Anti-inflammatory Effect of Biotin and Plant extracts

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Summary

Biotin is a water-soluble vitamin used as a skin conditioning agent and promotes the formation of intercellular lipid layers through increased lipid synthesis, which improves the skin's natural barrier function. The anti-inflammatory effects of biotin have been investigated using *in vitro* assay models, such as MTT assay, measurements of concentrations of nitric oxide(NO), prostaglandin E_2(PGE_2), and inhibition rate of 5-lipoxygenase(5-LOX). In comparison with biotin, other plant extracts were tested at the same time which were kudzu vine extract, sage extract, paeonia extract, and dipotassium glycyrrhetinate. Nitric oxide is a signal molecule with functions such as neurotransmission, local vascular relaxation, and anti-inflammation in many physiological and pathological processes. NO can cause apoptosis and necrosis of target cells such as keratinocytes and is generated from L-arginine by nitric oxide synthase (NOS). Prostanoids, including prostaglandins and thromboxanes, are generated by the phospholipase A_2/cyclooxygenase(COX) pathway, and leukotrienes are generated by the 5-lipoxygenase pathway from arachidonic acid. Prostaglandin E2 recently have been shown to be beneficial in the resolution of tissue injury and inflammation, also has been implicated as an immunosuppressive agent and plasma levels of PGE_2 are elevated in patients sustaining thermal injury. Lipoxygenase metabolites from arachidonic acid have been implicated in inflammation, anti-inflammatory activity of the raw materials was evaluated *in vitro* by the offered inhibition of lipoxygenase.

Key Words : Biotin, Anti-inflammation, Nitric oxide, PGE_2, Lipoxygenase, MTT assay, Plant extracts

Introduction

Biotin is a co-factor in the fixation of CO_2. Four biotin enzymes have been found in animal and human tissues ; pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and 3-

methyl crotonyl-CoA carboxylase.(1,2) Cowan *et al.* (3) reported the immunologic studies on a kindred affected by biotinidase deficiency, which showed failure to develop specific antibodies in response to immunization and abnormal *in vivo* and *in vitro* cell-mediated response to antigen, and selective IgA deficiency. Fisher *et. al.* (4) found a different pattern of immuno-deficiency in a patient with biotinidase deficiency. These reports imply that patients with biotinidase deficiency are affected on both cellular and humoral immunity. Biotin is used as a hair-conditioning and a skin-conditioning agent in many cosmetic products at concentrations ranging from 0.0001% to 0.6%, Fiume reported that biotin is safe as used in cosmetic formulations based on toxicological data.(5)

Nitric oxide(NO) is an important cellular mediator produced in keratinocytes and macrophages from arginine by nitric oxide synthase(NOS) during inflammatory reactions in the skin.(6) During inflammation, NO mediates cytotoxicity and nonspecific host defense. In addition, NO regulates blood flow to the injured tissue. Excessive production of NO can lead to edema, prolonged inflammation, and injury by promoting the infiltration of macrophages and lymphocytes into the tissue.(7) Keratinocyte-derived NO may play an important role in inflammation and in controlling microbial invasion and wound healing in the skin.(8) Many studies have suggested that a reduction of NOS activity and subsequent NO production might be beneficial in abrogating inflammation. In this regards, NO inhibitors have proven to be effective in reducing tissue damage in several inflammatory disease models.(7,9)

The activation of phospholipase A_2 induces the mobilization of fatty acids from the membrane lipid pool, in particular arachidonic acid, for the synthesis of lipidic mediators at the site of cellular damage. Arachidonic acid is metabolized by two major pathways to pro-inflammatory mediators: the cyclooxygenase pathway and the 5-Lipoxygenase pathway.(10, 11) (Fig. 1) Prostaglandins are pro-inflammatory mediators resulting from metabolic degradation of arachidonic acid originating from membrane phospholipid. The most important products of enzyme cyclooxygenation of arachidonic acid are prostaglandin D2, E2, F2a, tromboxane A2 and prostacyclin.(12) The first step in the 5-LOX cascade consists of activation of the enzyme by 5-LOX-activating protein (FLAP), which leads to the formation of the leukotriens : 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-hydroxyeicosatetraenoic acid (5-HETE) (13). J. Baumann, *et. al.* showed some flavonoids were predominant inhibitors of either cyclooxygenase or lipoxygenase and proved to be a good tool for the elucidation of the arachidonic acid metabolism.(14)

