

Plasma Somatomedin in Children with Perthes' Disease

In-Young Ok, M.D.

Orthopaedic Dept. of Catholic Medical College and Center, Seoul, Korea

— 국문초록 —

Perthes' 환자의 혈장 Somatomedin 치

가톨릭 의과대학 정형외과학교실

옥 인 영

Perthes' disease는 발병원인이 아직 미상으로 되어 있으며, 여러가지 설중에 Burwell등은 골성장의 지연과 골성 연령의 retardation의 소견이 있는 것으로 미루어 내분비 계통의 이상이 동반된다고 하며, 성장ホルモン중 Somatomedin의 관여를 주장하였으며 이들은 쥐나 닭의 연골세포를 이용하여 Somatomedin 치를 측정하였다. 저자는 최근 Somatomedin bioassay에 더 sensitive한 토끼의 연골세포와 아직까지 아무도 시행하지 않은 사람의 연골세포를 조직배양하여 이들을 이용한 Perthes' 환아와 대조군으로 같은 연령의 아이들의 혈장내 Somatomedin치를 측정 비교하여 아래와 같은 결과를 얻었다.

- (1) 사람의 연골세포 조직배양시 D.M.E.M(Dulbeccos Modification of Eagles Medium), M.E.M. (Minimum Essential Medium), Waymouth's 배지중 D.M.E.M.배지에서 가장 빠른 세포의 성장을 볼 수 있었으며 M.E.M.배지에서 가장 세포성장이 늦었다.
- (2) 가토의 연골세포를 이용한 Somatomedin bioassay에서 대조군인 정상아에서는 0.89 ± 0.56 u/ml이었고 Perthes' 환아에서는 0.56 ± 0.37 u/ml 으로 Perthes' 환아에서 정상아보다 낮은 Somatomedin 치를 나타내었다.
- (3) Somatomedin치는 두군에서 모두 골성 연령 증가에 따라 증가하는 경향이 있다.
- (4) 사람의 연골세포를 이용한 Somatomedin bioassay에서는 혈장량이 증가함에 따라 [3 H] thymidin incorporation의 증가가 없었다.

Key Word : Legg-Perthes disease, Somatomedin, Plasma in children.

INTRODUCTION

Perthes' disease is an osteochondritis of the capital epiphysis of the femur.

It occurs more commonly in boys than girls and usually affects children of 3-10 years¹⁾. The story of Perthes' disease in the 70 years since its first description is, in a sense, that of orthopaedic surgery in miniature.

The majority of writers have maintained that

1) Former Fellow, Nuffield Orthopedic Center, Oxford University, England.

2) 본 논문은 Oxford대학 Nuffield Orthopedic Center에서 이루어 졌음.

Perthes' disease occurs in healthy normal children in whom no conclusive evidence of metabolic deficiency is found. This statement was made by Cavanagh et al²⁾, and after more than 70 years, it is still the prevailing opinion.

However, Burwell et al³⁾ have already studied skeletal age retardation and abnormalities of skeletal growth in children with Perthes' disease. His observations caused him to reevaluate certain aspect of the endocrine function of such children with particular reference to growth hormone (somatotropin) and somatomedin.

This paper examines the serum somatomedin

activity in Perthes' disease and compare it with that of suitable controls.

METHODS AND MATERIALS

(1) Preparation of human chondrocyte

Human cartilage was taken during the removal of polydactyly and iliac bone graft and removal of tarsal coalition in children.

The cartilage was placed in sterile 0.15 M saline. All procedures were conducted aseptically in a clean air laminar-flow cabinet. Specimens were cleaned of muscle and fibrous tissue and synovial tissue. Thin slices of cartilage were then removed from the articular surface with a scalpel and placed in Petri dishes containing sterile saline. Growth plate cartilage was not used.

The pieces of cartilage were sliced into approximately 1-2 mm lengths. The entire cartilage was treated with collagenase and trypsin. The cartilage segments were shaken for up to 30 minutes at 37°C. The trypsin solution was washed out by Waymouth's solution. Two to ten pieces of cartilage were put into three different media which contained 4 ml. of Waymouth's medium with 10% foetal calf serum or 4 ml. of D.M.E.M. with 10% foetal calf serum or 4 ml. of M.E.M. with 10% foetal calf serum.

