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Anti-inflammatory Potential of *Rikkosan* Based on IL-1β Network Through Macrophages to Oral Tissue Cells

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Abstract. *Rikkosan is a traditional Kampo medicine using the control of oral pain caused by dental caries, pulpitis, periodontitis and stomatitis. In order to provide evidence for its clinical effects, we herein investigated whether Rikkosan inhibits the production of pro-inflammatory substances in human and mouse models of inflammation. Rikkosan alone did not induce prostaglandin E_2 (PGE_2) production, but inhibited interleukin-1β (IL-1β) (5 ng/ml)-stimulated PGE_2 production in human gingival fibroblasts and human periodontal ligament fibroblasts, with a selectivity index higher than 4.0 and 4.3, respectively. Rikkosan alone dose-dependently stimulated tumor necrosis factor-α (TNF-α) production, reaching a peak level slightly lower than that attained by lipopolysaccharide (LPS) at 0.4 mg/ml in mouse macrophage-like RAW264.7 cells. At a higher concentration of Rikkosan (4 mg/ml), TNF-α production, however, declined significantly regardless of the presence or absence of LPS. Rikkosan dose-dependently inhibited IL-1β production by LPS-stimulated RAW264.7 cells, with a selective index of 7.6. Five constituent extracts of Rikkosan, either alone or in combination, showed similar effects on TNF-α and IL-1β productions in activated RAW264.7 cells, but to lower extents than that of Rikkosan. These results demonstrated that Rikkosan inhibited both IL-1β production by LPS-activated macrophages and PGE_2 production by IL-1β-stimulated human gingival fibroblasts and human periodontal ligament fibroblasts, suggesting that anti-inflammatory effects of Rikkosan may partially be generated by the inhibition of these pro-inflammatory substances via the IL-1β network through macrophages to oral tissue cells.*

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Rikkosan is Kampo medicine (Japanese traditional medicines) prescribed for the alleviation of oral pain generated by dental caries, pulpitis, periodontitis, stomatitis and tooth extraction. *Rikkosan* is a cinnamon color powder with slightly sweet and bitter taste, and is composed of five major constituent herb extracts, *Kanzo*, *Shoma*, *Ryutan*, *Saishin* and *Bofu* (Table I) (1). We previously investigated the mechanism of anti-inflammatory effects of *Rikkosan* using mouse macrophage-like RAW264.7 cells (1-3). In the arachidonic acid (AA) cascade, two enzymes are involved in the production of prostaglandin (PG) E_2. PG synthesis begins with the liberation of AA, the prime precursor, from membrane phospholipids by phospholipase (PL) A_2. Subsequently, cyclooxygenase (COX) catalyzes the rate-limiting reactions for PG synthesis comprising the bis-cyclooxygenation of AA to form PGG_2 and the peroxidative reduction of PGG_2 to PGH_2 (4). Cytoplasmic $PLA_2α$ is activated by pro-inflammatory cytokines or growth factors and catalyzes AA from the cell surface membrane (5, 6). COX has two isoforms, COX-1 (constitutive isoform) and COX-2 (inducible isoform) (7). We reported that low concentrations (0.04-0.4 mg/ml) of *Rikkosan* stimulated PGE_2 production, whereas a higher concentration (4.0 mg/ml) *Rikkosan* inhibited lipopolysaccharide (LPS)-stimulated PGE_2 production, suggesting the presence of both stimulator(s) and inhibitor(s) of PGE_2 production in *Rikkosan* (2). Western blot analysis demonstrated that *Rikkosan* and all its constituent herb extracts enhanced the intracellular concentration of COX-2 protein, whereas *Rikkosan* did not significantly affect the expression of PLA_2 protein (2, 3). *Rikkosan* dose-dependently inhibited the COX-2-catalyzed PGE_2 production and a higher concentration was required to inhibit the COX-1-catalyzed PG production (3).

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Table I. *Five ingredients of Rikkosan (RKS) and their major components.*

Constituent herb extract	Major components	Content*
Kanzo	Glycyrrhizin, glabric acid, liquiritin, licoricone, licoflavone	1.5
Shoma	Cimigenol, dahurinol, acerinol, β-sitosterol, cimicifugoside	2.0
Ryutan	Gentiopicroside, trifloroside, swertiamarin, gentisin	1.0
Saishin	β-Pinene, eucarvone, 1,8-cineol, *l*-asarinin, higenamine	2.0
Bofu	Deltoin, bergapten, psoralen, hamaudol, cimifugin	2.0

*RKS (7.5g) contains the 1.5g of dried extract composed of five constituent herb extract at the indicated ratio (w/w).

