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ARTICLE

Effect of Sulfated β-cyclodextrin, a Water Soluble Cycloamylose, on the Promotion and/or Inhibition of Angiogenesis

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Previous studies have reported that sulfated β-cyclodextrin, a naturally occurring cycloamylose built up from six to eight glucopyranose units, when administered alone promotes angiogenesis, but administered with an angiostatic steroid inhibits angiogenesis in the cick embryo bioassay. In our experiments sulfated β-cyclodextrin has been shown to posses many properties unrelated to its classical functions in the promotion and inhibition of angiogenesis that were not previously described. We studied the angiogenic and angiostatic properties of β-cyclodextrin in a subcutaneosus plastic sponge model in mice. We realized two set of experiments. In each set mice were randomized into five groups (n= 5 mice). The first group was treated with sulfated β-cyclodextrin (200 ng), the second group was treated with sulfated β-cyclodextrin (2000 ng), the third group received unsubstituted β-cyclodextrin (2000 ng), the fourth group was treated with sulfated β-cyclodextrin (20 000 ng) and the last group was used as a control group. In all groups compounds were administered intraperitonally 4 days after subcutaneous implantation of a sterile polyvinyl sponge on day 0, controls were not treated. Cyclodextrin administered alone at low drug concentration (200 ng) promoted angiogenesis and increased the development of venules in the sponge matrix. However, cyclodextrin administered at high drug concentration (2000 and 20 000 ng) reduced the vessel index in the sponge and areas of microhemorraghes were observed. From our results we propose that β-cyclodextrin contains both a promoter and an inhibitor of angiogenesis and that the activation of both is drug concentration dependent. (Pathology Oncology Research Vol 8, No 1, 47–53, 2002)

Keywords: angiogenesis, cancer, sulfated β-cyclodextrin

Introduction

With the identification of several pro-angiogenic molecules such as vascular endothelial cell growth factor, the fibroblast growth factors, and the angiopoietins, and the recent description of specific inhibitors of angiogenesis such as platelet factor-4, angiostatin, endostatin, and vasostatin, it is recognized that therapeutic interference with vasculature formation offers a tool for clinical application in various pathologies.

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Whereas inhibition of angiogenesis can prevent disease with excessive vessel growth such as cancer and metastases, stimulation of angiogenesis would be beneficial in the treatment of diseases such as coronary artery disease, gastro-duodenal ulcers, critical limb ischemia in diabetes and organ transplantation.[12] Folkman et al.[8] have reported that sulfated β-cyclodextrin administered with an angiostatic steroid inhibits angiogenesis at 100 to 1000 times the effectiveness of heparin in the chick embryo bioassay and also augments the anti-angiogenic effects of angiostatic steroids against corneal neovascularization in rabbits when sulfated β-cyclodextrin and a steroid are inserted into the cornea or applied topically as eyedrops. In contrast, they observed that β-cyclodextrin administered alone stimulated vessel growth to 164% of the growth of

untreated controls and vessel density to 303 % above the control level in rabbit corneas.[8]

Cyclodextrins are naturally occurring, water-soluble cycloamyloses built up from six to eight glucopyranose units[1] with highly hydrophilic and hydrophobic substituents, which have the ability to interact with a hydrophobic steroid and to adsorb to endothelial cells, respectively.[2] The internal molecule provides a hydrophobic cavity at the center and a hydrophilic outer surface. Steroids and other hydrophobic molecules with appropiate physiochemical structures and properties can form complexes with β-cyclodextrins;[2] an inclusion complex between hydrocortisone and β-cyclodextrin has been demonstrated.[9]

Previous studies have demonstrated that the subcutaneous injection of betamethasone together with quinacrine and indomethacin results in tumor regression to 30% to 70% of the tumor growth of untreated controls in AJ strain mice. Histopathological examination identified tumor vessels in regression and morphological features of apoptosis of endothelial cells.[13]