The flasks were put into a jar which filled up with 5% CO₂ gas. The jar was kept in the incubator at 37°C. The medium was changed twice a week. When the chondrocytes were confluent they were passaged by trypsinization.

Culture was continued until these cells were used for somatomedin assay.

(2) Preparation of rabbit chondrocytes

New Zealand white rabbits (350-700 g) were sacrificed, the knee joints and costal cartilage were removed and placed in sterile 0.15 M saline. All subsequent procedures were conducted aseptically in a clean air laminar flow cabinet.

Thin slices of cartilage were then removed from the articular surfaces with a scalpel and placed in Petri dishes containing sterile saline. The thin layer of cartilage immediately adjacent to the secondary ossification centre that contains hypertrophic chondrocytes which do not respond to somatomedin (Ashton and Francis, 1975) was not removed. The absence of these hypertrophic chondrocytes was confirmed by histological examination. Growth plate cartilage was not used.

The entire cartilage was then divided between two sterile plastic containers each containing 5 ml 0.25% (w/v) trypsin in Waymouth's medium. The cartilage segments were shaken for up to 1 hour at 37°C, the optimum incubation time, was determined for every new batch of trypsin used.

The trypsin solution was then replaced by 10 ml. 0.5% (w/v) collagenase solution was replaced with a further 10 ml. of the same medium and the incubation continued for a further 18 hours.

Subsequently, undigested material was allowed to settle and chondrocytes were isolated from the supernatant by centrifugation at 600 r.p.m. for 10 minutes.

The cell pellet was resuspended and washed twice in Waymouth's medium. The chondrocytes were then dispersed in approximately 4 ml. of Waymouth's medium to give $4 \times 10^4 \pm 0.8 \times 10^4$ cells/ml and counted in a haemocytometer.

(3) Estimation of bone age

The bone age was calculated from X-ray of pelvis, hands and ankle joints.

The age intervals were calculated from the secondary ossification centre shown on the X-rays and the mean figure taken.

(4) Preparation of plasma

10 mls of blood were collected in heparinized tubes from nine children with Perthes' disease and nine normal children who were age and sex matched. The plasma was separated aseptically

Table : Somatomedin bioassay with rabbit chondrocyte results for plasma and statistical results in children with Perthes' disease and normal children

Perthes' Disease				Normal		
Sex	Case No.	B.A. (yr)	S.A. (u/ml)	Case No.	B.A. (yr)	S.A. (u/ml)
Boy	1	14.0	0.31	1	13.5	0.56
	2	9.5	0.37	2	13.5	0.41
	3	9.5	0.49	3	11.0	1.80
	4	9.5	1.40	4	9.5	0.90
	5	9.5	0.38	5	9.5	0.90
Girl	6	13.5	0.79	6	13.5	1.70
	7	9.5	0.33	7	9.5	1.30
	8	5.0	0.21	8	9.5	0.57
	9	9.5	0.77	9	9.5	0.38
Mean		9.1	0.56±0.37	Mean	10.1	0.89±0.56

B.A. : Bone age, S.A. : Somatomedin Activity

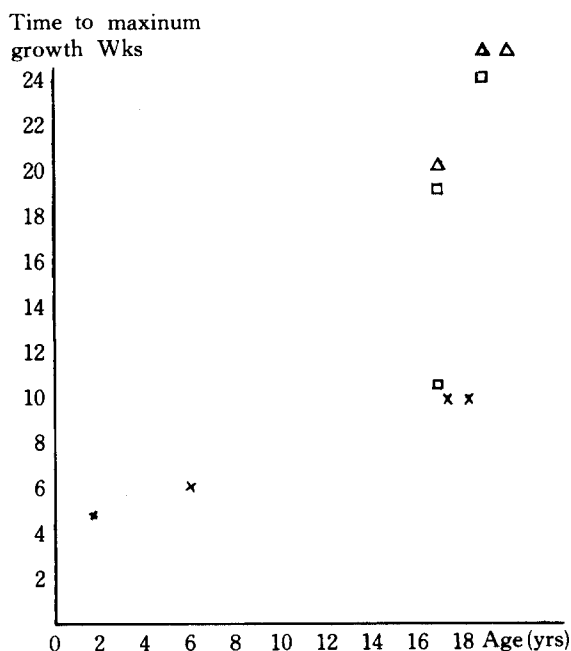


Fig. 1. Relation of growth rate of human chondrocyte to age in D.M.E.M. (x), M.E.M. (Δ) and Waymouth's (□) medium.

within four hours. Supernatants were frozen at -20°C until assay.

