deoxynucleotide (ODN) successfully decreased the expression of target gene. Therefore, if antisense ODN against IGFBP-2 gene could suppress its expression under malnutritional conditions, physiological activities of IGF-I might be kept to the level of normal animals. In the present study, we have examined the effect of antisense IGFBP-2 ODN administration on tissue IGFBP-2 gene expression in fasted chicks.

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MATERIALS AND METHODS

One hundred single-comb White Leghorn male chicks from a local hatchery (Hattori Yokei Ltd, Nagoya, Japan) were fed a commercial chick mash diet (Pre-chick, crude protein 215 g/kg, metabolizable energy 12.1 kJ/g; Marubeni Shiryou Ltd, Tokyo, Japan) from hatching until 3 days of age in electrically heated brooders. At this age, after the birds were fasted for 8 h, 56 birds of uniform body weight were selected and divided evenly into 8 experimental groups of 7 birds each. The birds were placed in stainless steel metabolism cages in a temperature-controlled (29±1°C) room. Continuous illumination was provided. Twenty-eight chickens in 4 experimental groups were directly injected with antisense IGFBP-2 ODN using a microsyringe. Fourteen chicks in half of antisense groups were injected into the liver, and the remaining 14 birds were injected into the cerebroventricle. The antisense IGFBP-2 ODN used in this study was phosphorothioated and its sequence was 5'-CCG ACC CCG CCG AGC GCC AT-3' (Rikaken Co. Ltd., Nagoya, Japan). The Antisense IGFBP-2 ODN binds to complementary sequences of IGFBP-2 mRNA and inhibits translating IGFBP-2 mRNA to peptide. The ODN was dissolved in 0.15 M NaCl solution containing 0.1% Evans Blue and then mixed with DMRIE-C reagent (Life Technologies, Frederick, MD, U.S.A.) (1:1). The DMRIE-C reagent is a cationic liposome to transfer exogenous genes into cytoplasm. The amount of antisense

IGFBP-2 ODN injected was 50 μg for each chicken. Twenty-eight control birds were injected with the mixture of 0.15 M NaCl solution containing 0.1% Evans Blue and DMRIE-C regent. At 2 or 6 h after injection, 7 birds each were anesthetized by diethyl ether, and then the liver and brain were removed. Tissue samples were frozen immediately in liquid nitrogen and were stored at -80°C until analyzed.

Total RNA was extracted from tissue samples by using a commercial total RNA extraction reagent TRISOL^{R} Reagent (Life Technologies, Frederick, MD, U.S.A.), and then poly-(A) RNA was isolated from total RNA with Oligotex-dT30 (Takara Shuzo Co. Ltd., Kyoto, Japan). Thereafter, to detect tissue IGFBP-2 mRNA, northern hybridization was conducted according to the procedure described by Sambrook et al. (1989). In this assay, 2 μg of poly-(A) RNA sample was used. Chicken IGFBP-2 cDNA (Schoen et al., 1995) was generously donated by Dr. T. J. Schoen (Laboratory of Retinal Cell and Molecular Biology, National Eye Institute, National Institute of Health, Bethesda, MD, U.S.A.). The chicken IGFBP-2 cDNA fragment was isolated from plasmid vector pBluescript KS+ by cutting with Not I/Sal I. In the present study, to certify that equal amounts of total RNA samples were loaded onto agarose gels for electrophoresis, chicken ribosomal protein S17 mRNA was also measured. Chicken ribosomal protein S17 cDNA (Trueb et al., 1988) was generously gifted by Dr. B. Trueb (Laboratorium fur Biochemie I, ETH Zentrum, Zurich, Switzerland). Chicken ribosomal protein S17 cDNA was isolated from plasmid vector pUC19 by cutting with Eco RI. The ^{32}P-labelled probes were synthesized according to the protocol of a random primer DNA labelling kit (Takara Shuzo Co. Ltd., Kyoto, Japan). The intensity of chicken IGFBP-2 and ribosomal protein S17 bands was measured using a bio-imaging analysis system (BAS 2000, Fuji Photo Film, Co. Ltd., Tokyo, Japan).

Statistical analysis was performed by one-way ANOVA followed by Duncan's multiple range test (Duncan, 1955) using the General Linear Model Procedures (GLM ; SAS/STAT Version 6, SAS Institute, Cary, NC). Differences between means were considered to be significant at $p<0.05$. Regression equation was also calculated using GLM.