Therefore, in a previous study we administered sulfated β-cyclodextrin with betamethasone in mice with a sterile saline impregnated –sponge and in mice with a tumor cell-impregnated sponge. The hypothesis we wished to test was that sulfated β-cyclodextrin carries betamethasone to the endothelial cell surface of tumor vessels where it is adsorbed by strong hydrophilic bonding, enhancing or facilitating the rapid uptake of the angiostatic steroid into endothelial cells. If true, the carrier function of cyclodextrin may minimize the toxic side effects of betamethasone by reducing its dosage. We could demonstrate a potentiation by β-cyclodextrin of betamethasone induced inhibition of tumor angiogenesis and induction of apoptosis. Nevertheless, we could not demonstrate a potentiation by β-cyclodextrin of betamethasone induced inhibition of inflammatory angiogenesis and induction of apoptosis.

However, in our experiments sulfated β-cyclodextrin has been shown to posses many properties unrelated to its classical functions in the promotion and inhibition of angiogenesis that were not previously described. Cyclodextrin when administered alone had concentration-dependent angiogenic and angiostatic properties. Hence in the present study we studied the effect of β-cyclodextrin on angiogenesis at different concentrations in mice.

Materials and Methods

Experimental animals and sponge implants

Female AJ strain mice (*Mus musculus*) aged 2 month and weighing 25 g were obtained from the Animal Center of the Department of Molecular Pharmacy, Faculty of Medicine, University of Chile, Santiago de Chile, and were used in all experiments. The mice were housed 10 per cage, with free access to food and water. Mice were anaesthetized, and a small piece (3 mm^2) of a steril polyvinyl sponge impregnated with 5 ul of sterile saline or 5 ul of ascitic fluid containing TA3-MTX-R tumor cells was implanted subcutaneously in the dorsolateral region of the thorax as described by Freeman et al[10] of each mouse. Diazepam, atropine and ketamine were used for anesthesia.

The tumor used was mouse ascitic tumor TA3-MTXR, a tumor resistant to methotrexate obtained from tumor TA3 of female AJ strain mice (*Mus musculus*)[13] (Department of Molecular Pharmacy, University of Chle, Chile). The tumor TA3-MTXR was maintained by intraperitional inoculation of ascitic fluid containing tumor cells before use in the study. The inoculated mice were daily treated with 1,3 mg methotrexate before use in the study. The animals were kept in a steril environment and all experiments were performed according to the guidelines for animal handling of the University of Chile.

Experimental design – injection groups

Sulfated β-cycloxdextrin was obtained from Aldrich. Bethametasone was obtained from the Laboratory Chile S.A. We realized two set of experiments. In each set mice were randomized into five groups (n= 5 mice).The first group was treated with sulfated β-cyclodextrin (200 ng), the second group was treated with sulfated β-cyclodextrin (2000 ng), the third group received unsubstituted β-cyclodextrin (2000 ng), the fourth group was treated with sulfated β-cyclodextrin (20 000 ng) and the last group was used as a control group. In all groups compounds were administered i.p. 4 days after subcutaneous implantation of a sterile polyvinyl sponge on day 0, controls were not treated.

We starfed to treat the mice 4 days after s. c. implantation of the sponge because we determined a steep increase in the proliferative phase of granulation tissue development as previously described by Boyle and Mangan[4] and angiogenesis on days 5 to 6, after sponge implantation.

Mice were killed by cervical dislocation 48 hours after treatment on day 0, the sponges removed and fixed in 10% formalin. During the first set of experiments a sterile sponge impregnated with 5 μl of sterile saline was implanted subcutaneously in the dorsolateral region of the thorax and during the second set of experiments a sterile sponge impregnated with 5 μl of ascitic fluid containing 5 x 10^5 tumor cells was implanted.

Vessel staining, grading ,and counting

The sponges were fixed immediately after removal in 10 % formalin, trimmed and embedded in paraffin. Paraffin sections (4 to 6 μm in thickness) were prepared and stained with haematoxylin and eosin (HE) for histopathological examination identifying vessels in regression and morphological fea-

tures of apoptosis of endothelial cells. A vessel index was estimated using a 100 mm^2 grid counting in an independent manner small venules and microvessels in the most active areas of neovascularization. In each sponge 24 fields (each field of 25 mm^2) were scored by two independent observers in a blinded fashion. The vessels of representative sponge samples were highlighted by staining their endothelial cells immunocytochemically for CD31, a specific marker for vascular endothelium.

