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(54) Title: PHARMACEUTICALLY EFFECTIVE COMPOUNDS AND THEIR USE

(57) Abstract: The invention provides use of panaxadiol for the treatment of conditions requiring stimulation of angiogenesis but not stimulation of chemoinvasion and use of panaxadiol in the manufacture of medicaments for such treatments. A preferred panaxadiol is the naturally occuring ginsenoside Rb1.

Pharmaceutically Effective Compounds and Their Use

This invention relates to compounds which we have found to have angiogenic effects in particular circumstances, in particular the ginseng component Rb_1 and its derivatives, and other panaxadiol ginsenosides which act in the same way, and their use in certain therapeutic applications for which angiogenic response is valuable.

The root of Panax ginseng has been well known as a general "tonic" for many centuries. It is used for its anti-stress effects and for toning up the nervous system, and has been suggested for a variety of other purposes, including treatment of atherosclerosis, cerebrovascular diseases, liver dysfunction, post-menopausal disorders and hypertension.

Attele *et al* in Biochem. Pharmacol. Vol. 58, pp 1685-1693, 1999, review the various pharmacological effects which have been reported for ginseng.

Morisaki *et al* in Br. J. Pharmacol. (1995) 115, 1188-1193 surmise that saponin of ginseng *Radix rubra* influences angiogenesis. They state that orally administered ginseng has been reported to stimulate the repair of intractable skin ulcers in some patients. Morisaki *et al* suggest a mechanism of tube formation by saponin modifying the balance of protease/protease inhibitor secretion from HUVEC and enhancing the migration of HUVEC.

Kanzaki *et al* in Br. J. Pharmacol. (1998) 125,255-262, disclose some effects of saponin on wound healing. They report the Morisaki *et al* suggestion that saponin stimulates wound healing by the suggested mechanism above, but carry out further testing to clarify this. They surmise that saponin stimulates fibronectin synthesis through changes of TGF-β receptor expressions in fibroblasts.

Although these authors have surmised possible effects on wound healing of saponin of ginseng the mechanism of wound healing is so complex and so many factors are involved that the situation is far from certain.

White *et al* in Pharmacotherapy July 2001: 21(7): 773-7, "An Evaluation of the Hemostatic Effects of Hydrophilic, Alcohol and Lipophilic Extracts of Notoginseng" disclose that notoginseng extracts can have hemostatic effects. The same authors discuss use of notoginseng extracts for hemostatic effects in J. Clin. Pharmacol. 2000 Oct.: 40(10): 1150-3.

Separately, Sato *et al* in Biol. Pharm. Bull. 1994 May; 17(5):635-9, report that one ginsenoside, Rb_2, inhibited tumour growth and showed an anti-angiogenic effect.

Mochizuki *et al* in Bio. Pharm. Bull. 1995; Sep; 17 (9): 1197-202 disclose that saponin preparations 20(R)-and 20(S)-ginsenoside-Rg_3 inhibit the lung metastasis of tumour cells and suggest that this mechanism is related to inhibition of the adhesion and invasion of tumour cells and to anti-angiogenic activity.

Therefore, the effects of ginseng on angiogenesis are at present uncertain. As can be seen from above, it has been suggested both that it exhibits angiogenic effects and that it exhibits anti-angiogenic effects.

WO01/92289, published 6 December 2001, discloses intravenous preparations, skin preparations, mucosal preparations and cosmetics comprising Rb ginsensosides as skin tissue regeneration/reconstruction promoters or wound healing promoters and as fertiliser additives for use as plant tissue regeneration/reconstruction promoters. However, this publication does not discuss angiogenesis in particular. Many factors are involved in the wound healing process, of which angiogenesis is just one. Other steps can include fibroblast or keratocinyte growth, chemoattraction of inflammatory cells etc.

Our publication WO02/07732, published 31 January 2002, discloses that panaxatriol ginsenosides, in particular the naturally-occurring ginsenoside Rg1, may be used for stimulation of angiogenesis.

A known component in ginseng is the panaxadiol ginsenoside Rb_1. Rb_1 has a four trans-ring steroid skeleton and is a panaxadiol (see Figure 1a). The ginsenoside Rb_2 and the ginsenoside Rg_3 discussed by Sato and Mochizuki in the publication discussed above are also panaxadiols. Thus it would be expected that materials of this type are generally anti-angiogenic.

However we have found surprisingly that the ginsenoside Rb_1 and other panaxadiols may be used to stimulate angiogenesis in particular circumstances and indeed we have found that Rb_1 in particular is a highly potent angiogenic compound in certain specific circumstances.