In the present studies, we suggested that biotin has immunological and dermatological solutions and investigated anti-inflammation effect of biotin. To investigate anti-inflammation effect, some plant extracts were tested : kudzu vine extract, sage extract, paeonia extract, and dipotassium glycyrrhetinate which are known as anti-inflammatory agents. Test methods for the determination of anti-inflammation effect are as followed : the measurement of NO & PGE_2 concentration, inhibition rates of 5-lipoxygenase and MTT assay for the rate of cell viability.

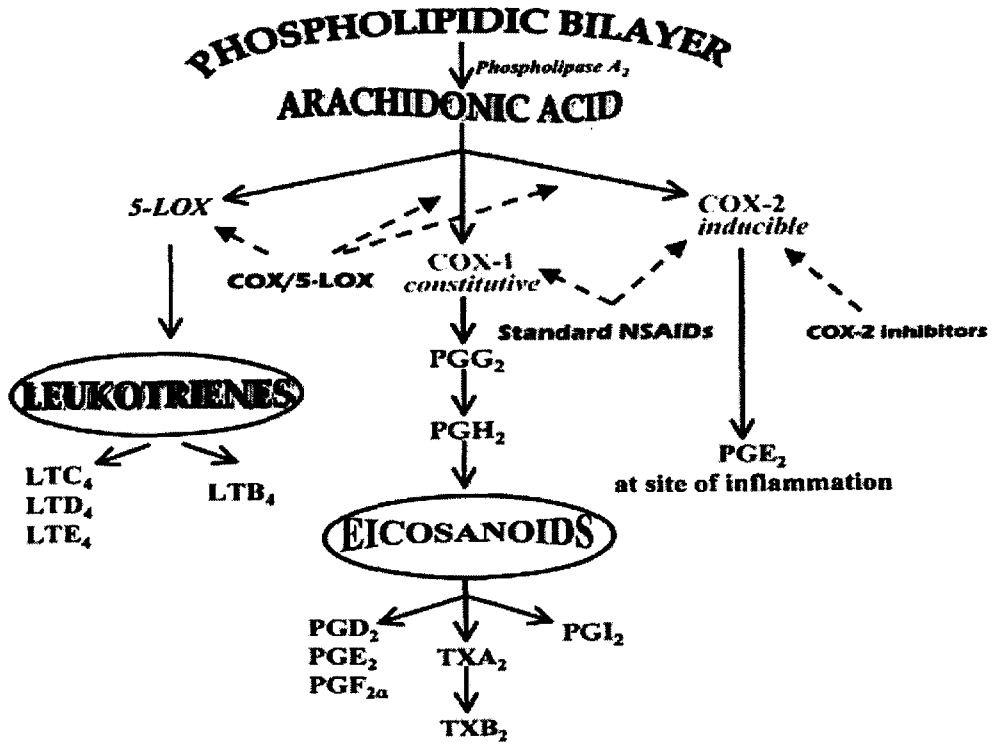

Fig. 1. Schematic pathways of the arachidonic cascade. Solid lines represent metabolite production; dotted lines represent inhibition.

Materials and Methods

1. Nitrite assay

NO from macrophages was assessed by determining the NO^{2-} concentration in the culture supernatant. Samples (100 ul) of culture media were incubated with 150 ul of Griess reagent (1% sulfanilamide, o.1% naphthylethylenediamine in 2.5% phosphoric acid solution) at room temperature for 10 min in 96-well microplate (1). Absorbances at 540nm were read using an ELISA plate reader. Standard calibration curves were prepared using sodium nitrite as a standard. (15)

2. Determination of PGE_2

Spent media was removed at the indicated time and the accumulated levels of PGE_2 in the media were determined using enzyme immunoassay kit from Cayman chemical(Ann Arbor, MI,

U.S.A.). After incubation for 10 min at 37℃, media was removed and subjected to enzyme immunoassay for the measurement of PGE_2 (16)