Sponges were frequently heterogeneous in their microvessel density, but the area of highest neovascularization were found by scanning the sponge sections at low power (using a 40 × objective) and identifying the areas with the highest number of microvessel staining on the HE and CD-31 sections. These areas of high neovascularization could occur anywhere in the sponge, but were most frequent at the margins of the sponge. After the area of highest neovascularization was identified small venules and individual microvessels were counted on a 400 × field (i.e. 40 × objective lens and 10 ocular lens). Any stained endothelial cell or endothelial-cell cluster that was clearly separate from adjacent microvessels, tumor cells, and other connective-tissue elements was considered a single, countable microvessel. A vessel index was estimated using a 100 mm^2 grid counting in an independent manner small venules and microvessels in the most active areas of neovascularization. In each sponge 24 fields (each field of 25 mm^2) in the sponge periphery and 24 fields (each field of 25 mm^2) in the sponge matrix were scored by two independent observers in a blinded fashion.

Vascular density for each sponge was expressed as the number of small venules and microvessels per 600 mm^2 in the sponge periphery, counting a total of 24 fields of 25 mm^2, and per 600 mm^2 in the sponge matrix, counting a total of 24 fields of 25 mm^2. Vascular density for each experimental group was expressed as the number of small venules and microvessels per 3000 mm^2 (5 × 24 fields of 25 mm^2) in the sponge periphery and per 3000 mm^2 (5 × 24 fields of 25 mm^2) in the sponge matrix.

Staining with antibody anti-CD31

Paraffin sections (4-6 μm) were lysed by tripsin (Sigma, Chemicals) for 20 minutes at 37°C. Tissue peroxidases of the tissue were inactivated by DAKO–S 2001 for 5 minutes at room temperature. The samples were then washed once with double distilled water and once with PBS buffer (pH 7,2-7,6). The proteins of the tissue were inactivated by 0,25% casein and submerged in DAKO-X 0909 for 5-20 minutes at room temperature.

The samples were then incubated for 15 minutes at 37°C with primary mouse antibodies anti-CD31 (Pharmigan: 100 μl) and DAKO-S 3022. The samples were then washed once with PBS and submerged in PBS buffer for 3 minutes at room temperature. The antibodies anti –CD31 were visualized incubating the samples with antibodies DAKO-E 0354 (Vector) and a streptavidin labeled HRP DAKO-P 0397 solution (ABC standard kit: Vector).

Analysis of apoptosis

The apoptotic rate was determined by in situ terminal deoxynucleotydil transferase (TdT) labeling with biotin-labeled dUTP in paraffin sections (4-6 μm) of representative sponge samples.[11] Paraffin sections were placed on a glass slides; glass slides were briefly treated with 0.01% poli-L-lysin solution (molecular weight 300.000, Sigma Chemical).

The samples were lysed by 20 ug/ml of proteinase K (Sigma Chemicals) for 15 minutes at room temperature and washed four times with double distilled water. Tissue peroxidase was inactivated by 2% H_2O_2 for 5 minutes at room temperature. The samples were then washed once with bidistillated water and submerged in 0.3 e.u./μl TdT equilibration buffer.

The Tunel reaction was carried out by incubating the samples for 1 hour at 37°C in a moist chamber with 0.3 U/ml TdT, 20 mmol/L biotin –labeled dUTP and TdT buffer. The samples were then transfered to PBS buffer for 15 minutes at room temperature, washed once with double distilled water and treated with 2% albumin solution for 10 minutes at room temperature, washed once with double distilled water and submerged in PBS for 5 minutes. The samples were then incubated for one hour at 37°C with a avidin-biotin labeled peroxidase solution (1:10-1:20), washed once with bidestillated water and stained with AEC for 30 minutes at 37°C. Samples of rat small intestine mucous membrane were used as positive controls; the small intestine mucous membrane of rats present high apoptotic rates.