Thus according to a first aspect of the invention we provide a method of stimulating angiogenesis in circumstances which do not require chemoinvasion in a subject by the use of a panaxadiol ginsenoside, preferably Rb_1. The invention also provides the use of a panaxadiol ginsenoside, preferably Rb_1, in the manufacture of a medicament for treatment of a human or other subject by stimulation of angiogenesis, for a condition which requires stimulation of angiogenesis but does not require stimulation of chemoinvasion.

As discussed above, this conclusion is surprising, firstly in view of the general uncertainty about the angiogenic effects of ginseng as a whole, with Kanzaki *et al* and Morisaki *et al* suggesting that ginseng extract may have angiogenic effects but Sato *et al* and Mochizuki *et al* suggesting that certain components (and thus ginseng itself) have an anti-angiogenic effect. It is particularly surprising in view of the fact that the specific components stated to have an anti-angiogenic effect are panaxadiols. It has not been realised previously that Rb_1 is in fact a potent angiogenic compound which can be used in circumstances where chemoinvasion is not required.

Rb_1 is in the panaxadiol or 20(s)-protopanaxadiol group of ginsenosides (Gillis, Biochem. Pharmacol., Vol.

54, pp1-8, 1997). We believe that other panaxadiol ginsenosides also exhibit equivalent angiogenic effects. The discussion below concerns Rb_1 but is applicable to other angiogenic panaxadiols which show the same effect.

Panaxadiols have the structural formula in Figure 1 (b). They are based on the triterpene dammarane structure. Some panaxadiols are naturally-occurring ginsenosides.

In the formula 1(b) R_2 is H and each of R_1 and R_3 may independently be H or any organic group having up to 30 carbon atoms. They may be the same or different. In particular they may be sugar-containing groups. The sugar groups may be 5-ring sugars or 6-ring sugars and may for instance be selected from glucose, maltose, fructose, xylose, rhamnose and arabinose. They may alternatively be alkyl, alkenyl or alkynyl so that R_1O- and/or R_3O- are ethers. Alternatively they may be acyl groups so that the R_1O- and/or R_3O- groups are esters. For instance R_1 and R_3 may be fatty acyl, saturated or unsaturated. Preferably R_1 and R_3 are sugar-containing groups. Preferably they contain only sugar moieties but they may be derivatives of sugars.

Preferably each of R_1 and R_3 has not more than 24, preferably not more than 18 and particularly preferably not more than 12 carbon atoms.

Compounds of the Formula 1(b) may have any stereochemical structure. Preferably the stereochemical structure of the 4-ring skeleton is trans-trans-trans as in naturally occurring Rb_1.

Within any structure for which the steroid ring stereo chemistry is defined, the stereochemistry at C20 may be R or S. Thus any defined skeleton stereostructure can produce two enantiomers. The S-configuration is preferred. Panaxadiols may be used in the form of a single enantiomer or a non-racemic mixture of enantiomers or as the racemate. Panaxadiols having the structural formula of a naturally-

occurring ginsenoside preferably also have the stereochemistry of the naturally occurring ginsenoside.

Mixtures of panaxadiols may be used.

The compounds used in the invention differ from panaxatriols in that in the latter the group R_2 is not H but OR. In one aspect of the invention panaxadiol is preferably used without panaxatriol. Thus in this case the use of non-purified ginseng or saponin of ginseng is excluded. In one preferred aspect of the invention we use only one or two active constituents, each of which is a panaxadiol.

Naturally-occurring panaxadiols for use in the invention may be obtained from various ginseng species. These include *Panax ginseng* (often described as Oriental ginseng), *P. quinquefoliens* (often described as American ginseng), *P.notoginseng* and *P.japonicus* C.A.Mey (often described as Japanese ginseng). Preferably they are obtained from *P.ginseng* or *P.notoginseng*, preferably *P.ginseng*.

All of the naturally-occurring ginsenosides have the same stereostructure of the four-ring steroid skeleton. The structure is trans-trans-trans.

The panaxadiol may be naturally occurring, eg Rb_1, Rb_2, Rc or Rd, preferably Rb_1. It may be formed by synthesis or, preferably, by purification of a panaxadiol ginsenoside from ginseng. Purification of Rb1 and other naturally occurring panaxadiols from ginseng may be achieved by known methods. Derivatives of the formula in Figure 1(b) may be used. These will have equivalent functionality. That is, any derivatisation does not significantly reduce the angiogenic effect. Generally functionally equivalent derivatives have the same or a greater angiogenic effect than Rb_1 (or other panaxadiol). If it is a lesser effect it is generally not less than 70%, usually not less than 80%, of the effect of Rb_1.

