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# Vascular Changes in Relation with Tumor Regression Induced by Combined Oral Administration of Heparin and Hydrocortisone†

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=Abstract= The oral administration of heparin and hydrocortisone inhibited the tumor growth and induced the regression of the solid myeloma implanted on the back of BalbC mice. The inhibition of tumor growth was dependent on the regimen, which was suggested by the reactivation of tumor growth with interruption of the theraphy. The histologic analysis of those tumors revealed intravascular deposition of fibrillary eosinophilic substances and necrosis of tumors as well as the thickening of the vascular wall. The present experiment confirmed the tumor inhibition effect of Folkman's heparin and hydrocortisone treatment and found that the regimen could induce the vascular change in addition to the proposed angiogenesis inhibition.

Key words: ***Vascular change, Tumor regression, Heparin, Hydrocortisone***

## INTRODUCTION

Angiogenesis, in general, and tumor-induced angiogenesis in particular, have received much attention in the last decade (Folkman 1972, 1986; Folkman and Cotran 1976; Schor and Schor 1983). Angiogenesis is a complex process which involves tissue disruption and reorganization, cellular growth and changes in the composition of the fluid environment, and the extracellular matrix. The general features of capillary growth could be summarized as invasion of endothelial cells with vascular basement membrane and locomotion toward the angiogenic stimuli, linear alignment, lumen formation, capillary loop formation and microvascular pericyte incorporation (Ausprunk and Folkman 1977; Gross *et al.* 1983; Kalebic *et al.* 1983). This phenomenon of angiogenesis is related not only to the progressive tumor growth (Folkman *et al.* 1983), but also to the physiological and pathological neovascularization as in menstruation, trophoblast implantation, embryonic development, wound healing, rheumatoid diseases and diabetic retinopathies (Schor and Schor 1983). Therefore, elucidation of angiogenesis mechanism may affect a broad spectrum of biological researches. Especially, if the tumor growth could be controlled through suppression of tumor angiogenesis, the mechanism of tumor angiogenesis might be one of the utmost urgent targets to be solved in relation to the cancer control. In this aspect, Folkman's original hypothesis that solid tumors are angiogenesis-dependent (Folkman 1972) has impacted deeply the cancer research. However, the methodological difficulties of research in angiogenesis hampered the progress in this field. Only recently, some successes in *in vitro* culture of endothelial cells (Gimbrone *et al.* 1974; Folkman *et al.* 1979), linear quantitation of capillary growth by use of rabbit cornea (Gimbrone *et al.* 1974) or egg's chorioallantoic membrane (Folkman 1985), and the use of inert polymeric pellets implanted into tissues to provide sustained release of macromolecules with angiogenic or anti-angiogenic activity (Langer and Folkman 1976) have led the angiogenic researches to the fruitful results. In those endeavors, the coincidental finding of tumor regression caused by heparin in the presence of hydrocortisone attracted our deep concern. Folkman and his colleagues asserted that the

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tumor regression was probably due to the angiogenesis inhibition effect of the above treatment (Folkman *et al.* 1983). So far, we have tried to develop a tumor model system in which we might be able to stimulate the growth or induce the regression of tumor experimentally only by use of physiological means. Therein, we have adopted a hormone-dependent tumor system such as mammary cancer model (Park *et al.* 1985, 1986). The tumor growth and regression model, modulated by the physiological substances such as heparin and hydrocortisone, may be another good candidate for our purpose to study the biochemical and genetic behavior of cancers. Therefore, the attractive idea of angiogensis-dependent tumor growth model prompted us to confirm the Folkman's idea, whether the tumor of any kind could be regressed by heparin and hydrocortisone treatment with their modes of action. Moreover, we could not find any report of observation on histological changes, caused by the heparin and hydrocortisone regimen. In the present experiment, we have tested the effect of heparin and hydrocortisone on the mouse myeloma system, to see if the treatment causes the regression of the tumor and what kind of subsequent histological changes could be followed.

## MATERIALS AND METHODS

### 1. Materials

BalbC mice were purchased from the Seoul National University animal breeding house and the myeloma cell line was generously donated by Dr.H.K.Chung, Dept. of Biochemistry, College of Medicine, Seoul National University. Heparin as sodium heparin injection U.S.P. form, and hydrocortisone as sodium hydrocortisone succinate injection, U.S.P. form, were purchased from Upjohn Co., Kalamazoo, Michigan, U.S.A.

### 2. Animal tumor model

The solid myeloma tumors were induced by inoculation of myeloma cells ($2.2 \times 10^7$ cells per head) on both of the scapular regions of mice and the variation of the tumor size was monitored by measuring the long and short diameters of the tumor masses with Vernier calipers.