RESULTS

The intensity of bands of hepatic IGFBP-2 mRNA visualized by Northern blot analyses is shown in figure 1. The hepatic IGFBP-2 gene expression was decreased to approximately 30% of the control at both 2 and 6 h after injection of antisense IGFBP-2 ODN.

Influence of antisense IGFBP-2 ODN on brain IGFBP-2 mRNA levels is shown in figure 2. There was no significant difference in the brain IGFBP-2 gene expression between control and antisense groups at 2 h after injection. However, after 6 h of injection with antisense IGFBP-2 ODN, brain IGFBP-2 mRNA level was decreased to approximately 60% of the control.

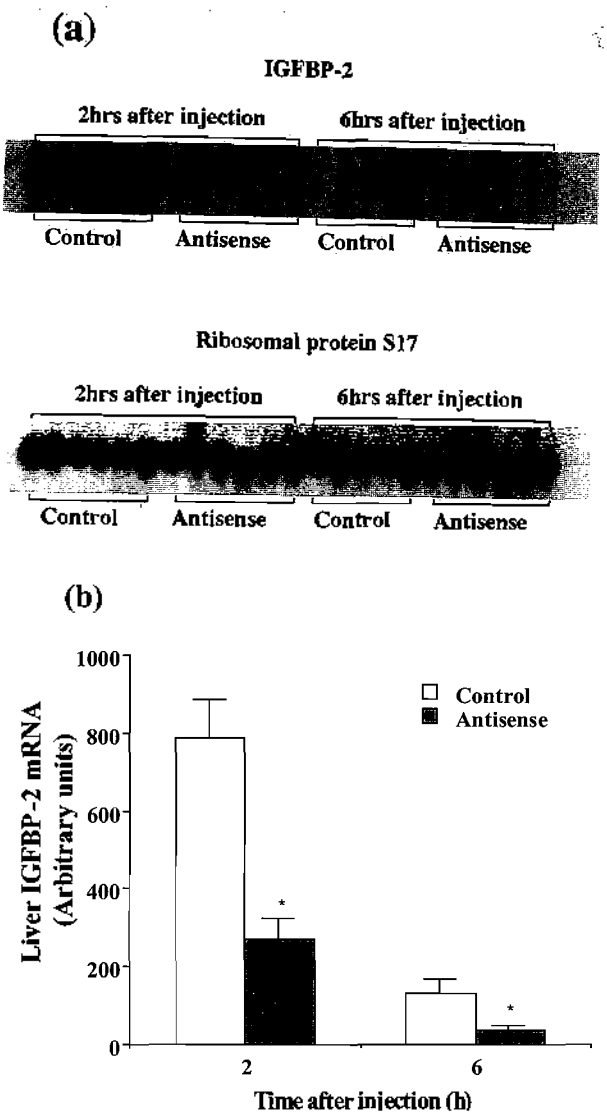

Figure 1. (a) Northern blot analysis of IGFBP-2 and ribosomal protein S17 mRNA in the liver of chicks. Poly-(A) RNA (2 μg) was applied to each lane and detected with ^{32}P-labeled chicken IGFBP-2 and ribosomal protein S17 cDNA. **(b)** Influence of antisense IGFBP-2 oligo nucleotide on IGFBP-2 mRNA level in the liver of chicks. Chickens were injected with or without antisense IGFBP-2 oligo nucleotide (in mixture of 0.15 M NaCl solution and DMRIE-C), and were killed at 2 or 6 h after the injection. Control birds were injected with the mixture of 0.15 M NaCl solution and DMRIE-C as a vehicle. Vertical bar represents mean ± SEM of 5-7 birds. * Significantly different from control within the same time at $p<0.05$.