The standard plasma consisted of a pool of plasma collected from six adult male volunteers (mean age 27 years) between 10.0 and 11.0 a.m. After separation the supernatant was divided into 2 ml. aliquot for freezing. The standard was assigned an activity of 1 unit somatomedin/ml.

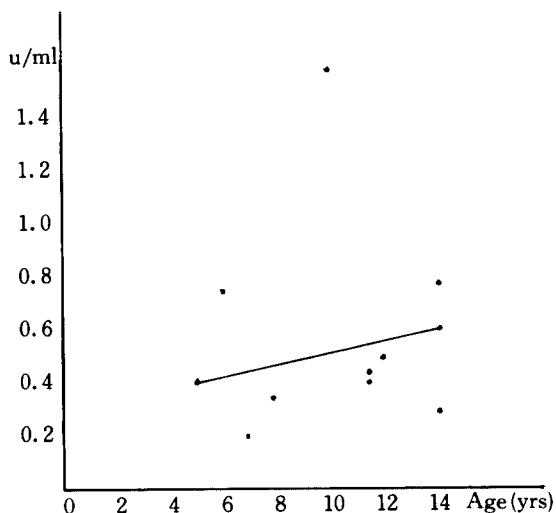


Fig. 2. Relation somatomedin activity to bone age in Perthes' disease children.

(5) Assay procedure

The frozen plasma was prepared by thawing and then spun at 2500 r.p.m. for 10 minutes. The supernatant was kept and the precipitate was discarded. Test and standard plasma to final concentration of 2.5%, 5% and 10% were added in duplicated to 1.9 ml. supplemented Waymouth's medium containing $2\mu\text{M}$ - $[^3\text{H}]$ thymidine ($1\mu\text{Ci/ml}$) in sterile plastic tubes. Chondrocyte suspension ($100\mu\text{l}$) containing 400,000 to 600,000 cells was dispensed to each tube. In addition, four blank tubes were prepared: two containing

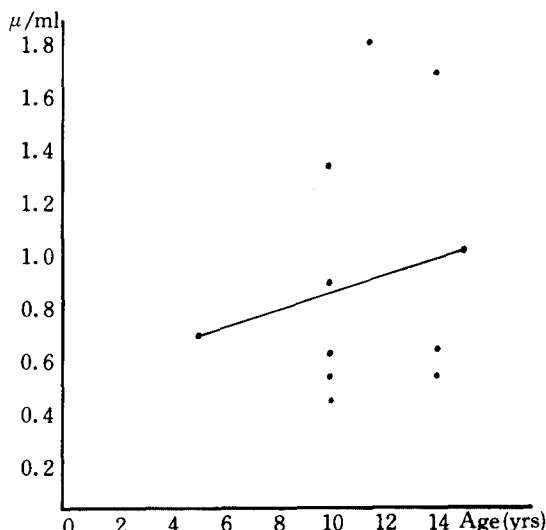


Fig. 3. Relation of somatomedin activity to bone age in normal children.

no cells and two containing cells in the absence of plasma. The tubes were stoppered and incubated at 37°C for 48 hours.

After incubation 1 ml. of cold DNA solution (2 mg/ml) and 3 ml. cold TCAC (trichloroacetic acid) were added to each tube to lysis the cells and precipitate cellular DNA. The tubes were stoppered, mixed, stored overnight at 4°C in a fridge. The tubes were then centrifuged at 2000 r.p.m. and the supernatant removed with a vacuum pump. The dried precipitate was dissolved in 2 ml. formic acid and 50 μ l samples from each tube were added to 4 ml. scintillation fluid. The incorporated tritium was then counted in a scintillation counter.

Statistical analysis was carried out as previously described¹¹.

RESULTS

Growth rate of human chondrocyte

The growth of human chondrocytes in three different media (D.M.E.M., M.E.M. Waymouth's) were compared. In D.M.E.M. the growth rate of human chondrocyte is much faster than other media. In the flask containing human chondrocyte from an 18 year old patient, the cells were

confluent at 10 weeks compared with more than 20 weeks in MEM and Waymouth's media. In D.M.E.M. the growth rate was also age dependent. In the flask, which had human chondrocytes from a 2 year old patient, the cells were already confluent at 5 weeks (Fig. 1).

