

# 学 位 論 文 の 要 旨

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学 位 論 文 名	Effect of Ageing on Healing of Bilateral Mandibular Condyle Fractures in a Rat Model
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## 論 文 内 容 の 要 旨

### INTRODUCTION

The mandibular condyle is frequent site of maxillofacial fracture. The management of condylar fracture is important to restored mandibular ramus height by an open reduction. In children in particular, conservative treatment is usually advocated because surgical treatment of condylar fracture tends to cause growth disturbances rather than eliminate them. In a previous study of unilateral mandibular condylar fracture rats revealed that the conservative treatment could be feasible in child unilateral condylar fracture. However, there have been no detailed experimental studies on the healing of bilateral condylar fracture. In an effort to explore the feasibility of conservative treatment of dislocated bilateral condylar fracture in a younger clinical population, this study aimed to elucidate the effect of ageing on condylar remodelling after experimental bilateral condylar fracture in rats.

### MATERIALS AND METHODS

Male Sprague-Dawley rats aged 3, 6, and 36 weeks old ( $n = 25/\text{cohort}$ , total  $n = 75$ ) were divided a fracture group ( $n = 12$ ) and sham control group ( $n = 12$ ), with 1 rat from each cohort used as a normal unoperated control.

**Surgical procedures** In the fracture group, a 1-cm incision was made over the mandibular

angle parallel to the inferior border of the mandible on each side under general anesthesia. The bilateral condylar neck was performed horizontal osteotomy at lowest level of the sigmoid notch. In the sham control group, the condylar neck was exposed in the same manner and the wound was closed.

**Tissue preparation** The control rats were killed immediately, while the fracture and sham control groups were prepared for microscopic evaluation at 1, 2, 4, and 8 weeks ( $n = 3/\text{week}$ ) after fracture. All sections were dehydrated in a graded series of ethanol and processed for routine paraffin embedding. Four-micron-thick, coronal, midcondylar sections were cut. Routine haematoxylin-eosin (HE) staining was done on the first section, and the second section was stained with Azan.

**Immunohistochemistry** Cell proliferation was evaluated by the bromodeoxyuridine (BrdU) labelling index (LI). Osteochondrogenesis was assessed by the expression of Indian hedgehog (Ihh), type X collagen, and osteocalcin in the condylar head.

**Quantitative analysis** BrdU-labelled and non-labelled cells in the intermediate cell layers of both condyles in the fracture and sham control groups were counted with the aid of a squared eyepiece graticule at a magnification of  $400\times$ . For each animal, four coronal mid-condylar sections were prepared and at least 1,000 cells per section were counted to obtain the average LI (labelled cells/total cells counted  $\times 100\%$ ).

The number of Ihh-positive cells was also counted in a zone of flattened chondrocytes or a zone of hypertrophic chondrocytes by the same method as for the BrdU-labelled cells.

## **RESULTS AND DISCUSSION**

Post operation, in 3- and 6-week-old rats, mean body weight continued to increase both the fracture and sham groups. In 36-week-old rats, body weight decreased in both the fracture and sham groups from immediately to postoperative week 1, then gradually increased in both groups.

In histological findings, at postoperative week 1, the condylar fragment was deviated and hypertrophy of the condylar cartilage was clearly apparent in all age cohorts. In 3-week-old rats, at postoperative week 2, the condylar fragment had returned to its normal position. At postoperative week 4, the segmental gap was bridged and the characteristics of the condyle were normal. In 6-week-old rats, at postoperative week 2, the condylar fragment was still deviated and

boney gap was observed. At postoperative week 4, union by immature bone trabeculae was observed and the condylar process was centralized. At postoperative week 8, the normal characteristics of the condyle and condylar process were well preserved. In 36-week-old rats, at postoperative week 2, part of the condylar fragment was absorbed and deformity of the condylar cartilage. At postoperative week 4, the displacement of the condylar fragment and diastasis of the fracture edge were observed. However the condylar process had been formed in the direction of median of temporal fossa. At postoperative week 8, the condylar process was repositioned in the temporal fossa.

BrdU LI values in the fracture groups recovered by 45.8% in 3-week-old rats, 36.0% in 6-week-old rats, and 17.9% in 36-week-old rats at 8 weeks after fracture. The number of Ihh-positive cells in the fracture groups significantly increased up to 2 weeks after fracture, then gradually decreased until 8 weeks after fracture.

In the fracture groups, except for weak type X collagen expression at postoperative week 1 in 6-week-old rats, the findings were similar to those of the 3- and 6-week-old rats in the sham control groups. In 36-week-old rats, weak type X collagen expression was seen at postoperative week 2 and 4.

Regarding the expression of osteocalcin, in the fracture groups, there were no osteocalcin-positive cells in the marrow until expression similar to the sham control groups at postoperative week 4 and 8 was seen in the 3- and 6-week-old rats. In 36-week-old rats, expression was invariant over time.