The structures of the naturally-occurring ginsenosides Rb_2, Rc and Rd are known. In each of these R_1 is Glc^2-Glc-.

In Rb_2 the group R_3 is Glc^6-ara(p) and in Rc the group R_3 is Glc_6-ara(f). In Rd the group R_3 is Glc.

Other naturally-occurring panaxadiols which may be used include compounds commonly described as notoginsenosides. These are found naturally in the leaves and seeds of the species *Panax notoginseng*. The notoginsenoside-R4 has the formula 1(b) in which R_1 is Glc2-Glc and R_3 is Glc6-Glc6-Xyl.

The steroid ring stereochemistry is the same in this naturally-occurring notoginsenoside as in the ginsenoside Rb_1. It exists naturally in the 20S form.

Suitable panaxadiols of the formula in Figure 1(b) may be derived using methodologies such as quantitative structure activity relationships (QSAR) and comparative molecular field analysis (CoMFA) so that molecules having equivalent or increased effectiveness over naturally-occurring ginsenosides may be devised using the naturally-occurring ginsenosides as starting points. This methodology is discussed for instance by Richon and Young, in "An Introduction to QSAR Methodology", Network Science, 2000. Software packages such as QSAR with CoMFA, available from the company Tripos Inc may be used, but other products are available.

It is also possible to use such techniques to derive compounds not of the Formula 1(b) but which show equivalent functionality to Rb_1 or other panaxadiol. In particular, the CoMFA methodology, a three-dimensional QSAR technique, is useful. For instance, techniques are known for defining the steric and electronic features of a compound necessary to ensure optimal supramolecular interactions with a specific biological target structure. This ensemble of steric and electronic features is known as a pharmacophore. Similar techniques may be used to define other molecules having the steric and electronic features provided by Rb_1 or other naturally-occurring ginsenoside.

In the invention at least one panaxadiol is used. The invention relates to use of panaxadiols and thus use of

non-purified ginseng or saponin of ginseng is preferably excluded. Preferably the only active constituents of the pharmaceutical composition used are panaxadiols. Preferably only one or two active constituents are used, each of which is a panaxadiol.

In the treatment methods Rb_1 may be applied to the subject in any convenient manner.

Preferred vehicles include liposomes, microsomes or microsponges within which the Rg_1 may be contained and which may be injected for instance intravenously, intramuscularly or subcutaneously. Cationic liposomes and stealth liposomes with or without antibody coating may be used.

Other suitable vehicles include implants, such as collagen implants, in a suitable form for application to the subject. Implants may be made of natural (eg collagen, fibrin or gelatin), synthetic (eg methylcellulose, ELVAX, tetradecacyclodextran) or semi-synthetic materials. They may be applied to a wound or ulcer for which healing is required. Other application forms include topical application, eg as a cream, gel, transdermal patch or time/controlled drug release system. The Rb1 may be used impregnated in materials which are introduced to the body of the subject for other reasons, eg sutures (eg made of catgut) or vascular grafts.

Applications for which the angiogenic activity of Rb_1 is beneficial include those in which angiogenesis can take place without chemoinvasion, which is believed to be inhibited by Rb_1. Angiogenesis requires four phases, (a) destruction of the basement membrane by proteases secreted by endothelial cells, (b) migration/chemoinvasion of endothelial cells, (c) proliferation of endothelial cells, (d) tube formation by endothelial cells.

Thus applications for Rb1 include the late phase of wound healing where chemoinvasion has already taken place by otherwise normal physiological processes. Rb1 may be used in combination with other angiogenic factors or

compounds where Rb1 is used to promote late stage angiogenesis (other angiogenic factors or compounds having been used to promote early stage angiogenesis). The invention may also be used in the maintenance of the established neo-vasculature, in treatment of patients recovering from myocardial ischaemia and diabetic nephropathy.

Rb1 and other panaxadiols showing equivalent functionality may also be used to increase graft or implant survival, promote bone growth and healing from fractures and the healing of diabetic ulcers, pressure ulcers, cerebrovascular ischaemia and angina.

Rb1 may also be used for maintenance therapy for preventing hair loss by promoting or maintaining an existing angiogenic response.

For instance, Rb1 can be used in treatment of patients having suffered from myocardial ischaemia. Growth factors can be supplied to the patient, either by treatment directly with growth factors or with use of gene therapy which elevates expression of growth factors. Rb1 may then be used to maintain the newly grown vessels. Similarly, diabetic nephropathy, which is characterised by a regression of blood vessels, may be treated with Rb1.