3. Lipoxygenation of arachidonic acid

Biotin and other anti-inflammatory materials were tested against the lipoxygenation of arachidonic acid by soybean lipoxygenase. Incubations were carried out at 25℃ for 10 min in a mixture containing 50 mM sodium phophate buffer pH 7.0, 0.44 mM arachidonic acid, 25㎍ soybean lipoxygenase (4000 I.U.) and test drug in a total volume of 1.5 ml. The reaction was stopped by the addition 1 ml of 20 % (w/v) trichloroacetic acid and 2 ml of 0.67 % (w/v) thiobarbituric acid were added. The mixture was gently boiled for 10 min, cooled to room temperature and centrifuged ag 4000 g for 5 min. The absorbance at 535 nm of the supernatant was measured. All samples were tested as triplicates against buffer, blanks of acidified lipoxygenase were substracted. (14) All reagents were obtained from Sigma.

4. MTT assay

HaCaT cells were seeded at $7.0x10^3$ and samples were added in 96 well plate. After incubation for 2 days (37℃, 5% CO_2), MTT(3-(4,5 dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide) were added. After more incubated for 4hrs, centrifugated and removed supernatants and then added DMSO (Dimethyl Sulfoxide). The optical density (at 540 nm) was measured by microplate reader.

Results and Discussion

1. The decrease effect of nitric oxide

We measured the concentration of nitric oxide, Table Ⅰ, Fig.2 show the results. To examine the decreasing effect by each materials, Lipopolysaccharide(LPS) was used as a stimulating agent which induce nitric oxide from normal cells. First of all, biotin shows noticeable decreasing effect of nitric oxide depends on the concentrations, particularly at 0.05%, it decreased from 27.48 μM to 2.29 μM. Because biotin is slightly soluble in water, 0.05% was maximum testing concentration. Also, kudzu vine extract shows similar effect, however considering testing concentrations difference, it was relatively low effect on NOS inhibition. The case of other plant extracts have no effect, even at high concentration.

		NO (μM)	PGE_2 (ng/mg protein)
control		1.19	4.9
LPS(10㎍/ml)		27.48	31.12
paeonia ext	0.10%	29.98	25.32
	0.50%	29.43	16.21
	1.00%	26.68	9.26
kudzu vine ext	0.10%	26.28	17.94
	0.50%	22.13	13.04
	1.00%	15.34	7.44
sage ext	0.10%	27.88	27.6
	0.50%	25.23	27.17
	1.00%	23.28	23.33
Biotin	0.001%	26.48	29.36
	0.01%	16.69	25.63
	0.05%	2.29	14.39
DPG-K2	0.01%	29.53	26.46
	0.10%	32.38	27.2
	0.50%	37.43	22.4

Table Ⅰ. Decrease effects of NO and PGE2 by biotin and plant extracts. paeonia ext, kudzu vine ext, sage ext : %(v/v), and Biotin, DPG-K2 : %(w/v). Raw 264.7 cell, LPS 10㎍/ml, 24hrs incubation