### 3. Treatment of the animals

Tumor bearing animals were divided into three groups : namely, the first group as control, the second group as hydrocortisone and heparin treatment group, and the third group as treatment interruption group. To the control group, the physiological saline was supplied. To the experimental group, both of hydrocortisone and heparin were orally administered, mixed in the drinking water at the concentration of 200 unit/ml and 0.45 mg/ml, respectively. And to prevent the opportunistic infection due to steroid use, the antibiotics (ampicillin 100 $\mu$g/ml, tetracycline 25 $\mu$g/ml) were supplemented to all the drinking water of three groups.

### 4. Histologic analysis of the tumor samples

The histologic changes, caused by the treatment were visualized by hematoxylin-eosin staining and fibrin staining by phosphotungstate.

## RESULTS

### 1. Effect on tumor growth

As in Fig. 1, the tumor growth was shown to be affected by the combined oral administration of heparin and hydrocortsone. The tumors of control group expanded logarithmically (Fig. 1A), while those of heparin and hydrocortisone group stopped their growth and actually regressed (Fig. 1B). The dependency of regression or growth inhibition of the tumors was confirmed again by the interruption of the heparin and hydrocortisone treatment, which resumed and reactivated tumor growth (Fig. 1C). The rebound tumor growth by interruption of treatment was more prominent than the control tumor growth.

### 2. Histological analysis

As in Fig. 2, in the control group, they showed the well preserved myeloma pattern throughout the experimental period. There are a few blood vessels scattered in the neoplastic tissues containing fibrillary eosinophilic substances. In contrast, the tumors treated with heparin and hydrocortisone, showed the varying degrees of tumor necrosis from mild and moderate (Fig. 3), to severe state (Fig. 4,5). Interestingly, the blood vessels in the tumor masses of the treatment group were filled with fibrillary substances and/or inflammatory cells, and capillary endothelial thickening were observed. The eosinophilic fibrillary substances, filled in the blood vessels, were negative in phosphotungstate staining, which suggested them not to be the fibrin.

## DISCUSSION

The selective control measure of angiogenesis, if established, might have a tremendous impact on biology, especially on cancer research. In this regard, the hypothesis that solid tumors are angiogenesis-dependent (Folkman 1972) and the haphazard finding that heparin in the presence of hydrocortisone inhibits the neovascularization (Folkman *et al.* 1983) attracted the increasing interest not only from an oncologic view point but

