学 位 論 文 の 要 旨

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学 位 論 文 名　Effects of Restriction of Fetal Jaw Movement on Prenatal Development of the Temporalis Muscle

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論 文 内 容 の 要 旨

INTRODUCTION

Mechanical strain regulates cell proliferation, differentiation and maturation, all of which are related to growth and development, therefore, masticatory activity and jaw movement are thought to be related to the morphology of the jaw and jaw-related muscles. Jaw movement is responsible for changes in both the volume and length of masticatory muscles, as determined by direct measurements of muscle and jaw movements. Temporalis muscle is one of the three main jaw-closing muscles (masseter, temporalis, medial pterygoid muscles). The combination of its broad origin on the temporal fossa and the inner surface of the temporal fascia and its limited insertion on the anterior border and medial surface of the coronoid process allows differential activation and variation in the line of action. Previously we reported that prenatal jaw movement is an important mechanical factor for the development of the mandibular condylar cartilage and articular disc, however, the effects of jaw movement on the development of the temporalis muscle in the prenatal period was not clarified. The present study was therefore designed to investigate the role of the fetal jaw movement on the development of the temporalis muscle.

MATERIALS AND METHODS

Jcl:ICR female mice were used for jaw movement restriction of their embryos by *exo utero* surgery system. At embryonic day (E)15.5 we sutured mandible and maxilla of the embryo by 8-0 nylon, sham-operation was also done by passing the needle in the

mandible and maxilla without making a knot, and then we kept the embryos for *exo utero* development. We prepared 4 groups of embryos, i.e., E18.5 sham-operated group, E18.5 sutured group, E18.5 *in utero* control group, and E17.5 *in utero* control group. At E18.5 embryonic heads were fixed with Bouin's solution, 5 μm-thick serial sagittal sections were made, and hematoxylin and eosin (HE) staining was done for histological and computerised histomorphometric study. Temporalis muscles were prepared for transmission electron microscopical examination to evaluate the maturity of the muscle fibers at the subcellular level and to observe and count satellite cells, a type of stem cells related to the differentiation of muscle fibers from the late gestation. To examine the expression of myogenic regulatory factor-6 (*Myf-6*) mRNA, we used quantitative real-time RT-PCR at E18.5.

RESULTS AND DISCUSSION

The HE staining of the histological section of the temporalis muscle revealed that in the E18.5 *in utero* control and E18.5 sham-operated groups, the muscle fibers were generally thick, straight, homogenous, and were arranged in groups or bundles, and the spindle-shaped nuclei were peripherally placed. In the E18.5 sutured group, the muscle fibers were thin and not straight (i.e., curly or wavy), muscle fibers appeared scattered with more extracellular space, and the nuclei were large, round, and generally centrally situated. The developmental characteristics of the muscle fibers of the E17.5 *in utero* control group were generally similar to those of the E18.5 sutured group, except that curly and wavy muscle fibers were not observed in the E17.5 *in utero* control group. There were no significant differences in the total volume of the temporalis muscle, muscle fiber volume between the E18.5 *in utero* control and E18.5 sham-operated group, while the muscle fiber volume was significantly higher in these groups than in the E17.5 *in utero* control group. At E18.5, both the total muscle volume ($p < 0.05$) and the muscle fiber volume ($p < 0.01$), but not the connective tissue volume, were significantly lower in the E18.5 sutured group than in the E18.5 *in utero* control or E18.5 sham-operated group. Our present histological observations suggested that the development of the temporalis muscle was more delayed in the E18.5 sutured group with respect of the timeline of development than in the E18.5 *in utero* control and the E18.5 sham-operated groups.

TEM analysis revealed that in the temporalis muscles of the E18.5 *in utero* control and E18.5 sham-operated groups the muscle fibers were condensed, arranged in bundle, contained fewer fibroblasts in-between and there was less extracellular matrix/space than in the E18.5 sutured group. The spindle shaped nuclei of the muscle fibers were peripherally situated, and the mitochondria were of regular size and shape, contained more and parallel cristae and exhibited greater electron density in the E18.5 *in utero* control and E18.5 sham-operated groups than in the E18.5 sutured group. In the E17.5 *in utero* control and E18.5 sutured groups, density of muscle fibers was low, scattered

arrangement was seen, and there were more fibroblasts as well as more extracellular matrix/space than in the E18.5 *in utero* control and E18.5 sham-operated groups. In the E18.5 sutured group, muscle cell nuclei were large and centrally situated, mitochondria were expanded in volume and contained fewer cristae, and lower electron density of cell nuclei and mitochondria were observed than in the E18.5 *in utero* control and E18.5 sham-operated groups. Further muscle cells frequently contained generally electron-dense inclusion bodies of various sizes in the E18.5 sutured group. TEM study suggested that, in the E18.5 sutured group, the muscles tended to be less mature than those of the E18.5 *in utero* control and the E18.5 sham-operated groups, although some immature muscle fibers were also identified in the latter groups. Histological and TEM findings, taken together, suggested that jaw movement restriction might have delayed muscle differentiation and progress to maturity and might possibly have had other abnormal effects on the muscle development.

We therefore examined whether or not myogenic regulatory factors were affected by the jaw restrictive treatment. *Myf-6* was selected from among a number of candidate factors, because *Myf-6* mRNA begins to be expressed in the masticatory muscles of the mouse embryo from E13-15, and its expression increases steeply until to the time of birth. However, in the present study, *Myf-6* expression in the temporalis muscle did not significantly differ between the E18.5 sutured and E18.5 sham-operated groups, suggesting that the genetic commitment to myogenesis was not affected by the present procedure.

We found that the number of satellite cells was significantly greater in the E18.5 *in utero* control group than in the E17.5 *in utero* control group, which is consistent with the finding that these cells started to appear as undifferentiated stem cells at the end of the second wave of myogenesis at E17.5. However, we found no significant differences between the E18.5 *in utero* control, E18.5 sham-operated and E18.5 sutured groups. Therefore, these findings may correspond to the result regarding *Myf-6* expression, although our TEM observations were limited to a very small area.

Although no significant changes were observed at the *Myf-6* level or in the frequency of satellite cells in the E18.5 sutured group, the present morphological findings suggested that the development of the temporalis muscle was delayed by approximately one day. Certain abnormalities were observed in the muscle fibers of the E18.5 sutured group, which suggests that jaw movement restriction indeed induced developmental differences from the E18.5 *in utero* control and the E18.5 sham-operated groups.

CONCLUSION

The histological, histomorphometric analyses and TEM findings from the present *in vivo* study using the *exo utero* method suggested that the fetal jaw movement plays a role in the proper development of the temporalis muscle in the prenatal period.