Periodontitis is defined as the inflammation of periodontal supporting tissue caused by specific pathogenic microorganisms, resulting in advanced inflammatory infiltration, the destruction of the gingival connective tissue and the periodontal ligament, and alveolar bone loss (5). LPS derived from an oral bacterium such as *Porphyromonas gingivalis* stimulates not only macrophages, but also human gingival fibroblasts to produce pro-inflammatory cytokines, such as interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α (TNF-α) and these cytokines damage the periodontal tissue (8-10). PGE_2, likely to be involved in the pathogenesis of periodontal disease, is produced upon stimulation with these cytokines by gingival fibroblasts and smooth muscle cells (11). Among these cytokines, IL-1β was clearly associated with the severity of periodontitis and thereby the level of IL-1β is considered to be a useful diagnostic marker of periodontal diseases (12, 13). Using cultured human gingival fibroblasts (HGFs) and human periodontal ligament fibroblasts (HPLFs), we found that stimulation of these cells by IL-1β produced higher amounts of PGE_2 than when induced by LPS (14, 15). We have reported the anti-inflammatory effect of various herbal medicines such as *Sasa senanensis* Rehder extract on HGFs and HPLFs stimulated with IL-1β (15). There have been no experimental reports to examine the anti-inflammatory effect of *Rikkosan* on gingivitis and periodontitis. In order to elucidate the possible preventive or therapeutic effect of *Rikkosan* on inflammatory diseases, we investigated whether *Rikkosan* inhibits PGE_2 production by IL-1β-stimulated HGFs and HPLFs, and TNF-α and IL-1β production by LPS-stimulated RAW264.7 cells.

Materials and Methods

Materials. Rikkosan (Lot No. 2990110010) was obtained from Tsumura Corp., Tokyo, Japan. The five ingredients of *Rikkosan* and their major components are listed in Table I. The following chemicals and reagents were obtained from the indicated companies. Dulbecco's modified Eagle's medium (DMEM), phenol red-free DMEM: Invitrogen Corp, Carlsbad, CA, USA; fetal bovine serum (FBS): Gemini Bio-Products, Woodland, CA, USA; dimethyl sulfoxide (DMSO): Wako Pure Chem. Ind., Osaka, Japan; *Escherichia coli* LPS (Serotype 0111:B4) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT): Sigma Chem. Ind., St. Louis, MO, USA; PGE_2 Express enzyme immunoassay (EIA) Kit: Cayman Chemical Co, Ann Arebor, MI, USA; and Mouse IL-1β EIA Kit, TNF-α EIA Kit: R&D Systems, Minneapolis, MN, USA. Culture plastic dishes, 6-well, 24-well and 96-mircowell plates were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

Assay for viable cell number. HGFs and HPLFs were established from the periodontal tissues of the first premolar extracted from a mandible, as described previously (16, 17). The life span of HGFs and HPLFs was about 40 population doubling level (PDL), and the cells at 10-20 PDL were used in the present study. HGFs and HPLFs were cultured in DMEM supplemented with 10 % heat-inactivated FBS, 100 units/ml penicillin G and 100 μg/ml streptomycin sulfate under a humidified atmosphere with 5% CO_2. HGFs and HPLFs were inoculated at 1:3 split ratio in 96-microwell plates, and incubated for 48 h to achieve complete adherence of the cells. Near confluent HGFs and HPLFs were cultured for 24 h in fresh medium containing different concentrations of *Rikkosan*. The relative viable cell number of the attached cells was determined by MTT method. In brief, the cells were incubated for 1 hour with 0.2 mg/ml MTT. The cells were dissolved in 0.1 ml DMSO and the absorbance at 540 nm of the cell lysate was determined. From the dose-response curve, the 50% cytotoxic concentration (CC_{50}) was calculated.

Measurement of PGE_2 production. HGFs and HPLFs were inoculated at 1:3 split ratio in 24-well plates, and incubated for 48 h to achieve complete adherence of the cells. These cells were incubated for 24 h in fresh culture medium containing different concentrations of *Rikkosan* in the presence or absence of IL-1β (5 ng/ml). The culture supernatant was collected by centrifugation, and the PGE_2 concentration determined by EIA kit. The limit of quantitation of PGE_2 was 15 pg/ml. The 50% inhibitory concentration (IC_{50}) for the inhibition of PGE_2 production was calculated and the selective index (SI) was determined by the following equation: $SI=CC_{50}/IC_{50}$ (18).