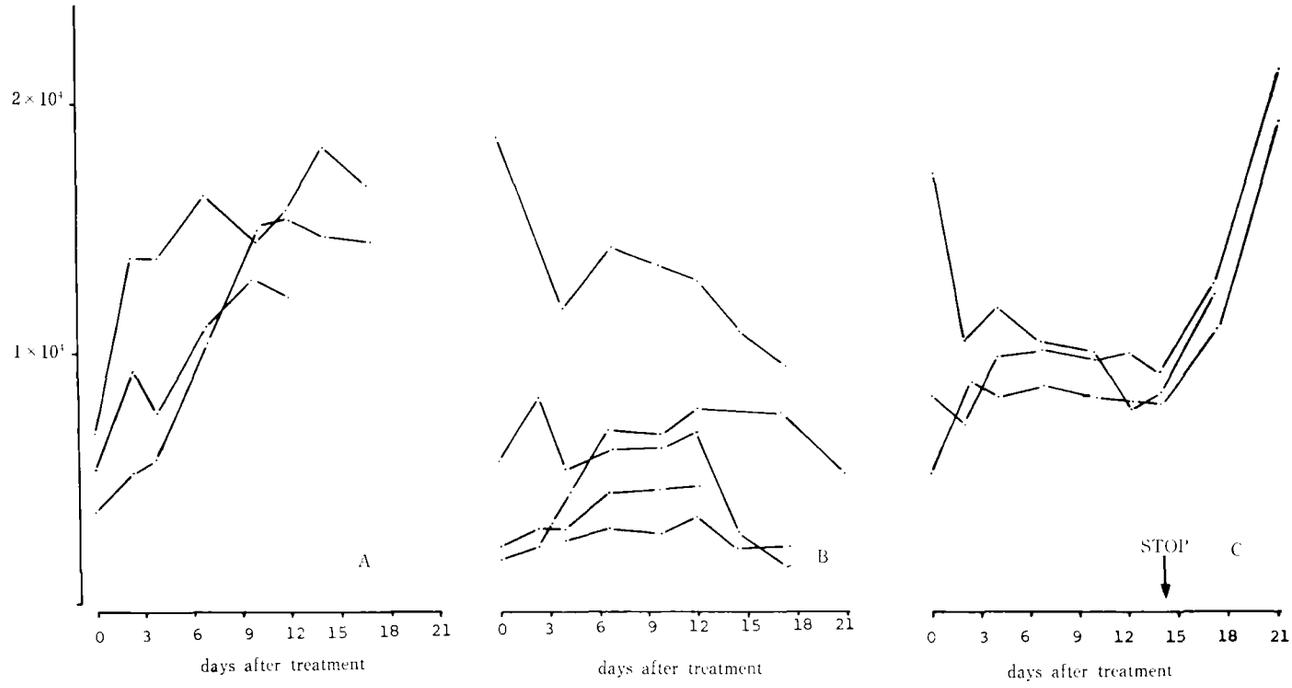


Fig. 1. Tumor regression by combined oral administration of hydrocortisone and heparin: Myeloma on Balb C mice; A. control group, B. hydrocortisone with heparin group, C. treatment interruption group, hydrocortisone and heparin treatment was stopped at 14 days of treatment initiation.

also of many other biological researches, such as ovulation, wound healing, arthritis and diabetic retinopathy (Folkman 1984). Folkman and his colleagues reported a series of works in relation to the effect of hydrocortisone and heparin, and they concluded that their tumor regression effects are based on angiogenesis inhibition. Actually, so far, heparin was believed to have angiogenic activity (Taylor and Folkman 1982). But by the study of Folkman's group, the heparin of a specific brand (Panheprin, Abbott) used with hydrocortisone caused the marked regression of tumors in reticulum cell sarcoma, Lewis Lung carcinoma, B-16 melanoma and bladder carcinoma of mice (Folkman *et al.* 1983). To explain its biochemical mechanism, they have tried to identify the essential elements in those regimens, and recently they have reported that the hexasaccharide fraction of the heparin mixture could be the substitute for the whole heparin effect (Folkman *et al.* 1983) and 11-alpha-epicortisol has the angiostatic activity (Folkman 1986). The effect of hydrocortisone is not clearly related with that of glucocorticoid activity. 11-alpha-epicortisol differs from hydrocortisone only in that the 11-hydroxyl group lying below the plane of the molecule. Inspite of their huge efforts, the phenomeon of tumor regression by heparin and hydrocortisone can not be explained in biochemical terms. Moreover, the relation between tumor regression and the assumed angiogenesis-inhibition by the treatment were not directly proven yet. Thereby, the purpose of the present experiment was to confirm the tumor regression effect of heparin and hydrocortisone treatment and to observe the subsequent histologic changes, especially focusing on the endothelial changes of the capillaries. With the myeloma model, we could confirm the growth-inhibition and regression-induction of the tumor by the combined oral administration of heparin and hydrocortisone (Fig. 1). Although the tumor regression by the regimen was marked, it was not so much prominent as originally suggested by Folkman *et al.* (1983). And the dependence of tumor regression on the regimen was confirmed by the interruption experiment after two weeks of treatment, which markedly reactivated the tumor growth, and an acceleration in tumor expansion was noted. The histologic analysis of the tumor samples showed that the regimen caused a prominent necrosis of the tumors and intravascular de-

posit of eosinophilic fibrillary substances as well as thickening of capillary endothelium (Fig. 2, 3, 4, 5). It was suggested that the fibrin deposited in the perivasular matrix of the tumor might play an important role in the angiogenic response, through activation of the clotting and/or fibrinolytic system (Dvorak *et al.* 1979; Ryan 1970; Cliff 1963). The apparent change of the endothelial bed of the tumor capillaries attracted our another concern, because the most vulnerable elements in tumors would be the vascular endothelium. Since a network of capillary blood vessels supplies all the nutritional needs of every tissue and organ in the body, the tumor tissue is not an exception. Especially for the rapidly growing tissues, every increase of the cell population must be preceded by an increase in new capillaries converging on the tissue (Folkman 1986). The essence of angiogenesis is the migration and proliferation of endothelial cells. Recently some anti-cancer measures were developed through attack on vascular endothelial cells by hyperthermia, interventive radiology, tumor angiogenesis inhibition (Denekamp 1984). Our results suggest that the Folkman's regimen of heparin and hydrocortisone inhibited the tumor growth and caused the concomittant vascular changes in the tumor tissues, such as intravascular deposit of eosinophilic fibrillary substances and endothelial thickening of the capillaries with the varying degrees of necrosis, probably in addition to the concomittant tumor angiogenesis inhibition, as suggested by Folkman *et al.* (1983).