We believe that these effects are demonstrated by the fact that Rb_1 shows angiogenic effects in the *in vitro* HUVEC model discussed below. However, it does not show angiogenic effects *in vivo* and we believe this is due to inhibition of chemoinvasion. Thus the invention is based on the realisation that Rb1 is inhibitory of one stage of the angiogenesis process but a promoter of a later stage. We believe that this has never previously been realised. This realisation enables the application of Rb1 in specific treatments where it is required to promote, for instance, the tube formation stage. This makes it a good candidate for timed therapeutic interventions, ie in promoting angiogenensis once chemoinvasion of endothelial cells has occurred.

Other angiogenic compounds which can be used in combination treatments include Rg1 (or other panaxatriol) as disclosed in our publication WO02/07732. For instance, Rg1 may be administered to promote the initial stages of wound healing and once chemoinvasion has taken place Rb1 may subsequently be administered to promote the later stages.

We also find that combinations of Rb1 with bFGF (basic fibroblast growth factor) and TNF (tumour necrosis factor) or VEGF (vascular endothelial growth factor) and TNF give particularly good and even synergistic results.

We also provide a process for the production of a pharmaceutical composition comprising providing ginseng or ginseng extract, obtaining from the ginseng or ginseng extract the ginsenoside Rb_1 in purified form, optionally derivatising the Rb_1, and combining the purified and optionally derivatised Rb_1 with a pharmaceutically acceptable excipient. Any derivatisation does not remove the functionality of the ginsenoside. This process may be applied to any other naturally-occuring panaxadiol in ginseng.

Such a composition may be used in the manufacture of a medicament for use in a method for the treatment of a human or animal subject by stimulation of angiogenesis and may be used in a method of treating a human or other animal subject by stimulating angiogenesis using a pharmaceutical composition of this type. The method may be any of those discussed above.

The subject treated may be any animal, preferably a mammal, and is usually a human.

The Rb_1 may be used in the form of any suitable pharmaceutical composition, generally provided by purifying Rb_1 from ginseng and combining it with a suitable pharmaceutical excipient. Pharmaceutical excipients can be chosen on the basis of general knowledge.

The pharmaceutical composition may be in a form suitable for oral administration, for instance tablets,

capsules or a gel. Alternatively, it may be an aerosol formulation. It can thus be sprayed onto any biological surface such as the skin or internal organs. A particularly suitable composition comprises Rb_1 in artificial or tissue-engineered skin or in slow drug release skin patches. Others include the forms such as sutures, vascular grafts and skin grafts discussed above. Compositions may also be in a form suitable for use in conjunction with gene-derived therapy. One suitable form is delivery in a polymeric or copolymeric matrix containing Rb1 and known angiogenic compounds, whereby the angiogenic promoters are released before the Rb1 and promote chemoinvasion and Rb1 is released later.

Dosages may be chosen by those skilled in the art using known methods. The dosage is chosen so that the panaxadiol is administered in an amount effective to stimulate angiogenesis in the subject. Suitable dosages may be from 1 μg to 100mg per kg per day, preferably from 25 μg to 500μg panaxadiol per kg per day, preferably from 5 μg to 100 μg panaxadiol per kg per day. Treatment may be carried out for any period of time and may be over a period of months or even years. However, generally treatments will be carried out for from 1 to 60 days, preferably 1 to 30 days.

Because of the novel finding that Rb_1 stimulates tube formation but inhibits chemoinvasion we also provide methods of treatment of a human or other animal subject by inhibition of chemoinvasion with a panaxadiol. We also provide use of a panaxadiol in the manufacture of a medicament for treatment of a condition requiring inhibition of chemoinvasion. Such conditions include chemo prevention of tumours, diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis, endometriosis, Crohn's disease and adiposity.

Features of the first aspect of the invention may be applied to this aspect of the invention where appropriate.

The invention will now be illustrated by reference to the following examples.

Examples

Example 1

We tested angiogenic effects by means of an *in vitro* assay similar to that described in Lauder *et al*, Angiogenesis 1998;2;67-80. In this assay, "wounded" human umbilical vein endothelial cells (HUVEC) are exposed to increasing concentrations of Rb_1. In their normal physiological state endothelial cells remain quiescent but proliferate in injured tissue, causing angiogenesis. In mimicry, human umbilical vein endothelial cells were allowed to reach a confluent state in a well containing a semi-circular coverslip following which a "wound" to the monolayer was imparted by removing the cover slip, and leaving an area denuded of cells.