Statistical analysis

ANOVA was used for multiple comparisons of treatments, using the statistic software SPSS. Parametric variables were compared by Student's paired t –test and the Tukey and the C.W. Dunett test were used for multiple comparisons of treated groups. The mean microvessel density values ± SD´s of the mean were determined for each test group of the first and second group of experiments.

Results

Determination of inflammatory and tumor angiogenesis in control mice

Figure 1. shows that in the sterile saline-impregnated sponge, the mean count of capillaries (in areas of highest neovascularization) in the sponge periphery during the first 4 days of sponge evolution was 12,8 per 600 mm^2.

Highest neovascularization in the surroundings of the sponge was reached at 5 days after the implantation of the sponge on day 0. The mean count of capillaries was 34,8 per 600 mm^2. Just after 5 days of sponge evolution the vessels started to invade the sponge matrix.

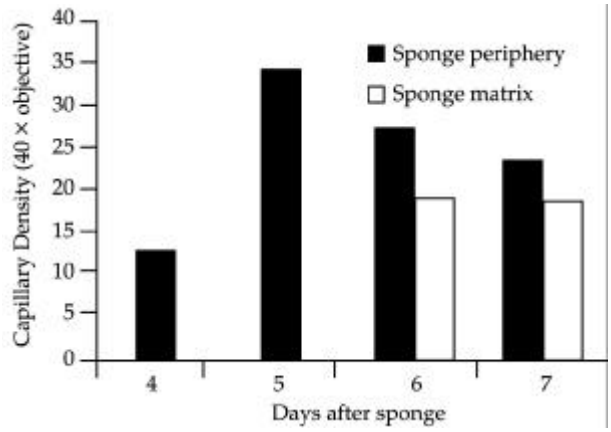

Figure 1. Determination of inflammatory and tumor angiogenesis in control mice

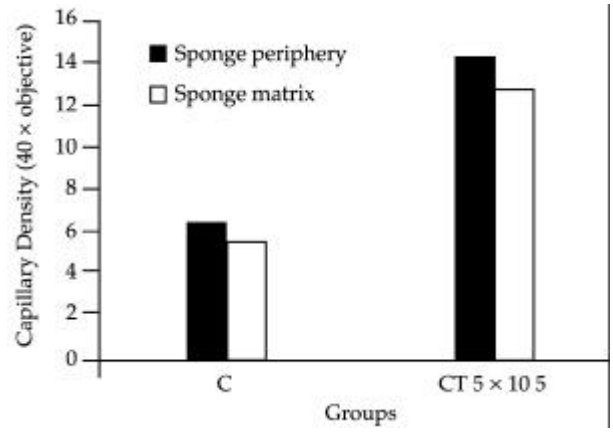

Figure 2. Inflammatory and tumor angiogenesis in control mice

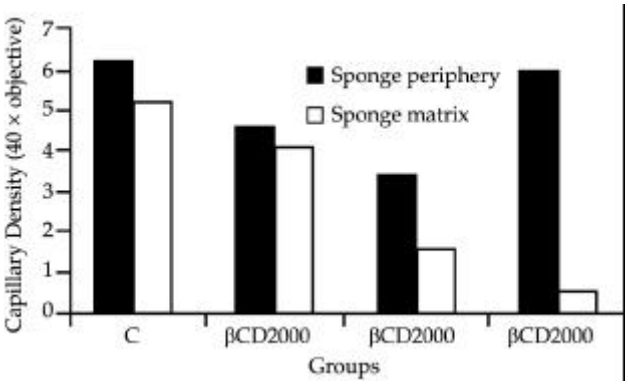

Figure 3. Comparison of microvessel density in the sterile saline-impregnated sponge between the groups of mice treated with sulfated-cyclodextrin (200 ng – 2000 ng and 20000 ng)

The mean count of capillaries in the sponge matrix was 19,3 per 600 mm^2. 7 days after the implantation of the sponge on day 0 microvessel density in the periphery and matrix of the sponge decreased to 23,5 capillaries per 600 mm^2 and 18,8 600 mm^2 fields, respectively. Inflammatory and tumor angiogenesis in control mice are compared in *Figure 2.*

