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PRELIMINARY COMMUNICATION

EFFECT OF ENDOTELON® (PROCYANIDOLIC OLIGOMERS) ON EXPERIMENTAL ACUTE LYMPHEDEMA OF THE RAT HINDLIMB

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ABSTRACT

Endotelon® (Procyanidolic Oligomers, Sanofi-Labaz Laboratories) are recognized "angioprotectors" for human venous insufficiency. Using an operative experimental model of lymphedema, we sought pharmacological evidence for potential lymphagogue activity of Endotelon®.

After surgical interruption of hindlimb lymphatics, rats developed for 7 days, peripheral edema that represented a mean 24% increase in volume compared to the nonoperated hindlimb (control). In this experimental counterpart of acute lymphedema, Endotelon® (400mg/kg/day p.o.) administered "prophylactically" (i.e., before, during, and 7 days after development of lymphedema) decreased rat hindlimb lymphedema by ~4 7%. When administered just before lymphedema became established (i.e., curatively), Endotelon® and Coumarin (another "angioprotector") failed to affect hindlimb edema.

These results suggest that Endotelon® may reduce or prevent postoperative acute edema.

The venous and lymphatic systems are functionally interdependent, constituting the drainage or return pathways for the circulation of blood and tissue fluid, respectively. The venous system primarily transports electrolytes, gases, and small molecules generated by tissue catabolism. The lymphatic system, on the other hand, which derives from the venous system (1) is the primary transport pathway for macromolecules, such as high molecular weight proteins and immunological cells, that are too large to enter venous capillaries directly (2). When the interstitial fluid load exceeds the capacity of lymphatic drainage, lymphatic insufficiency develops (3) and protein-rich lymph diffuses through the surrounding soft tissues (4). Edema is one long-term consequence and infection is another.

Endotelon® is made up of procyanidolic oligomers (OPC) that are extracted from the tannin of grape pips. The OPC stems from a 3,4 diol flavan monomer that has a double linkage (*Fig. 1*). The most potent molecular structures are those that are made of 2 to 5 units. They act on the vascular wall and more precisely on mesenchymal cells (5) as well as

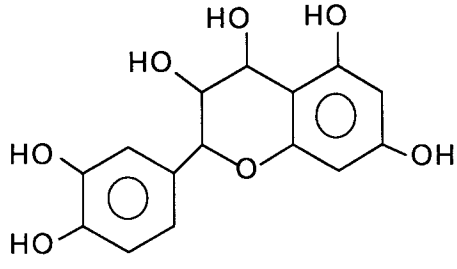

Fig. 1. The 3,4 diol flavan monomer.

on the extracellular matrix where they bind to fibrous proteins and prevent elastin and collagen degradation (6). Study of OPC activity against inflammatory mediators has shown

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that these oligomers are "angioprotectors" (7), and also act to block free radicals (7). In the human, a double blind study has shown some beneficial activity of Endotelon® when administered for venous insufficiency (8).

The aim of this study was to examine whether Endotelon® is a lymphatic angioprotector using an experimental model of acute lymphedema. After several preliminary experiments, we opted to use an animal counterpart of acute lymphedema as described in the dog and rat hindlimb (9,10) rather than the more difficult acute experimental lymphedema model in the rabbit ear (11,12).

Using this experimental model, we tested Endotelon® administered before, during, and 7 days after lymphedema developed (i.e., prophylactically) or alternatively for the first 4 days after surgical preparation of acute lymphedema (i.e., curatively) and compared the findings with Coumarin treatment, the angioprotector effects of which have been previously well described (4,13,14).