The HUVECs were plated in 24 well plates with a bisected cover slip in each well. On reaching confluent state, the cells were wounded by lifting off the cover slip at time zero. The wounded monolayer of cells was incubated in the presence of Rb_1 for a further 48 hours following which the cells were harvested and counted. Results are shown in Figure 1. They demonstrate that Rb_1 stimulates endothelial cell proliferation.

Example 2

In this experiment the effect of Rb_1 on tube formation by HUVECs and modulation by Nw-nitro-L-arginine methyl ester, L-NAME (an inhibitor of Nitric Oxide Synthase enzyme) were assessed. Cells were plated on extra-cellular matrix extracted from EHS murine sarcoma, and cultured for 16 hours in absence or presence of Rb_1. Where the NOS-inhibitor, L-NAME, was used, the cells were pre-incubated with L-NAME, which was maintained throughout the entire duration of the experiment. Endothelial cells incubated on extracellular matrix align and form tubular structures as seen in the photomicrographs of Figure 4. Results are in Figure 3.

Figure 4B shows pretreatment with 12.5 nM Rb_1. This induced a concentration-dependent formation of tubes, which were quantified by counting the branches formed per view field. Figure 4C shows the results of treatment with L-NAME (10^{-3}M). This did not alter the basal or tube formation.

Figure 4D shows that tube formation was unaffected by pretreatment with L-NAME. Concentration response of Rb_1 inducing angiogenesis is shown in Figure 4E.

Example 3

This experiment tests the effect of Rb_1 on chemoinvasion by HUVECs. Human recombinant HGF/SF(1nM) (Figure 5B) in the lower chamber, increased the chemoinvasion of HUVECs as compared with vehicle (Figure 5A). Pretreatment with Rb_1 inhibited the migration of the HUVECs towards HGF/SF. (Figure 5C)

Figure 5A shows cells migrated under control conditions. Figure 5B shows HGF/SF (1nM) facilitated HUVEC chemoinvasion and spreading. Figure 5C shows that Rb_1 (125nM) inhibits HGF/SF-induced chemoinvasion. Figure 5D shows the mean number of migrated cells per high power view field under different experimental conditions (mean $\pm$ SEM, n=4).

Example 4

This example demonstrates the effect of Rb1 in combination with both bFGF and VEGF/TNF.

An *in vitro* assay was carried out in accordance with the assay described by Koolwijk et al in Angiogenesis 4:53-60;2001, page 55. In this system tube formation in the presence of bFGF/TNF and in the presence of VEGF/TNF was assessed. The effect of adding Rb1 to the system was then also tested. Results are shown in Figure 6. These results clearly show a significant increase in tube formation in the presence of Rb1.

CLAIMS

1. Use of a panaxadiol in the manufacture of a medicament for the treatment of a condition requiring stimulation of angiogenesis but not requiring chemoinvasion.

2. A use according to claim 1 wherein the panaxadiol is a naturally-occurring ginsenoside, preferably Rb1.

3. A use according to claim 1 or claim 2 in which the treatment is selected from the group consisting of promotion of late stage angiogenesis, maintenance of established neo-vasculature, treatment of myocardial ischaemia, treatment of diabetic nephropathy, healing of diabetic ulcers or pressure ulcers, treatment of cerebrovascular ischaemia and angina, increase of graft or implant survival, promotion of bone growth, healing of fractures, and preventing hair loss by promoting or maintaining an existing angiogenic response.

4. A use according to any preceding claim in which the panaxadiol has been obtained by purification from ginseng.

5. A use according to any preceding claim in which the panaxadiol is used without panaxatriols.

6. A use according to any of claims 1 to 4 in which the panaxadiol is used in combination with a panaxatriol, preferably a naturally-occurring ginsensoside, more preferably Rg1.

7. A use according to any of claims 1 to 4 in which the panaxadiol is used in combination with tumour necrosis factor-alpha (TNF-alpha).

8. Use according to any of claims 1 to 4 in which the panaxadiol is used in combination with basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF).

9. A use according to claim 7 in which the panaxadiol is used in combination with either TNF-alpha and bFGF or TNF-alpha and VEGF.

10. Use of a pharmaceutical composition in the manufacture of a medicament for use in a method for the treatment of a

condition requiring stimulation of angiogenesis but not stimulation of chemoinvasion, wherein the composition has been prepared by (1) providing ginseng or a ginseng extract, (2) obtaining from the ginseng or ginseng extract a panaxadiol in purified form, optionally (3) derivatising the panaxadiol, and (4) combining the purified and optionally derivatised panaxadiol with a pharmaceutically acceptable excipient.

11. A use according to claim 10 in which the pharmaceutical composition comprises at least one component which is not naturally present in ginseng.