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=국문초록=

# 헤파린과 하이드로코티손의 병합경구투여에 의한 종양퇴화과정에서의 혈관변화

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헤파린과 하이드로코티손의 경구투여는 BalbC 흰쥐의 배부에 이식한 고형 myeloma의 성장을 억제하고, 퇴화를 유도하였다. 동 투여방법의 중단은 암성장의 촉진을 나타내어, 이러한 암성장 억제가 Folkman식의 헤파린과 하이드로코티손요법에 의존적임을 입증하였다. 동치료과정에서의 병리조직적 변화로는 혈관내 섬유상 · 호산성물질의 침착, 암조직의 괴사 및 혈관벽의 비후가 관찰되었다. 본 연구는 Folkman식의 헤파린-하이드로코티손 요법의 암성장억제 효과를 확인하고, 동 요법이 Folkman씨가 주장한 신생혈관생성억제 뿐 아니라, 기존의 암조직내 혈관들에도 변화를 초래하고 있음을 관찰하였다.

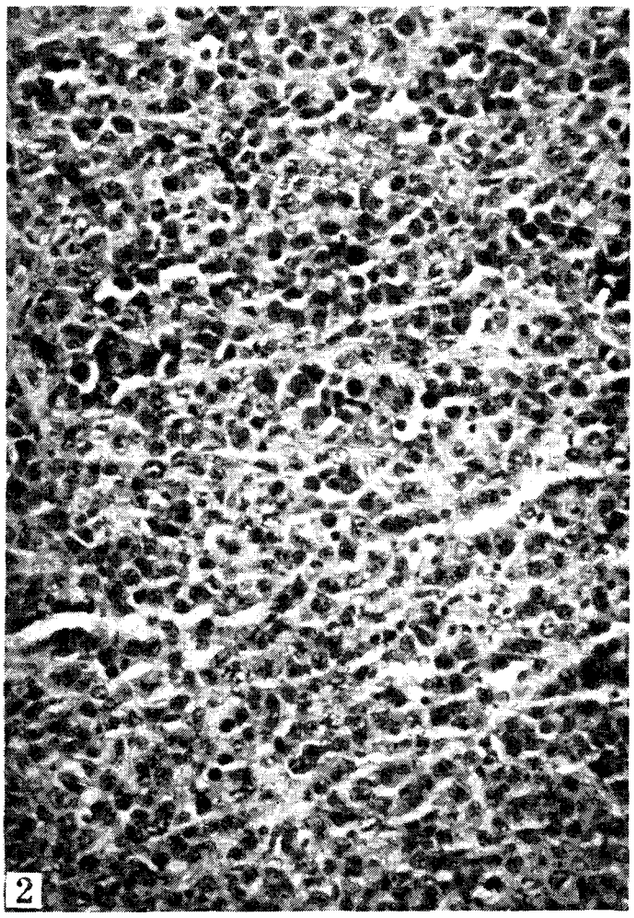
## LEGENDS FOR FIGURES

**Fig. 2.** Histopathologic pattern of solid myeloma of untreated group.

**Fig. 3.** Histopathologic pattern of solid myeloma after heparin and hydrocortisone treatment. Mild tumor necrosis and thickening of capillary vessels are becoming prominent.

**Fig. 4.** Histopathologic pattern of solid myeloma after heparin and hydrocortisone treatment. Severe necrosis of the tumors are prominent.

**Fig. 5.** Histopathologic finding of solid myeloma after heparin and hydrocortisone treatment. Thickening of capillary vessel is prominent.



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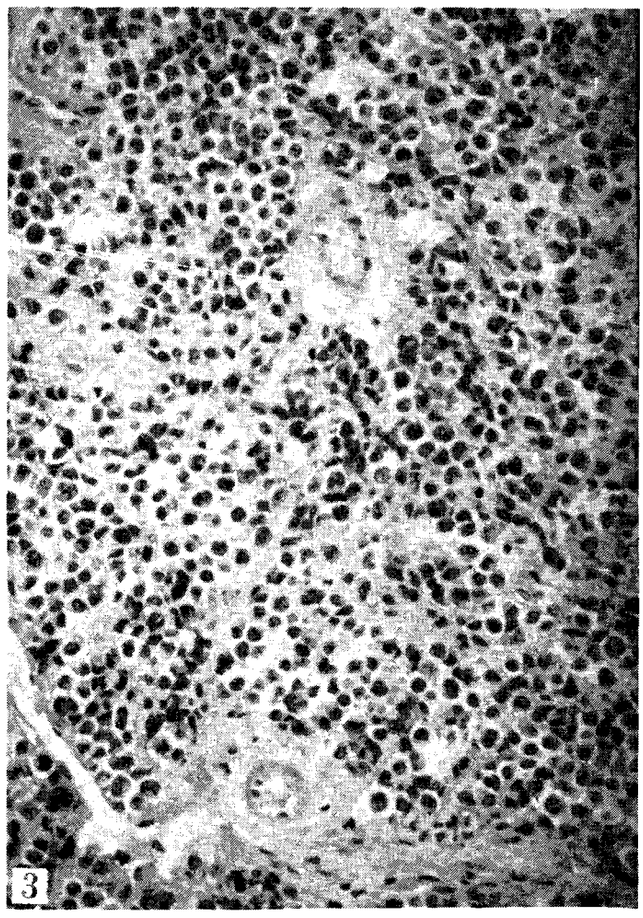