MATERIALS AND METHODS

Male OFA rats, approximately 300g body weight (IFFA Credo, France) were fed *ad libitum* and allowed free access to water during the experiment. They were housed individually with 12/12 light-dark cycle. To create acute lymphedema of the hindlimb, we used a method similar to that described by Wang (10) and Olszewski (9). Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Sanofi-Animal Health) 60mg/100g body weight. The right hindlimb was operated upon while the contralateral hindlimb served as the control. To visualize the peripheral lymphatics, we injected 0.5ml of a 0.5% patent blue solution (Sigma) dissolved in physiological saline into the subcutaneous tissue of the first interdigital space of the paw prior to shaving and sterilization. A circumferential incision, including skin and subcutaneous tissue, was made at mid-thigh. The femoral lymphatics were resected for 1cm and ligatured at both ends. The afferent and efferent lymphatics of the popliteal node were ligated before the latter was excised. The two skin edges were sutured to the underlying skeletal muscle taking care to ensure a 1cm gap between the wound edges. The incision site was then sealed with collodion to avoid direct contact with air. Rats were then allowed to recover in a warm environment and housed separately. Two types of treatment were studied: "prophylactic" and "curative."

Prophylactic treatment

Endotelon® (Sanofi-Labaz Laboratories) was solubilized in physiological saline to a final concentration of 25mg/ml and administered daily (400mg/kg). Each rat received 1.6ml/100g body weight by oral administration. Hindlimb lymphedema induction occurred on day 8 with each rat treated daily (except for day 8) from day 1 to day 16.

Group 0: Non-operative hindlimb (no lymphedema) control (n=8) received physiological saline only.

Group 1: Operated hindlimb controls (acute lymphedema) (n=6) received physiological saline. Lymphedema was induced on day 8.

Group 2: Non-operated controls (no lymphedema) (n=8) received daily Endotelon® (400mg/kg).

Group 3: Operated group (acute lymphedema) (n=6) received daily Endotelon® (400mg/kg). Lymphedema was induced on day 8.

Curative treatment

Endotelon® or Coumarin (Sigma) were administered orally daily at a dose of 400mg/kg. Endotelon® was dissolved in physiological saline to reach a concentration of 80mg/ml. Coumarin was dissolved in ethanol 50% to the same concentration. Rats received either one or the other drug orally 1.6ml/100g body weight, and were treated from day 1 to day 4 after surgical preparation for acute lymphedema (day 0).

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Group 0: Non-operated hindlimb (no lymphedema) control (n=8) received physiological saline only.

Group 1: Operated hindlimb (acute lymphedema) control (n=6) received physiological saline. Rats were operated upon on day 0.

Group 2: Endotelon® non-operated controls (no lymphedema) (n=8) received Endotelon® daily (400mg/kg).

Group 3: Endotelon® operated rats (acute lymphedema) (n=6) received daily Endotelon® (400mg/kg). Rats were operated upon on day 0.

Group 4: Coumarin non-operated controls (no lymphedema) (n=4) received daily Coumarin (400mg/kg).

Group 5: Coumarin operated rats (acute lymphedema) (n=3) received daily coumarin (400mg/kg). Rats were operated upon on day 0.

To evaluate the intensity of the lymphedema, we measured each day the volume of the right paw with a plethysmometer (Ugo-Basile) before operation and on each day thereafter. To better estimate the effect of Endotelon®, the results were expressed by measuring the area under the curve. The inhibition percent of the lymphedema was calculated by subtracting from the operative (acute lymphedema with saline only) control value the value obtained in the treated group (Endotelon® or Coumarin).

A Student-Welch test was used to evaluate the significance of the volume measurement of the limb between the non-operated control (no lymphedema) treated with physiological saline and the operated (acute lymphedema) groups treated with physiological saline, Endotelon® or Coumarin.

RESULTS

Pattern of lymphedema in non-treated control group

Fig. 2 shows the profile of experimental acute lymphedema in the rat hindlimb. Lymphedema developed during a 7-day period. The maximal increase in volume ($0.45cm^3$) occurred on day 2 (the volume increased from $1.883 \pm 0.030cm^3$ to $2.300 \pm 0.049cm^3$). Over the ensuing 5 days, the lymphedema decreased to the non-operative (no lymphedema) control group value.