12. A use according to claim 10 in which panaxadiols are the only pharmaceutical actives, and preferably the ginsenoside Rb1 is the sole pharmaceutical active.

13. A use according to any preceding claim in which the panaxadiol is applied to the subject to be treated encapsulated in liposomes or in an implant.

14. A use according to claim 13 wherein the treatment comprises injecting liposomes into the bloodstream or application of a implant to the surface of a wound or application of an implant to the surface of an ulcer.

15. A pharmaceutical composition which comprises a panaxadiol encapsulated in liposomes, microsomes, microsponges or a controlled drug release system.

16. A pharmaceutical composition which comprises a panaxadiol in an implant made of natural, synthetic or semi-synthetic material, preferably a collagen implant.

17. A pharmaceutical composition which comprises a panaxadiol impregnated in a polymeric device suitable for implantation in the human body.

18. A pharmaceutical composition according to any of claims 15 to 17 in which the panaxadiol is Rb1.

19. A method of treating a human or other animal subject comprising stimulating angiogenesis in circumstances which do not require chemoinvasion by using a panaxadiol.

20. A method of treating a human or other animal subject comprising stimulating angiogenesis in circumstances which

do not require chemoinvasion by using the panaxadiol ginsenoside Rb1.

21. A method of treating a human or animal subject by stimulating angiogenesis in circumstances which do not require chemoinvasion using a pharmaceutical composition, wherein the composition has been provided by providing ginseng or a ginseng extract, obtaining from the ginseng or ginseng extract a panaxadiol in purified form, optionally derivatising the panaxadiol, and combining the purified and optionally derivatised panaxadiol with a pharmaceutically acceptable excipient.

22. Use of a panaxadiol, preferably the naturally occurring ginsenoside Rb1, in the manufacture of a medicament for the inhibition of angiogenesis.

23. A use according to claim 22 in which the inhibition is by inhibition of chemoinvasion.

24. A method of treating a human or other animal subject by inhibition of angiogenesis using a panaxadiol, preferably the naturally occurring ginsenoside Rb1.

25. A method according to claim 24 in which the inhibition is by inhibition of chemoinvasion.

Fig.1(a).

Rb1

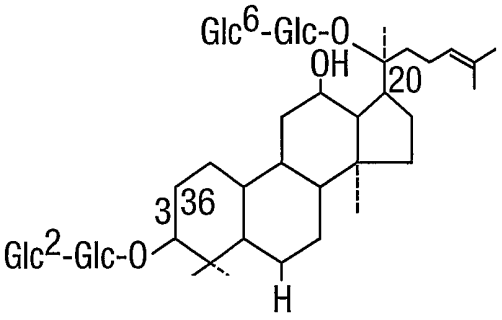


Fig.1(b).

Panaxadiol

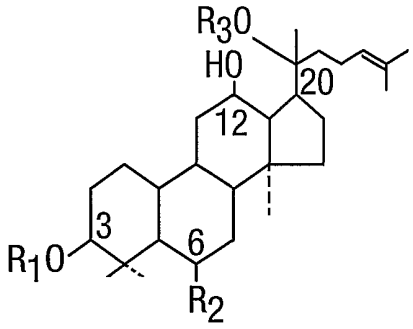


Fig.6.

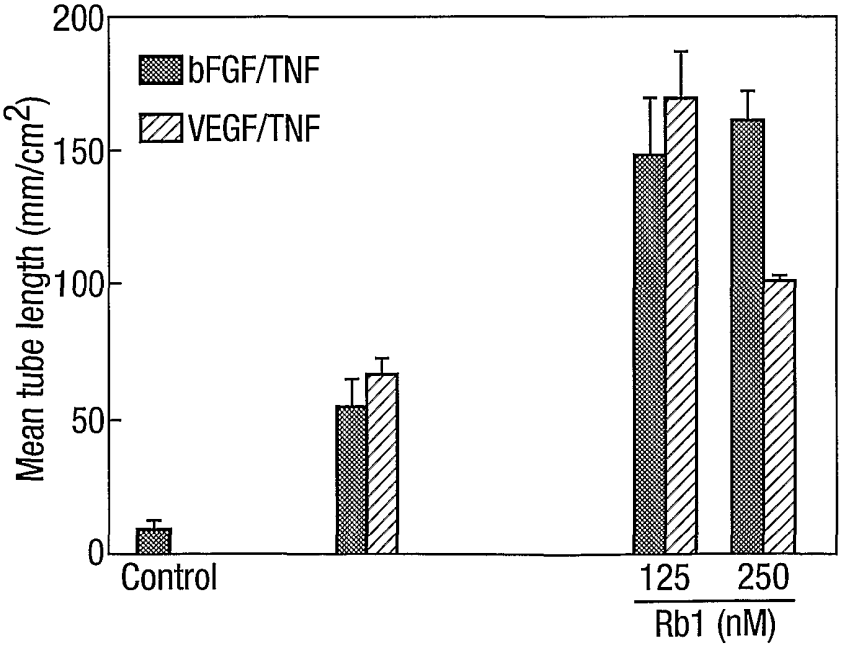


Fig.2.