Assay for TNF-α and IL-1β production. RAW264.7 cells (3×10^4 cells/well) were inoculated on 96-microwell plate, and incubated for 24 h to achieve complete adherence of the cells. The cells were incubated in fresh phenol red-free DMEM medium supplemented with 10% FBS containing different concentrations of *Rikkosan* or its components in the presence or absence of LPS (100 ng/ml). For the determination of IL-1β, LPS (1.0 and 10 μg/ml) was used as stimulator. The culture supernatant was collected by centrifugation, and TNF-α and IL-1β concentrations were determined according to the manufacturer's recommended procedures (1). The limit of

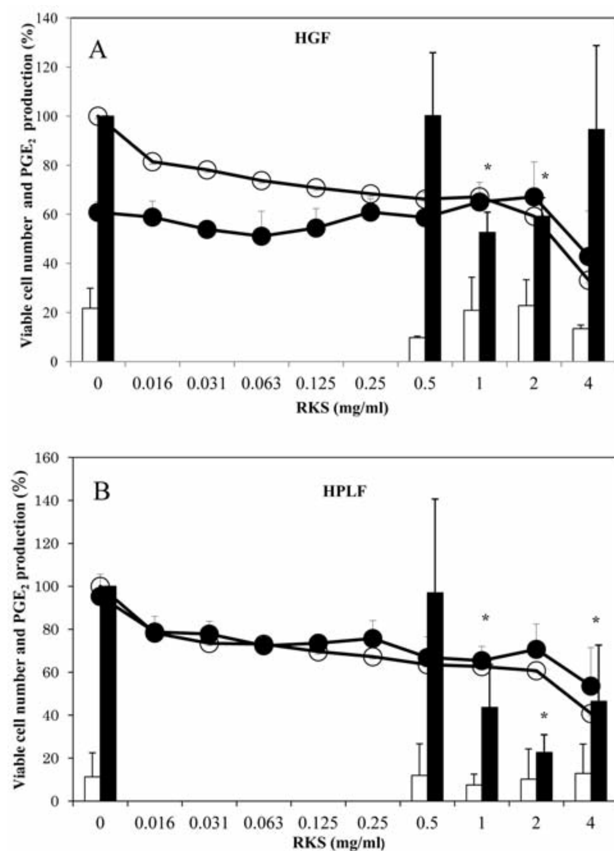

Figure 1. *Effect of Rikkosan (RKS) on the viable cell number and prostaglandin E_2 (PGE_2) production by interleukin-1β (IL-1β)-stimulated and unstimulated human gingival (HGFs) and periodontal ligament (HPLFs) fibroblasts. Near confluent HGFs (A), and HPLFs (B) were incubated for 24 h with 0.1 ml of fresh culture medium (in 96-microwell) containing the indicated concentrations of RKS in the presence (closed symbols) or absence (open symbols) of 5 ng/ml IL-1β. The viable cell number (circles) and extracellular PGE_2 concentration (bars) as a percentage of the control (without RKS) were determined. Each value represents the mean±SD of three independent experiments. *Statistically significant at a probability of p<0.05 versus IL-1β without RKS.*