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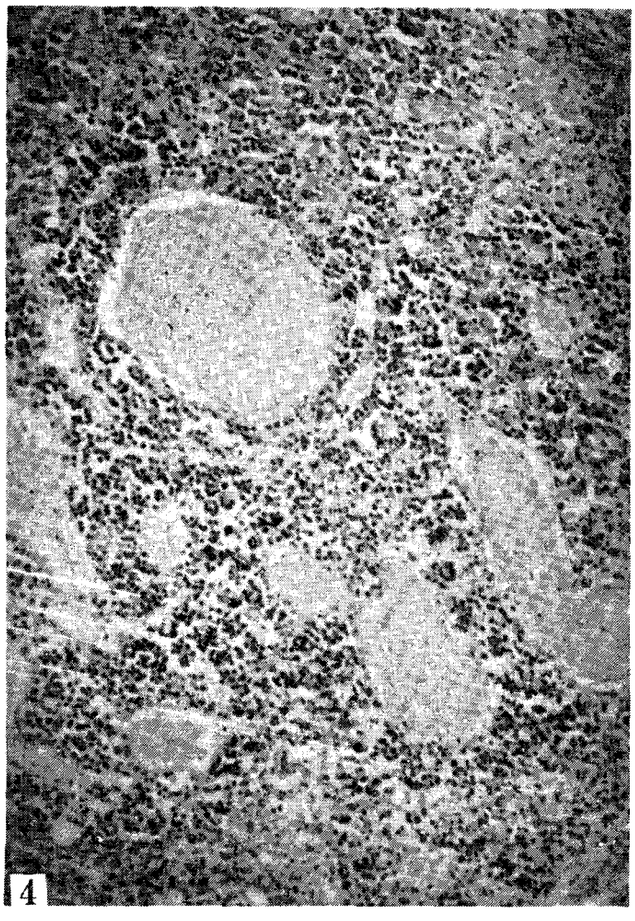

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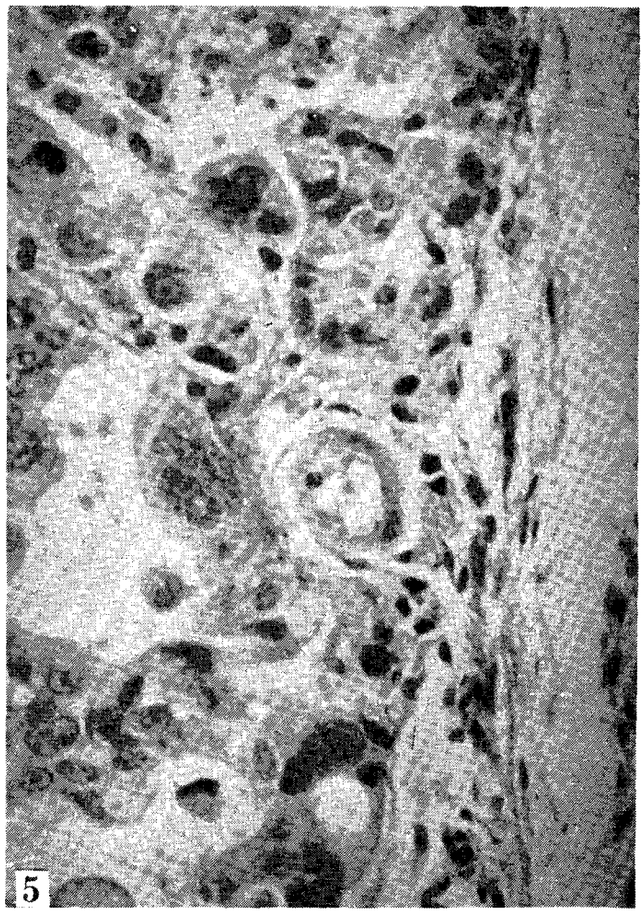

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