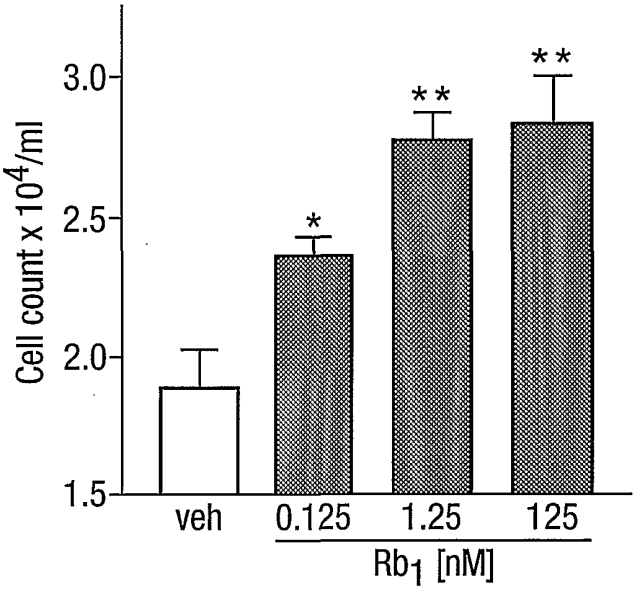

Cell count x 10^4/ml
3.0
2.5
2.0
1.5
*
**
**
veh
0.125
1.25
125
Rb_1 [nM]

Fig.3.

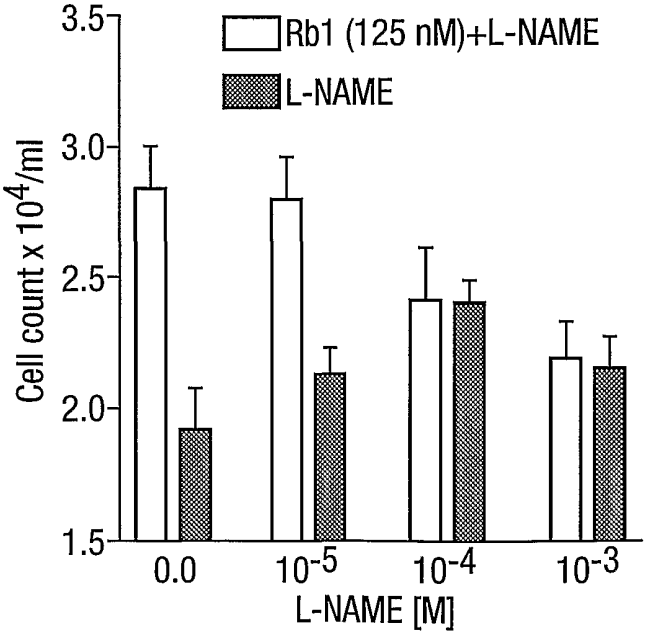

Rb1 (125 nM)+L-NAME
L-NAME
Cell count x 10^4/ml
3.5
3.0
2.5
2.0
1.5
0.0
10^{-5}
10^{-4}
10^{-3}
L-NAME [M]

Fig.4(a).

Control

Fig.4(b).

Rb1 [12.5 nm]

Fig.4(c).

L-NAME [10^{-3} M]

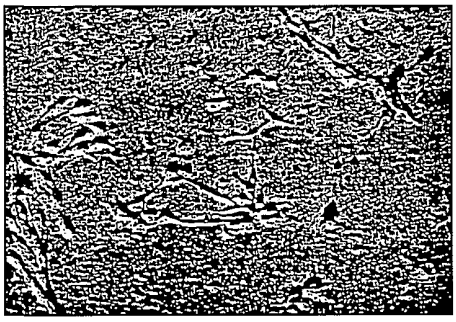


Fig.4(d).

Rb1 [125 nM]+
LNAME [10^{-3} M]

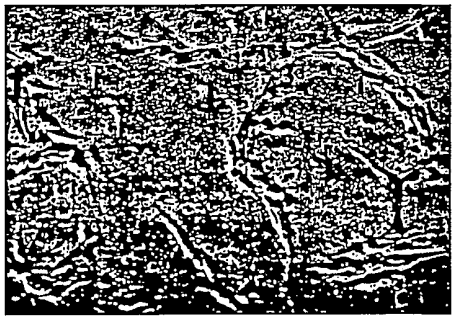


Fig.4(e).