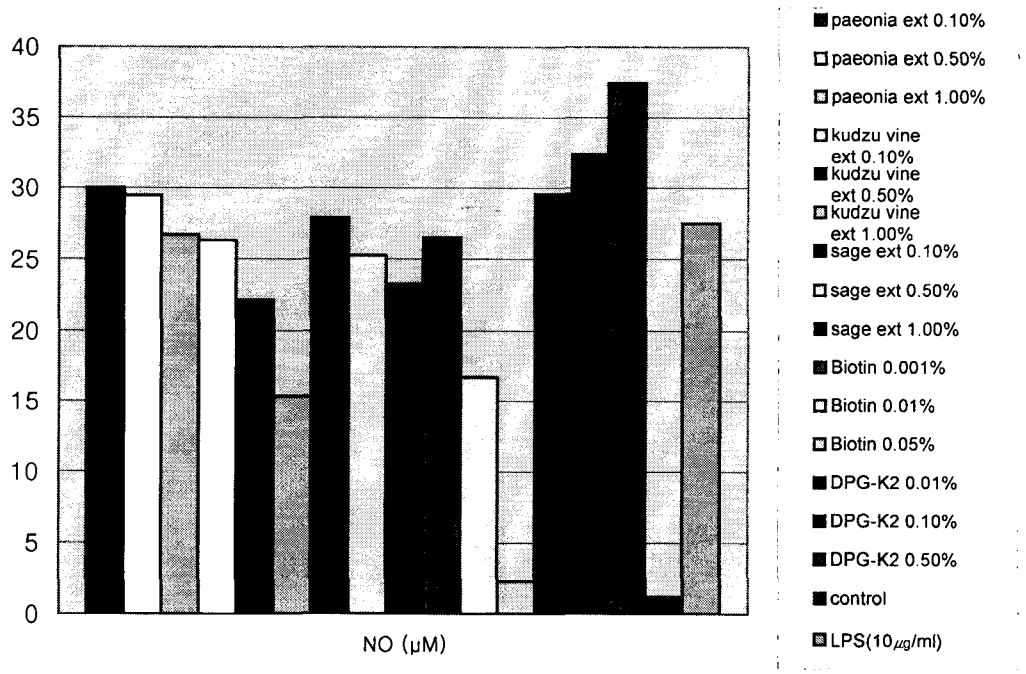

Fig. 2. The concentrations of nitric oxide by biotin and plant extracts

2. The results of PGE_2 measurement

The levels of PGE_2 were determined using enzyme immunoassay kit, Table Ⅰ, Fig.3 show the results. Among tested samples, kudzu vine extract was showed excellent efficacy through tested each concentrations, particularly the result of 1.0% approach almost control level. At high concentration, not only kudzu vine extract but also paeonia extract showed similar test data. Biotin has relatively low effect compared with above two extracts, but it could be significant result considering testing concentrations different. Sage extract and DPG-K_2 did not have effect on the inhibition of PGE_2 biosynthesis.

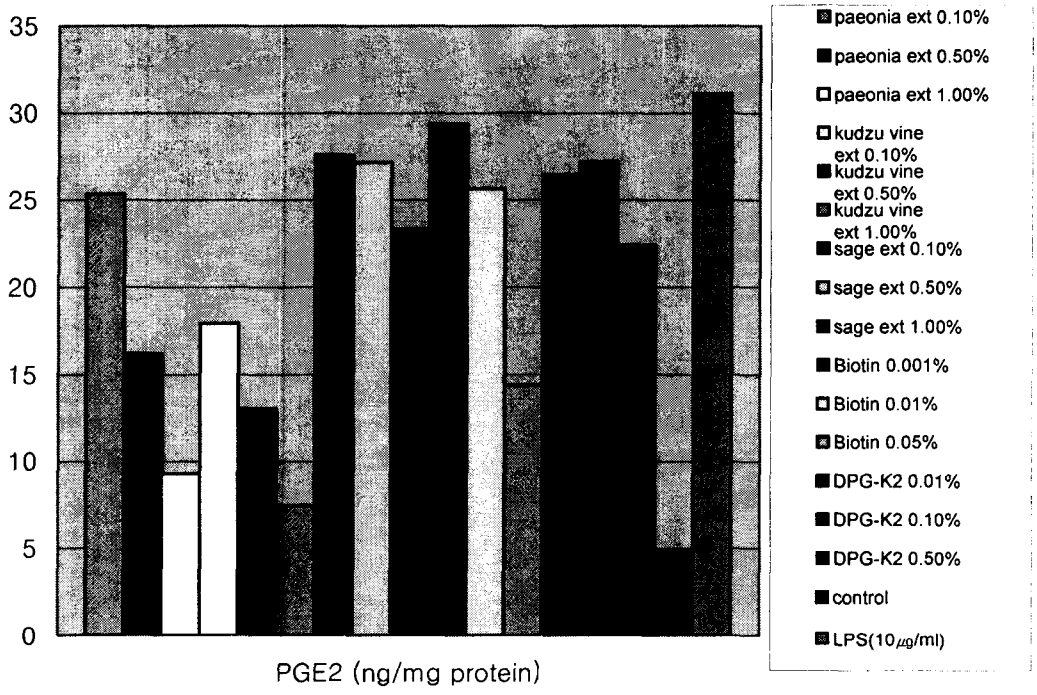

Fig. 3. The concentrations of PGE_2 by biotin and plant extracts

3. Inhibition of Lipoxygenase

We tested the effect of all our materials on the lipoxygenation of arachidonic acid by soybean lipoxygenase, Table Ⅱ shows the results. Each % rate was calculated as follows :

$$\text{Lipoxygenase \% inhibition} = \frac{(OD_{exp.} - OD_{control})}{(OD_{std.} - OD_{std.\ control})} \times 100$$