The tumor-cell impregnated sponge had a mean capillary–density grade of 13,68±7,93 in the sponge periphery and a density of 12,45±8,19 capillaries in the sponge matrix at 6 days of evolution, the sterile saline-impregnated sponge had a grade of 6,26±2,77 capillaries in the sponge periphery and a capillary - density of 5,23±2,61 in the matrix. Furthermore we found in the tumor-cell impregnated sponge many large dilated venules. These results show that tumor TA3-MTX-R is an "angiogenic tumor", which potentiates the inflammatory angiogenesis induced by subcutaneous implantation of the plastic sponge.

Comparision of microvessel density in the sterile saline–impregnated sponge between the groups of mice treated with sulfated β*-cyclodextrin (200 ng, sulfated* β*-cyclodextrin 2000 ngr) and sulfated* β*-cyclodextrin (20 000 ng)*

Figure 3 and show that β-cyclodextrin is likely to exert its antianiogenic effect in a concentration dependent way. *Figure 3* shows that βCDS 200 and βCDS 2000 inhibited capillary density in the periphery to 25,4 % and 25,8 % of that in untreated controls and suppressed capillary density in the sponge matrix to 22,18 % and 62,4 % of the density in control mice. However, βCDS 20 000 decreased capillary growth in the surroundings of the sponge to 3,2 % of the density in control mice and suppressed capillary-density in the sponge matrix to 87% of the density in control mice.

Figure 4. show that the development of venules in the sponge matrix was increased by βCDS 200 to 114 % of control mice; in contrast βCDS 200 inhibited venule growth in the periphery to 27,12% of that in control mice. However, βCDS 2000 and βCDS 20 000 inhibited venule density in the sponge matrix to 57,93% and 93,46% of that in untreated controls and suppressed venule- density in the periphery to 30,51 % and 25,41 % of the density in control mice.

*Tumor angiogenesis in mice treated with sulfated–cyclodextrin 2000 ng (*βCDS*) and unsulfated–cyclodextrin 2000 ng* (βCDNS) *for 48 hours.*

Tumor angiogenesis is compared between the groups of mice treated with βCDS 2000 and βCDNS 2000 in *Figures 5* and *6.* βCDS2000 and βCDNS2000 decreased capillary growth in the surroundings of the sponge implant to 31,36% and 52,12% of the density in control mice and

suppressed angiogenesis in the sponge matrix to 100% and 75,9% respectively of untreated mice. Mean capillary-density in the tumor-cell impregnated sponge in mice treated with βCDS 2000 and βCDNS 2000 on day 5 for 48 hours is summarized.

Figure 6. show that the development of venules in the periphery of the sponge was increased by βCDS2000 and βCDNS 2000 to 9,68% and 6,44% of the venule density in control mice; in contrast βCDS 2000 inhibited venule growth in the sponge matrix to 100% of that in control mice. However, βCDNS 2000 increased venule density in the sponge matrix to 1,22% of venule growth in untreated mice.

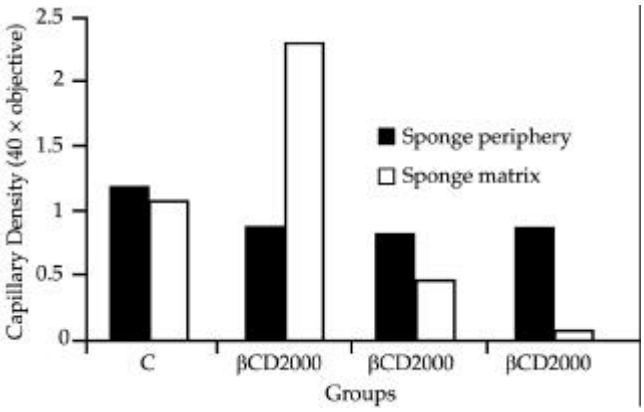

Figure 4. Mean venule-density in the sterile saline impregnated sponge in control mice treated with BCDS2000 and BCDNS2000 on day 5 for 48 hours