In all age cohorts, bilateral condylar fractures were restored by conservative treatment, but healing was delayed by ageing. BrdU LI values in the fracture groups were higher in younger rats at 8 weeks after fracture. The results of Ihh, type X collagen and osteocalcin expression indicated that osteochondrogenesis in the condyles recovered immediately after fracture in younger rats. Thus, the younger an animal with bilateral mandibular condylar fracture, the more satisfactory the TMJ condition without surgical treatment, but functional issues regarding ramus height and its consequences on occlusion have not been tested in this study.

### **CONCLUSION**

The findings of this study support the clinical concept of conservative treatment of bilateral condylar fractures in younger patients.

## 論文審査及び最終試験又は学力の確認の結果の要旨

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学位論文審査委員	主 査	内尾 祐司
	副 査	川内 秀之
	副 査	浦野 健

## 論文審査の結果の要旨

顎関節突起骨折の治療には外科療法と保存療法とがあるが、片側顎関節突起骨折では若年者に対する保存療法の有用性が実験的に証明されており、軟骨内骨化と骨膜反応による複合的な修復により骨折が治癒するといわれている。しかし、その詳細は未だ不明であり、しかも両側顎関節突起骨折(BMCF; bilateral mandibular condyle fractures)に対する検討もない。そこで、申請者は BMCF に対する保存療法の有用性と、加齢による影響を明らかにする目的で、ラットの BMCF モデルを作製して、骨軟骨骨化と下顎頭の細胞増殖活性の観点から検討した。75匹の Sprague-Dawley (SD)雄ラットを25匹ずつ若年群(3週齢)、成年群(6週齢)、老年群(36週齢)に分け、さらに各群を骨折群12匹、sham control 群12匹および control 1匹とした。全身麻酔下に歯科用バーにて BMCF モデルを作製し、骨折後1、2、4および8週にホルマリン還流固定と脱灰の後、骨折部を含む頭蓋前額断薄切パラフィン切片を作製した。骨折の治癒過程を hematoxylin-eosin染色と Azan染色で評価するとともに、軟骨細胞の増殖を Indian hedgehog (Ihh)陽性細胞数で、軟骨内骨化についてはX型 collagen (COL X)発現を免疫組織化学的に評価した。さらに、骨芽細胞の成熟をosteocalcin (OCN)の発現で、下顎頭の細胞増殖活性を bromodeoxyuridine (BrdU)標識率によって評価した。その結果、骨折部は若年群と成年群では骨折後4週で治癒したが、老年群では8週を要した。若年群では骨折後早期から肥大軟骨層の肥厚による下顎頭の変形を生じた。一方、老年群では骨膜からの骨形成による下顎頭の変形を認めた。成年群では軟骨の肥厚と骨膜反応が複合的に観察された。いずれの群でも、骨折の治癒とともに変形はほぼ消失していた。また、全群で小骨片の変形に一致して Ihh 陽性細胞数と BrdU 標識率の上昇を認めた。若年群と成年群では骨折後早期から COL X が発現する一方で、OCN は発現しないまま軟骨内骨化が促進した。しかし、老年群では COL X の発現はなく、OCN は骨折後1週より発現していた。また、骨折後8週における BrdU 標識率は年齢とともに低下した。以上より、BMCF に対する保存療法では加齢に伴い骨折治癒が遅延することや下顎頭の修復機序が異なることが明らかとなった。本研究は若年者のBMCFに対する保存療法の有用性だけでなく、骨折修復機序の年齢的相違を明らかにするもので、学位授与に値すると判断した。

## 最終試験又は学力の確認の結果の要旨

申請者はラット両側顎関節突起骨折を用いて、年齢が及ぼす骨折修復機序の相違を組織学的および免疫組織化学的に明らかにした。これは若年者における両側顎関節突起骨折に対する保存療法の有用性を実験的に証明しただけでなく、骨折修復機序の年齢的相違を解明した研究であることから学位授与に値すると判断した。(主査 内尾祐司)

申請者は両側顎関節突起の介達骨折のラットモデルを用いて、骨折修復の機序と加齢の影響について、病理組織学的検討を行い、保存療法の有用性を明らかにした。研究に必要な方法論の理解や臨床的な知識も十分であり、学位に値すると判断した。(副査 川内秀之)

申請者は組織学的および免疫組織学的手法を用いて、ラット両側顎関節突起骨折モデルにおける骨折修復機序の年齢による相違を明らかにし、さらに若年群における保存療法の有用性を明らかにした。学位論文審査時における質疑応答も的確で、豊富な関連分野の知識を有しており、学位授与に値すると判断した。(副査 浦野 健)

(備考) 要旨は、それぞれ400字程度とする。