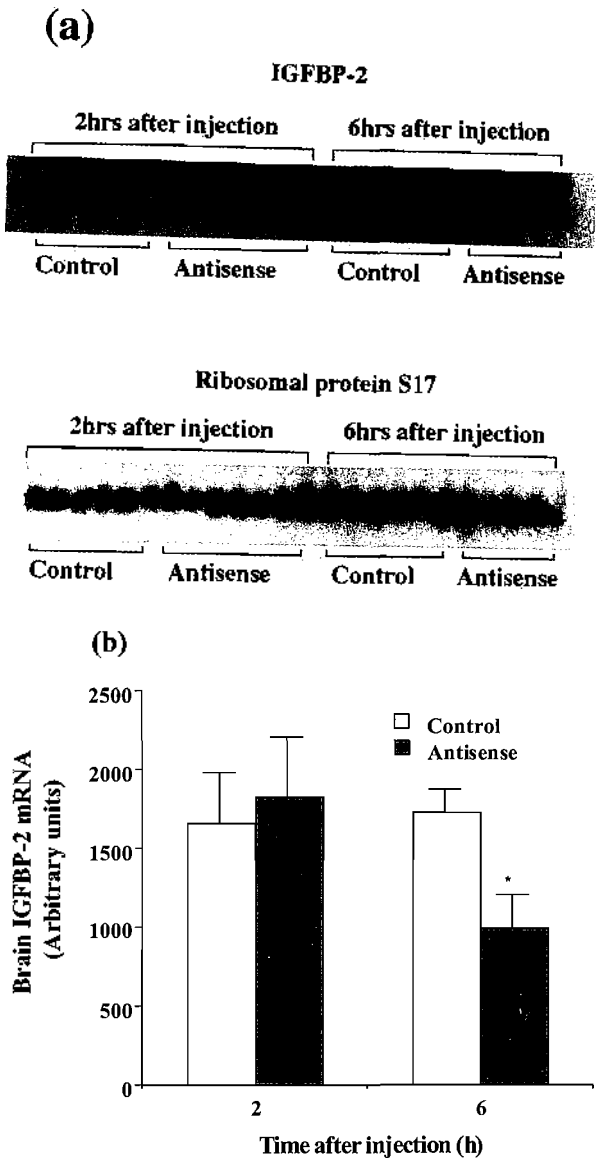

Figure 2. **(a)** Northern blot analysis of IGFBP-2 and ribosomal protein S17 mRNA in the brain of chicks. Poly-(A) RNA (2 μg) was applied to each lane and detected with ^{32}P-labeled chicken IGFBP-2 and ribosomal protein S17 cDNA. **(b)** Influence of antisense IGFBP-2 oligo nucleotide on IGFBP-2 mRNA level in the brain of chicks. Chickens were injected with or without antisense IGFBP-2 oligo nucleotide (in mixture of 0.15 M NaCl solution and DMRIE-C), and were killed at 2 or 6 h after the injection. Control birds were injected with the mixture of 0.15 M NaCl solution and DMRIE-C as a vehicle. Vertical bar represents mean ± SEM of 5-7 birds. * Significantly different from control within the same time at $p<0.05$.

DISCUSSION

In our previous study, we showed that the response to fasting was different between liver and brain, in which 2 d fasting increased liver IGFBP-2 gene expression but did not change brain IGFBP-2 gene expression (Nagao et al., 2001). As shown in figure 1, fasting successfully stimulated IGFBP-2 gene expression in the liver of control chicks and did not affect on brain IGFBP-2 gene expression. However, the hepatic IGFBP-2 mRNA of control chicks was decreased at 6 h after vehicle injection. In the present study, the age of chicks was 3 days and it was considerably younger than that used in our previous study. These results suggest that the response of IGFBP-2 gene expression to fasting might be modulated by the difference in the age of birds.

The hepatic IGFBP-2 gene expression was decreased to almost 30% of the control at 2 h after injection of antisense IGFBP-2 ODN (figure 1). As similar reduction in hepatic IGFBP-2 mRNA level was also observed at 6 h after injection, it was suggested that the effect of antisense ODN administrated in the present study was, at least, kept to 6 h after injection.

As shown in figure 2, brain IGFBP-2 gene expression was decreased to approximately 60% of the control at 6 h after injection of antisense IGFBP-2 ODN. However, only 2 h after ODN injection was not enough to suppress the brain IGFBP-2 gene expression (figure 2). It was reported that the effect of antisense ODN administrated into cerebroventricle was observed after 12 h of administration (Wan et al., 1998). In the present study, as the effect of antisense ODN was observed at 2 and 6 h after administration in the liver and brain, respectively, the time required for antisense ODN to exert the effect after injection might be depend on the difference in tissues.

In conclusion, we showed that the expression of IGFBP-2 gene in the liver and brain was successfully decreased by administrating antisense ODN and that response time against antisense ODN differed between liver and brain.

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