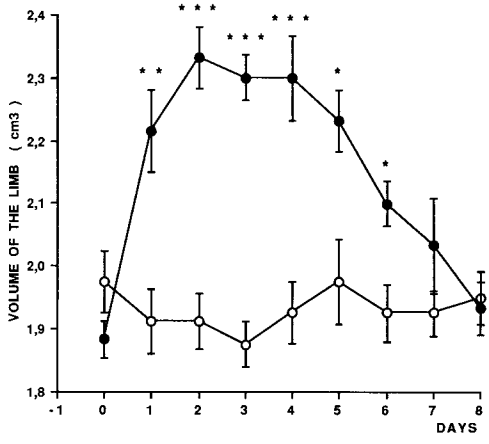

*Fig. 2. Volume of the rat hindlimb following surgical procedure. Nonoperated hindlimb controls (○), operated hindlimb controls (●). These rats received only physiological saline. Rats were operated upon at day 0. Values represent mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001 compared to nonoperated controls.*

Effect of Endotelon® used as prophylactic treatment

Fig. 3 shows the effects of Endotelon® administered prophylactically each day for 7 days, before the operative procedure and for 7 days after lymphedema induction. The values obtained with the Endotelon® in the non-operated (no lymphedema) controls was similar to that of physiological saline in the non-operative (no lymphedema) controls (data not shown). With Endotelon® prophylactic treatment, the maximal increase in volume of the limb was $0.304cm^3$ (the volume increased from $1.900 \pm 0.044cm^3$ to $2.216 \pm 0.054cm^3$). Thus, reduction in lymphedema was ~32% or using the measurement of the area under the curve, the reduction was ~47%.

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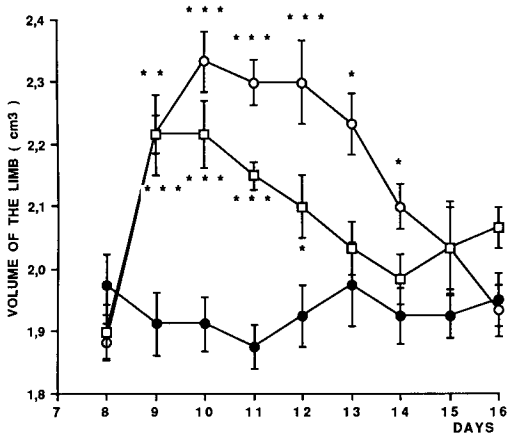

*Fig. 3. Prophylactic treatment with Endotelon®. Volume of the hindlimb 8-16 days; surgical preparation on day 8. Nonoperated controls (●), operated control group (○), operated controls receiving Endotelon® (day 1-16) (□). Values represent mean ± SEM *p < 0.05, **p < 0.01, ***p < 0.001 compared to nonoperated controls (no lymphedema).*

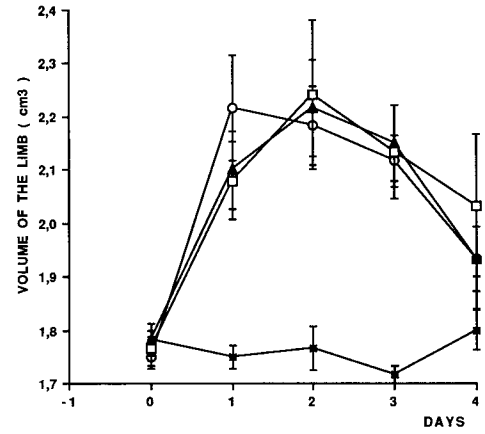

Fig. 4. Volume of the rat hindlimb following surgical preparation on day 0. Nonoperated controls (x), operated controls (lymphedema) with physiological saline (○), Endotelon® operated (lymphedema) group (▲), coumarin operated (lymphedema) group (□). Values represent mean ± SEM.

Effect of Endotelon® used as curative treatment

Fig. 4 shows that from day 1 (surgery) and for 3 days thereafter, Endotelon® and Coumarin failed to reduce hindlimb lymphedema. No effect of Endotelon® or Coumarin was observed on the volume of the control hindlimb (no lymphedema) (data not shown). We opted to stop the experiment before the end of the 7-day lymphedema period because the control-lymphedema was already in regression.