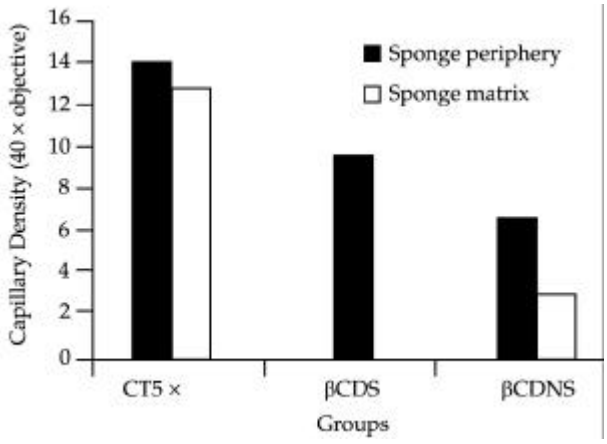

Figure 5. Mean capillary-density in the tumor-cell impregnated sponge in mice treated with BCDS2000 and BCDNS2000 on day 5 for 48 hours

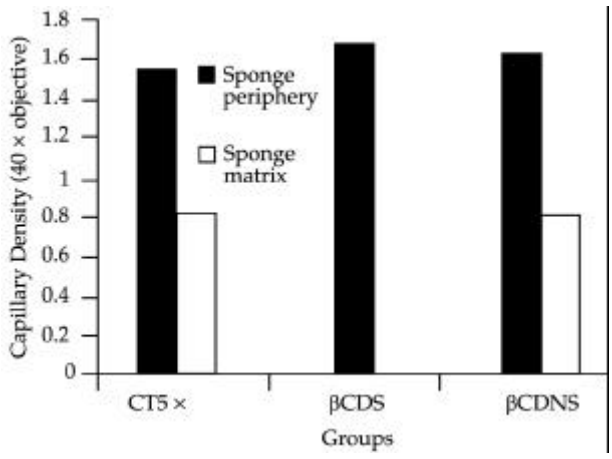

Figure 6. Mean venule-density in the tumor-cell impregnated sponge in mice treated with BCDS2000 and BCDNS2000 on day 5 for 48 hours

Discussion

We determined that sulfated β-cyclodextrin administered alone intraperitonally 4 days after sponge implantation on day 0 stimulated venule angiogenesis in the sponge matrix at low drug concentration (200 ng).The histopatological examination identified active areas of neovascularization with microvessels and venules throughout the sponge and the surrounding tissues of the sponge implant. The vessel index of venules in the sponge matrix was significantly higher than the vessel index of control mice with sterile saline–impregnated sponge. These results support the findings of Folkman et al.[8] They observed that – cyclodextrin can potentiate capillary growth when administered alone yet inhibit angiogenesis when administered with an angiostatic steroid.

There exists no definitive explanation of the angiogenic mechanism of β-cyclodextrin .

In our experiments sulfated β-cyclodextrin has been shown to possess many properties unrelated to its classical functions in the promotion of angiogenesis. We observed that sulfated β-cyclodextrin administered alone i.p. 4 days after sponge implantation on day 0 at high drug concentration (at 2000 and 20 000 ng) decreased the vessel index of capillaries and venules in the sponge periphery and matrix.

The histophatological examination identified vessels in regression, areas of microhemorrhages and morphological features of apoptosis of endothelial cells. The antiangiogenic property of cyclodextrin has not been reported by previously clinical studies.

From our results we propose that β-cyclodextrin contains both a promoter and an inhibitor of angiogenesis and that the activation of both is drug concentration dependent. Several other biological agents are known to exert their effects in a concentration dependent way. At higher concentrations antagonistic effects or loss of effects may occur, as has been described for TNF-α and granulocyte macrophage-colony-stimulating factor, for example.[12]

Nangia-Makker et al.[16] reported that galectin-3, a member of a growing family of carbohydrate – binding pro-

teins, induces endothelial capillary tube formation *in vitro* and angiogenesis *in vivo* and that the specifity of the effect is established by antibodies and competive sugars.