Even though most samples showed the effect of Lipoxygenase inhibition, it was not in proportion concentrations. Paeonia ext inhibited lipoxygenation of arachidonic acid predorminantly, the best

result was not at 5% but at 1%. Biotin showed no effect at 0.01%, but 33% inhibition rate at 0.05% which means it will be better LOX inhibitor if the solubility can increase. Though kudzu vine extract and sage extract showed good results at 1% and DPG-K_2, at 0.11%, those results was not significant and consistent.

		Lipoxygenase % inhibition
paeonia ext	0.10%	20.4
	1.00%	48.7
	5.00%	45.7
kudzu vine ext	0.10%	16.8
	1.00%	30.2
	5.00%	3.12
sage ext	0.10%	-21.2
	1.00%	22.6
	5.00%	15.6
Biotin	0.005%	-6.2
	0.01%	1.7
	0.05%	33.4
DPG-K2	0.05%	-14.9
	0.10%	38.7
	0.50%	-88.4

Table Ⅱ. Inhibition rate of lipoxygenase by biotin and plant extracts.

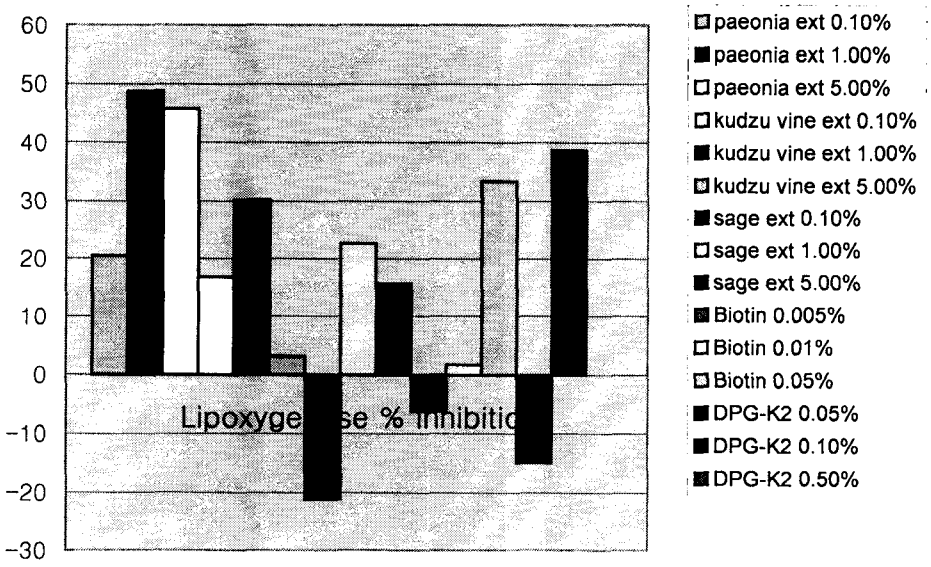

Fig. 4. Inhibition rate of lipoxygenase by biotin and plant extracts.

4. The results of MTT assay

MTT assay was used to examine cell viability, three types of treating samples to HaCaT cell were prepared : control, LPS added and LPS + each materials, TableⅢ and Fig. 5 represent the results. Among five materials, biotin showed remarkable cell viability increase effects which means biotin has low toxicity and anti-inflammation effect. From 0.01% concentration of biotin, the result was over the control level. Beside, kudzu vine extract and sage extract showed good results, about 90% level of cell viability.