DISCUSSION AND CONCLUSION

We produced experimental acute lymphedema in the rat hindlimb to evaluate the capacity of Endotelon® to reduce lymphedema when given orally at 400mg/kg/day. This experimental model causes a 24% increase in the volume of the limb compared to the nonoperated hindlimb. When administered prophylactically, Endotelon® reduced the maximal volume of the lymphedema by ~32% and overall inhibited swelling by ~47%. On the other hand, when given over the first 4 days after surgical preparation (i.e., curatively) Endotelon® and Coumarin failed to reduce the volume of the lymphedematous limb. Perhaps, lymphedema developed and regressed too quickly for a therapeutic advantage of these "angioprotectors" to occur since their actions take several days to develop. Used prophylactically, however, Endotelon® reduced acute lymphedema of the rat limb suggesting that this agent is a "protector" of lymphatic function. Perhaps Endotelon® may prove useful after operations to minimize or eliminate edema that commonly occurs, for example, after reconstructive procedures of the face (15) or mastectomy and axillary dissection in management of breast cancer.

The experimental protocol described by Wang (10) showed that after the regression of acute phase of lymphedema, a chronic stage of lymphedema often becomes reestablished 180 days post-surgery. Further study of the effects of Endotelon® on this more longstanding experimental model of lymphedema may be worthwhile.

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REFERENCES

1. Merlen, JF: Microcirculation et lymphatiques. Symposium de Lymphologie, Lymphatique et souffrance tissulaire, Association de lymphologie de langue française. (1981), 10.
2. Foldi, M, JR Casley-Smith: *Lymphangiology*. George Thieme Verlag, Stuttgart:New York (1983).
3. Foldi, M: Insuffisance circulatoire lymphatique: Lymphostase dont lymphoedème des membres. Symposium de Lymphologie, Lymphatique et souffrance tissulaire, Association de lymphologie de langue française. (1981), 18.
4. Foldi-Borcsok, E, JR Casley-Smith, M Foldi: The treatment of experimental lymphoedema. Angiologica 133 (1972), 229.
5. Gavignet-Jeannin, C, N Groult, N Godeau, et al: Effect of procyanidolic oligomers on cultured mesenchymal cells. I. Effect on attachment, proliferation and detachment of cells. Pathol. Biol. 37 (1989), 746.
6. Tixier, JM, G Godeau, AM Robert, et al: Evidence by *in vivo* and *in vitro* studies that binding of pycnogenols to elastin affects its rate of degeneration by elastase. Biochem. Pharmacol. 33 (1984), 3933.
7. Barbier, A, JP Maffrand, P Savi, et al: Activité angioprotectrice des Oligomères Procyanidoliques chez l'animal. In: *Endotelon et Unite Circulatoire Angio Dyn 88*, Libbey, J (Ed.) (1988), 31.
8. Henriet, JP: Endotelon dans les manifestations fonctionnelles de l'insuffisance veineuse périphérique. Etude E.I.V.E. Angiologie 5-74 (1988), 167.
9. Olszewski, J, Z Machowski, J Sokolowski, et al: Experimental lymphoedema in dogs. J. Cardiovasc. Surg. 9 (1968), 178.
10. Wang, GY, SZ Zhong: A model of experimental lymphoedema in rat's limbs. Microsurg. 6 (1985), 204.
11. Cunningham, DM, M Holding, RK Daniel: Microsurgical technics in experimental lymphoedema of the rabbit's ear. Surg. Gyne. Obst. 147 (1978), 877.
12. Huang, GK, YP Hsin: An experimental model of lymphoedema in rabbit ear. Microsurg. 4 (1983), 236.
13. Casley-Smith, JR: Benzopyrone de référence: la coumarine. Symposium International "Circulation d'échange et de retour" Vienne (1984).
14. Piller, NB: An integration of the modes of action of coumarin. Arzneim. Forsch./Drug Res. 27 (1977), 1135.
15. Baruch, J: Effet de l'Endotelon® dans les oedèmes post-chirurgicaux de la face. Annales de Chirurgie Plastique et Esthétique. 29 (1984), 393.

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