It has been demonstrated that sialylated fucosylated N-linked oligosaccharides, potential ligands for soluble E-selectin, were preferentially synthesized in endothelial cells undergoing tube formation.[14] In summary, these findings suggest that angiogenesis could be mediated by carbohydrate recognition.

According to Folkman et al.[7] heparin promotes angiogenesis *in vivo* and administered with cortisone inhibits angiogenesis on the chorioallantonic membrane (CAM) of the chick embryo. Furthermore heparin molecules are naturally found on the endothelial cell surface.[7] Sulfated β-cyclodextrin and several other saccharide carriers mimicked the angiostatic effect of heparin in combination with a steroid.[7]

On the basis of these findings and our results, we speculate that sulfated β-cyclodextrin binds to carbohydrate recognizing cell surface receptors where it may induce overexpression of integrins or other proangiogenic factors leading to endothelial cell migration and attachment. On the other hand, β-cyclodextrin administered at high drug concentration adsorbed to endothelial cells may induce apoptosis in endothelial cells.

We observed in the group of mice that were treated i.p. with unsubstituted β-cyclodextrin (2000 ng) a stimulation of tumor venule development throughout the matrix and the surroundings of the sponge implant. In contrast, sulfated cyclodextrin administered at 2000 ngr reduced the vessel index of venules in the sponge matrix to 100% of that of untreated controls.

The reasons for the difference between the effects of unsulfated and sulfated cyclodextrin are unclear. Possibly, unsubstituted cyclodextrin could not adhere to endothelial cells because the sulfate groups are necessary for the efficient adhesion of lycosaminoglycans to endothelial[2] and other cell surfaces.[18] We speculate that β-cyclodextrin tetradecasulfate binds to carbohydrate recognizing cell surface receptors where it may induce overexpression of integrins or other proangiogenic factors, leading to endothelial cell migration and attachment.

Both β-cyclodextrin latter at 2000 ng and 20 000 ng reduced angiogenesis in the matrix and periphery of the sponge implant. However, the decreased the vessel index of capillaries in the surroundings of the sponge to 3,2% of that of untreated controls while β-cyclodextrin dosed at 2000 ng decreased capillary–density to 25,8% of control mice. It is possible that once the endothelial cell is saturated by cyclodextrin the free circulating compounds of the drug may stimulate angiogenesis through the production of an angiogenic factor.

We have identified an important increase in the development of dilated venules in the sponge matrix of mice that were treated with cyclodextrin (200 and 2000 ng). In normal lymph nodes, specialized high endothelial venules (HEV) regulate lymphocyte extravasation from the blood. Bishop et al.[3] have identified high endothelial venules in sponge allografts .They react with mAb MECA 325 which identifies murine HEV, and bind lymphocytes. Many of the MECA 325 vessels found in sponge allografts were large, dilated vessels. Some of these vessels were found to have lymphocytes associated with the endothelial cells.

During the last years, several studies have demonstrated that endothelial cells respond to a variety of cytokines and angiogenic factors by altering their metabolic and cell surface characteristics, thereby enhancing leukocyte–endothelium, metastasis-endothelium and angiogenic factor-endothelia interactions.[14] From this we conclude that, in addition to direct promotion of angiogenesis, cyclodextrin by increasing the development of venules rich in endothelial cell surface adhesion molecules may regulate angiogenesis and facilitate the dissemination of metastasis by enhancing the angiogenic factor-endothelial and metastasis–endothelial cell interactions.

We observed that the administration of cyclodextrin at 2000 ng induced a greater reduction in the density of microvessels and venules in histologic sections in the tumor – impregnated sponge than in the sterile saline–impregnated sponge.

