EFFECT OF AGING ON THE PRODUCTION AND ACTION OF NITRIC OXIDE IN THE ARTICULAR CARTILAGE

**Introduction**

To understand the relation of nitric oxide(NO) to aging in the articular cartilage, we investigated the age-related change in the activity of nitric oxide synthase with nitrite assay. We also observed the change in the proliferative activity of chondrocytes after exogenous administration of NO using nonradioisotopic proliferation assay.

**Methods**

Forty New Zealand white rabbits were used, which comprised 4 age groups (1 month, 6 months, 1 year, and 3 years of age: 10 animals in each age group). Twenty rabbits were used for cartilage and cell culture, and another 20 rabbits for Western blotting and immunohistochemistry. The harvested chondrocytes were cultured in 96-welled plate. The concentration of cells was $1 \times 10^5$ for nitrite assay, and $1 \times 10^6$ for proliferation assay.

1) For nitrite assay, the spectrophotometric method based on the Greiss reaction was used. Changes in the level of nitrite were also measured after administration of lipopolysaccharide (LPS) (concentration: 1 microgram/ml) and N-nitro-L-arginine methyl ester (NAME) (concentration: 1 mg/ml).

2) Nonradioisotopic cell proliferation assay based on 5-bromodeoxyuridine uptake was used for cell proliferative assay. Change in the proliferative activity of chondrocytes was measured after administration of sodium nitroprusside (concentration: 1mM).

3) Endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) were detected from cartilage with Western blotting, and in situ with immunohistochemical staining. The student's T-test was used for statistical analysis.

**Results**

The nitrite concentration decreased with advancing age of rabbits. Chondrocytes from 1 month-old rabbits showed 2.1 times greater level (1.45 µM, S.D. 0.26 µM) than that of 3 year-old rabbits (0.69 µM, S.D. 0.18 µM). The difference was statistically significant (p<0.05). Chondrocytes from six-month-old rabbits and 1 year-old rabbits showed intermediate values, which were 1.25 µM (S.D. 0.29 µM) and 0.90 µM (S.D. 0.22 µM) respectively. Lipopolysaccharide significantly increased nitrite level for all of the groups (p<0.05). With LPS administration, the level was three fold greater for 1 month-old and 6 months-year old rabbits and four or five fold greater for 1-year old rabbits and 3 year-old rabbits than that of the control (Fig.1).

The proliferative activity was highest for the chondrocytes from 1 month-old rabbits, then decreased with the advancing age. Exogenous NO steeply decreased the proliferative activity in the chondrocytes from 1 month-old rabbits. The decrease was much milder in the chondrocytes from other age groups (Fig.2). The eNOS protein were detected at 140kDa and iNOS protein at 130kDa in all the age group. There was a definite tendency for both proteins to decrease with advancing age of rabbits (Fig.3).

**Discussion**

Increased production of nitric oxide is demonstrated in osteoarthritic cartilage while it is decreased in normal senescent articular cartilage[1]. The osteoarthritic cartilage shows some feature of immature cartilage such as decreased DNA synthesis and cell proliferation, and increased synthesis of chondroitin-4 sulfate[2]. In that context, the increase of NO production in the osteoarticular cartilage may be related to reverting to immature cartilage. The age-related change in the production and the action of NO might suggest a clue to the pathogenesis of osteoarthritis.

**Reference**

2. Mankin et al., Orthopaedic Basic Science: 1-44, AAOS, 1994

Fig.1 Levels of nitrite in chondrocyte cultures from rabbits of different age.