Somatomedin assay with rabbit chondrocytes

Rabbit chondrocytes offer a more sensitive assay method than chick chondrocytes and use mammalian rather than avian cells.

Somatomedin levels were compared in children with Perthes' disease and normal children at various ages. The mean somatomedin activity of normal children (0.89 ± 0.56) was greater than in children Perthes' disease (0.56 ± 0.37) (Table 4).

Somatomedin was significantly correlated with bone age in children with Perthes' disease or normal children (Table 4). But the trends between somatomedin activity and age in Perthes' disease or normal children are similar to Rayner and Burwell's results.

Somatomedin assay with human chondrocytes

The use of human chondrocytes, which had not previously been attempted, maybe a more appropriate method. However, there was no increase in (³H) thymidine incorporation with increasing plasma dosage with human chondrocyte assay.

DISCUSSION

The early suggestion that Perthes' disease was due to hypothyroidism was not confirmed by subsequent work. However, Laron¹⁰) in an endocrinologic evaluation of 35 children with active or healed Perthes' disease who also had growth retardation and/or bone age retardation of more than two years, found two children with isolated thyroid stimulating hormone deficiency and one child with primary hypothyroidism. La-

ron and his colleagues concluded that it was improbable that thyroid deficiency is a primary determinant in the aetiology of Perthes' disease.

Pituitary hormone does not act directly on the skeletal tissue but current evidence suggests, through somatomedin, a polypeptide hormone, which is produced primarily, although not exclusively, in the liver. In view of this possibility, somatomedin deficiency might account for the impaired skeletal growth and maturation of Perthes' disease.

Burwell⁽⁴⁾ and also Rayner⁽¹¹⁾ et al, measured the serum somatomedin in boys with Perthes' disease and compared it with that of suitable controls. Rayner reported elevated levels of somatomedin in the affected children in the age range of 5-11 years. The control boy's somatomedin values showed a significant positive correlation with both chronological age and bone age, but no correlation could be demonstrated in the Perthes' boys.

Burwell et al reported elevated levels of somatomedin in affected children in the age range of 3 to 5 years. The boys with Perthes' disease showed the same age-dependent increase in serum somatomedin with increasing age as in normal boys with the suggestion of an elevated somatomedin value in the younger patient.

The fact that the somatomedin increase at that time in growth when the velocity of growth in the bones is decreasing suggests that there may be a decreasing ability on the part of the tissues to respond to somatomedin. Such a postulated age-dependent change in the sensitivity of growing skeletal tissues to somatomedin before puberty could come about in one of two possible ways: first, by a reduction in the number of receptor sites on target cells, second, by reduction in the affinity of such receptor sites to the hormone.

Receptor sites to somatomedin have been found not only on isolated chondrocytes, but also

on fat cells and liver membranes⁽⁹⁾. Although there is no evidence as yet that somatomedin receptor sites on growth plate cells change in number or affinity with age. There are reports of an age-dependent change in the sensitivity of costal cartilage to somatomedin during growth⁽⁹⁾.

Our objective in this work has been to measure plasma somatomedin in children with Perthes' disease using three different assay techniques: We have used a sensitive mammalian rabbit chondrocyte bioassay method. We have also attempted an assay using human chondrocyte which may be more appropriate. In our somatomedin assay with rabbit chondrocyte, mean somatomedin activity of normal children was greater than in Perthes' children. This finding would not support the Burwell's receptor theory. But the trends between somatomedin activity and age in Perthes' disease or normal children are similar to Rayner and Burwell's result.

In somatomedin assay with human chondrocytes, there was no increase in thymidine incorporation with increasing plasma dosage with human chondrocyte assay.

There may be two possible reasons. First the nature and character of human chondrocyte may be changed during the period of explant culture. A number of passage (up to 4 times) were necessary in order to generate sufficient cells from the cartilage specimens which were obtained. This effect may be minimised if more rapidly growing younger cartilage can be obtained. Alternatively if sufficient cartilage can be obtained freshly isolated cells, prepared as far as rabbit chondrocyte would be preferable.

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