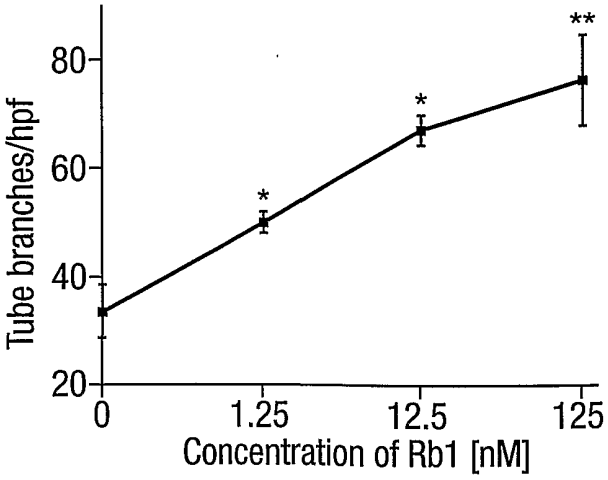


Fig.5(a).

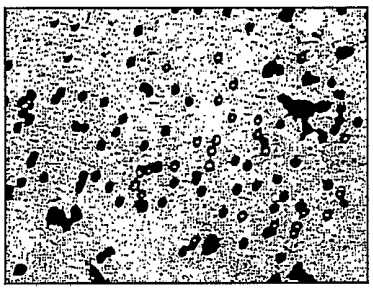


Fig.5(b).

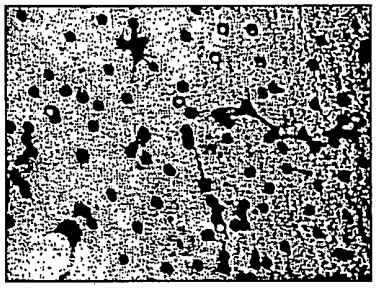


Fig.5(c).

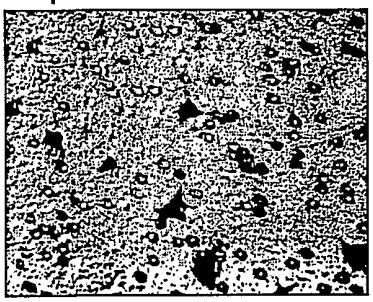
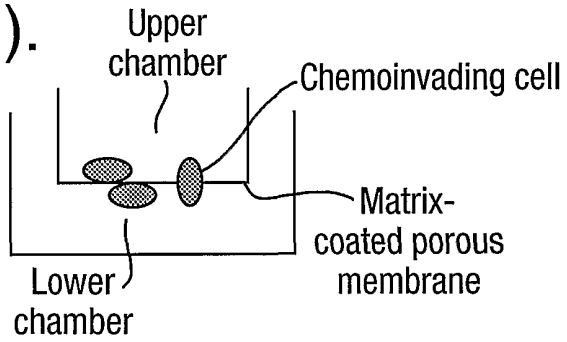
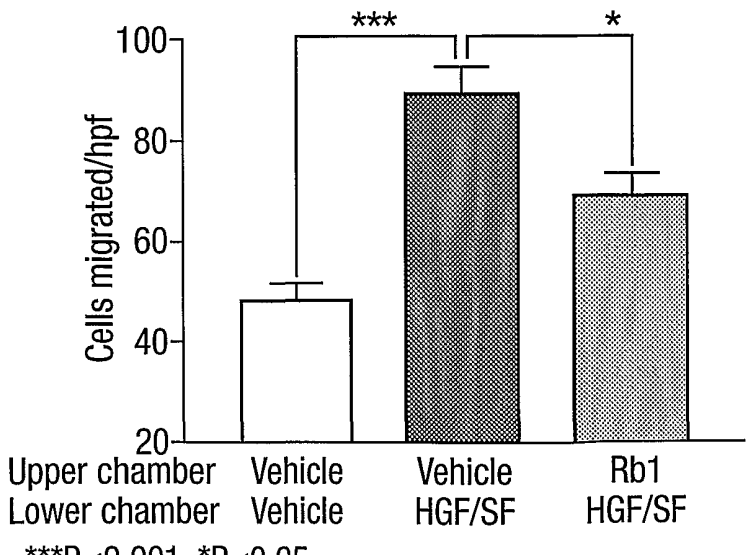


Fig.5(d).





***P<0.001, *P<0.05