EFFECTS OF IPRIFLAVONE ON COLLAGEN SYNTHESIS OF OSTEOBLAST-LIKE CELLS(MC3T3-E1 CELL LINE)

Yun-Seok, Yang D.D.S., M.S.D.

Visiting Scholar, Department of Oral and Maxillofacial Surgery
University of Washington, Seattle, washington

Ipriflavone이 골 세포주(MC3T3-E1 cell line)의 Collagen합성에 미치는 영향

양윤석 D.D.S., M.S.D.*

Visiting Scholar, Department of Oral and Maxillofacial Surgery
University of Washington, Seattle, washington

Ipriflavone(IP)은 골흡수 억제효과와 골형성 촉진효과를 지니는 약물로 보고되어 왔다.

이러한 IP의 특성때문에 골의 치유를 촉진시키기 위한 약물로서 구강외과 영역에서 쓰일 수도 있으리라 생각되었다.

이에 저자는 그동안 보고되어온 IP의 골 형성 촉진효과가 실제로 나타나는지를 확인하고 또한 어떤 농도에서 나타나는지를 알아보기위해 IP를 서로다른 농도로 하여 골세포주(MC3T3-E1 cell line)의 배지에 넣은후, 골 형성의 지표로 쓰일수있는 collagen합성정도를 보고자 하였으며 이 자료를 앞으로의 *in vivo* 동물실험 연구의 기초자료로 사용코자 하였다.

본 연구에서 IP의 골형성 촉진효과를 collagen 합성정도의 측정을 통해 확인하였고, 특히 IP이 10^{-7}M농도일때 현저한 collagen합성의 증가를 관찰 하였으며 앞으로의 동물실험등을 통해 구강외과 영역에서의 사용가능성에 대해 좀더 연구해 보고자 한다.

I. Introduction

Ipriflavone(7-isopropoxyisoflavone), a natural isoflavone derivative, is known to prevent bone loss in various types of animal models of experimental osteoporosis[5,6] and is also known to be effective for the treatment of osteoporosis in human[7].

As for the mechanism by which IP exerts its effect on the bone, both direct inhibition of osteoclasts by modulation of interecellular free calcium[8], and indirect inhibition on osteoclasts recrutiment and differentiation mediated by osteoblasts[1] have been demonstra-

ted.

However, observations from other studies indicate an effect of ipriflavone also on bone formation[4].

Early studies showed a stimulatory efect of ipriflavone on collagen systhesis in wholeorgan culatures of human otosclerotic auditory ossicle samples[2,3]. More recently, a direct role of ipriflavone and its metabolites in modulating the synthetic and growth properties of osteoblastc cells was demonstrated[4].

Several studies have also shown that the ipriflavone inhibits the activity of cyclicadenosine-3', 5',-monophosphate(cAMP) phosphodiesterase to increase the intercellular cAMP level[9] in bone-forming cells thereby IP enhances the activities of osteoblasts. Because of the peculiar characteristics of ipriflavone which has both osteoclastic inhibition and osteoblastic stimulation activities, I thought it could be used as a good agents for facilitation of bone healing in oral and maxillofacial surgery.

In this study, I investigated the effect of ipriflavone on bone formation through the observaton of the collagen type I systhesis using SDS-PAGE.

I also investigated the concentration of ipriflavone in which the increase of collagen synthesis was shown as a guidline for the future *in vivo* experimantal animal study.

II. Materials and Methods

Cell cultures

MC3T3-E1 cells[11] were maintaned in α-Minimum Essential Medium(α-MEM, GibcoBRL, USA) suplemented with 10% fetal bovine serum(FBS, GibcoBRL, USA).

When cells were confluent, cells were incubated with Ipriflavone at concentrations indicated for 24thours.

Sodium Dodecyl Sulfate(SDS)-Poly Acrylamide Gel Electrophoresis(PAGE)

Cells were pulse labeled with the α-MEM containing 10μCi/ml of [^{3}H] proline (Amersham, USA), 1% FBS and various Ipriflavone for 24 hours. The radioactivity of whole cell proteins was determined by trichloracetic acid precipitation[12].

The samples with the same amount of radioactivity were digested with pepsin solution [13] and analyzed by SDS-PAGE[14]

III. Results

Ipriflavone at 10^{-7}M exhibited a strong stimulatory effect on collagen synthesis, but ipri-

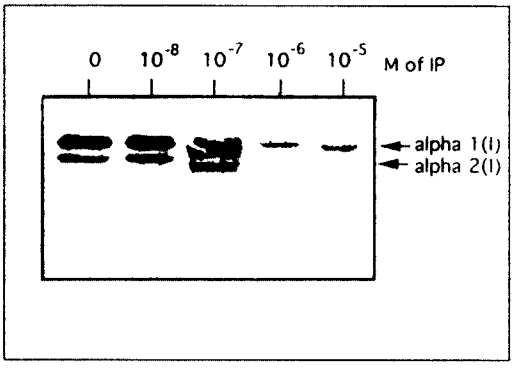

Fig. 1. Effect of different concentrations of IP on collagen synthesis of osteoblast-like cells(MC3T3-E1 cell line).

Table 1. Effect of ipriflavone on the synthesis of collagen

Concentration of Ip	0	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
Collagen synthesis	−	−	↑ ↑	↓	↓

flavone at 10^{-6}M, 10^{-5}M showed a inhibitory effect on collagen synthesis.(Fig. 1)

Ⅳ. Discussion

Collagen, the most abundant protein in connective tissue, represents 90% of the organic matrix of bone.

The collagen synthesis has been known as a good marker for the bone formation.

Therefore, I used the degee of collagen synthesis as a marker for the interpretation of the effects of Ipriflavone(IP) on the osteoblast-like cells.

In this study, I observed that IP stimulate the collagen systhesis of osteoblast-like cells. This confirms previous in vitro observations on IP's stimulation of collagen systhesis by human otosclerotic auditory ossicel samples[2,3].

This study showed that the concentration which stimulate the collagen synthesis of osteoblast-like cells was 10^{-7}M. We also observed that the higher concentration (10^{-6}M, 10^{-5} M) inhibited the collagen systhesis of osteoblast-like cells.

These date are not coincident with previous study(Sziklai et al : 1985) which showed that the concentration of IP which stimulated the osteoblasts-like cells was 10^{-5}M, but coincident with the study(Ribari et al : 1987) in the that 10^{-5}M reduced the collagenous protein synthesis.

These differences may be due to differences in culture conditions, cell species, and duration of the exposure of the cells to IP.

PGE_2 that is secreted by osteoblasts is currently the effective stimulator of bone resorption and enhances the resorptive activity of osteoclasts. It also play a role in collagen synthesis : low concentrations cause a decrease in collagen synthesis and high concentrations an increase[10].

Ribari et al[3] reported that IP 10^{-5}M decreased the collagen synthesis but increased collagen synthesis inhibited by low concentration of PGE_2.

Therefore, I think that the different concentration of PGE_2 which was secreted by osteoblast-like cells may be also a reason for the different results.

In conclusion, this study suggests that IP stimulates collagen synthesis of osteoblast-like cells in 10^{-7}M concentration.

Furthermore, IP may be used as a agent that facilitate bone healing in oral maxillofacial surgery.

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