		Rate of cell viability (%)
control		100.0
LPS(10㎍/ml)		42.5
LPS + paeonia ext	0.10%	57.8
	0.50%	44.5
	1.00%	34.5
LPS + kudzu vine ext	0.10%	62.5
	0.50%	96.1
	1.00%	96.4
LPS + sage ext	0.10%	54.5
	0.50%	83.5
	1.00%	77.4
LPS + Biotin	0.001%	88.9
	0.01%	123.0
	0.05%	115.4
LPS + DPG-K_2	0.01%	65.8
	0.10%	57.4
	0.50%	41.6

TableⅢ. Rate of cell viability(%) by biotin and plant extracts.

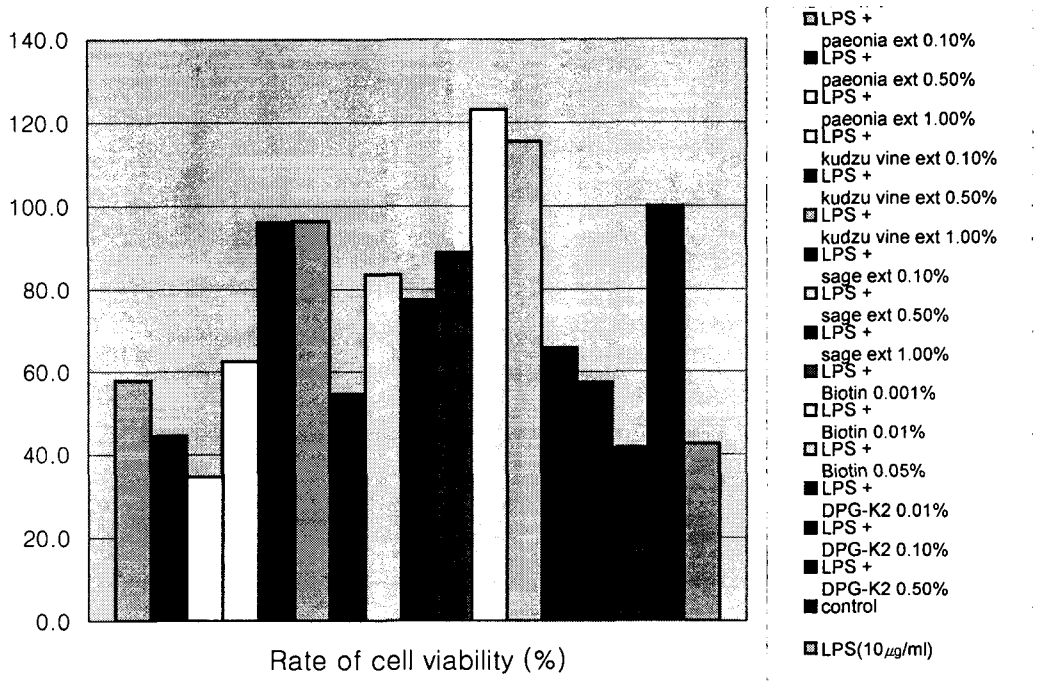

Fig.5. Rate of cell viability(%) by biotin and plant extracts.

Conclusion

We suggested biotin has anti-inflammatory effect considering immunological and dermatological factors, so the effects were investigated by measurement of nitric oxide, prostaglandin E_2, lipoxygenase inhibition rate and cell viability. The other vegetable origin samples were prepared to compare the effect which were kudzu vine extract, sage extract, paeonia extract, and dipotassium glycyrrhetinate and are known as anti-inflammation agent. From the experiments, we confirmed biotin has excellent anti-inflammation agent. NO can cause apoptosis of keratinocytes and may play an important role in inflammation and in controlling microbial invasion and wound healing in the skin, biotin showed noticeable decrease effect of nitric oxide. Related with peroxidation of arachidonic acid, concentrations of PGE_2 and lipoxygenase inhibition rates were measured, LOX was inhibited by biotin significantly, but decrease effect of PGE_2 was average level. Even increase effect of cell viability by biotin through MTT assay was so remarkable which means biotin has improvement effect of toxicity and inflammation. Compared with biotin, plant extracts were tested with same methods. Kudzu vine extract showed good effects on decrease of PGE_2 and increase effect of cell viability, paeonia extract, on decrease of PGE_2 and increase effect of lipoxygenase inhibition. Sage extract and DPG-K_2 did not represent any particular anti-inflammatory effects.

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