In conclusion, in this report we have demonstrated that sulfated β-cyclodextrin is a molecule with angiogenic and angiostatic properties. Cyclodextrin when administered at 200 ng can promote angiogenesis yet destroy capillaries and venules when administered at 2000 or 20 000 ng. Thus, sulfated β-cyclodextrin has a therapeutic potential as a promoter of angiogenesis when administered at low drug concentration, or as an angiogenesis inhibitor when administered at high drug concentration, or with an angiostatic steroid. It is important to emphasize that the systemic administration of cyclodextrin could induce angiogenesis in an otherwise dormant, undetected, tumor nodule, or may interfere with the normal malignant cell surveillance. Therapeutic angiogenesis may also hasten the development of artherosclerosis in patients with pre-existing disease and at risk for progression. Therefore we propose local administration of cyclodextrin in patients with ischemic disease.

Acknowledgement

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References

1. *Bender ML, Kaniyama M:* Cyclodextrin Chemistry. Springer Verlag, Berlin, 1978.
2. *Bergeron RJ:* In Inclusion Compounds, Arwood JL, Davies JED, MacNicol DD (eds); Academic Press, New York, Vol. 3, pp 391-443, 1984.

3. *Bishop DK, Jutila MA, Sedmak DD, et al*: Lymphocyte entry, NTO Inflammatory Tissues *in vivo*. Qualitative Differences of High Endothelial venule-like vessels in Sponge Matrix Allografts vs Isografts. J Immunol 142:4219-4224, 1989.
4. *Boyle EA, Mangan FR:* The effect of a novel, non-steroidal anti-inflammatory compound, nabumetol (BRL14777) , on cellular infiltration into 24-hour polyvinyl sponge implants in the rat , compared with some steroidal and non-steroidal anti-inflammatory drugs. J Pharm Pharmacol.34:570-575, 1982
5. *Carmeliet P, Rakesh JK:* Angiogenesis in cancer and other diseases. Nature 407:249-256, 2000.
6. *Dierendonck JH:* A new method to detect apoptosis in poraffin sections: in situ end-labelling of fragmented DNA. J Histochem Cytochem 41, 7, 1993.
7. *Folkman J, Langer R, Linhardt RJ, et al:* Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. Science 221:719-725, 1983.
8. *Folkman J, et al:* Control of Angiogenesis with Synthetic Heparin Substitutes. Science 243:1490-1493, 1989.
9. *Frank SG, Kavaliunas DR:* Investigation of the beta-cyclodextrin hydrocortisone inclusion compound. J Pharm Sci 72:1215, 1983.
10. *Freeman PC, Mangan FR, Wathius DK:* Some effects of hydrocortisone on the early development of the rat cotton pellet granuloma. Biochem Pharmacol 29:372-373, 1979.
11. *Gavrieli Y, et al:* Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119: 493-501, 1992.
12. *Griffioen AW, Molena G:* Angiogenesis: Potentials for Pharmacologic Intervention in the Treatment of Cancer, Cardiovascular Diseases, and Chronic Inflammation. Pharmacological Rev 52: 238-268, 2000.
13. *Guerrero A, et al:* Actividad antineoplαsica ejercida por quinacrina oral sobre tumors malignos transplantables en ratón. Revista Chilena de Cancerología 2:119-122, 1992.
14. *Koch AE, Halloran MM, Haskell C:* Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. Nature 376:517-519, 1995.
15. *Lubec G:* Interactions of N-CAM with heparin–like molecule. Nature 323: 743-474, 1986.
16. *Nangia–Makker P, Hanjo Y, et al:* Galectin-3 Induces Endothelial Cell Morphogenesis and angiogenesis. Am J Pathol 156:899-909, 2000.
17. *Nguyen M, Stubel NA, Bischoff J:* A role for sialyl Lewis–X/A glycoconjugates in capillary morphogenesis. Nature 365:267-269, 1996.
18. *O'Reilly MS, et al:* Angiostatin: A circulating endothelial cell inhibitor that suppresses angiogenesis and tumor growth. In: Cold Spring Harbor Symposia Quant Biol 59:471-482, 1994.
19. *Wijsman JA, Janker RR, Keijzer R, et al:* A new method to detect apoptosis in paraffin sections : In situ end- labelling of fragmented DNA. J Histochem and Cytochem 41:7-12, 1993.