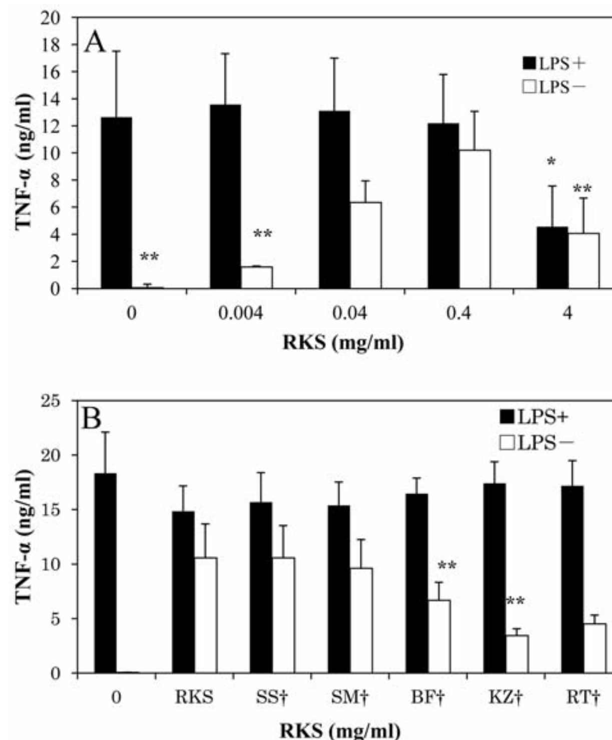

Figure 2. *Effect of Rikkosan (RKS) and its five constituent herb extracts on tumor necrosis factor-α (TNF-α) production by lipopolysaccharide (LPS)-stimulated and unstimulated RAW264.7 cells. RAW264.7 cells were incubated for 24 h without (LPS−) or with (LPS+) 100 ng/ml LPS in the presence of the indicated concentrations of RKS (A) or 0.4 mg/ml each of its five constituent herb extracts (B), and the TNF-α concentration in the medium was determined. Each value represents the mean±SD of three independent experiments. Statistically significant at a probability p<0.05: *versus with LPS without RKS, **versus without LPS with 0.4 mg/ml of RKS). †Adjusted to the concentration present in 0.4 mg/ml of RKS.*

quantitation of these cytokines was 4.8 pg/ml. We confirmed that the culture medium did not contain detectable amounts of IL-1β and TNF-α, and *Rikkosan* did not affect the colorimetric reaction of EIA for the determination of IL-1β and TNF-α (data not shown).

Statistical analysis. The mean values and standard deviations were calculated. One-way analysis of variance was performed to detect significant effects of the variables. Bonferroni's multiple comparison test was used at a *p*-value of 0.05.

Results

Effect on growth and PGE_2 production in human oral normal cells. *Rikkosan* was weakly cytotoxic against both HGFs and HPLFs with and without IL-1β (5 ng/ml) stimulation (HGF: CC_{50}>4 mg/ml without IL-1β, CC_{50}>4 mg/ml with IL1-β; HLPF, CC_{50}=3.078 mg/ml without IL1-β, CC_{50}>4 mg/ml with IL1-β) (Figure 1, Table II). When HGFs and HPLFs were stimulated with IL-1β, PGE_2 production and release into the culture medium increased from 0.2 to 1.3 ng/ml, and 0.2 to 3.7 ng/ml, respectively (data not shown). *Rikkosan* alone did not induce significant PGE_2 production in both HGFs and HPLFs cells. *Rikkosan* inhibited the PGE_2 production by IL-1β-stimulated HGFs most efficiently at 1 mg/ml, but the effect was less at higher concentrations (2-4 mg/ml) (Figure 1A). Similarly, *Rikkosan* inhibited PGE_2 production by IL-1β-stimulated HPLFs most efficiently at 2 mg/ml, the effect decreasing at higher concentration (4 mg/ml) (Figure 1B). The SI was >4.0 for HGF and >4.3 for HPLFs (Table II).

Effect on TNF-α production on activated mouse macrophages. Addition of *Rikkosan* dose-dependently stimulated the production of TNF-α, reaching a peak level

Table II. *Inhibition of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) production by Rikkosan in human gingival (HGF) and periodontal ligament (HPLF) fibroblasts, cells and mouse macrophage-like cells (RAW264.7).*

Cell	IL-1β	CC_{50} (mg/ml)	IC_{50} (mg/ml)	$SI=CC_{50}/IC_{50}$
HGF	With	>4	0.99 (PGE_2 production)	>4.0
	Without	>4		
HPLF	With	>4	0.94 (PGE_2 production)	>4.3
	Without	3.078		
RAW264.7	With	3.915	0.518 (IL-1β production)	7.6
	Without	>4		

The 50% cytotoxic concentration (CC_{50}) values against RAW264.7 cells are cited from our previous report (1). IC_{50}, the concentration that inhibited the PGE_2 or IL-1β by 50%.

slightly lower than that attained by LPS at 0.4 mg/ml in mouse macrophage-like RAW264.7 cells. At a higher concentration of *Rikkosan* (4 mg/ml), TNF-α production, however, declined significantly regardless of the presence or absence of LPS (Figure 2A). Similarly, *Rikkosan* stimulated PGE_2 production, reaching a peak level comparable with that attained by LPS, while a higher concentration of *Rikkosan* inhibited PGE_2 production in RAW264.7 cells (2).

In order to investigate the effect of the constituent herb extracts of *Rikkosan*, the concentration was set to reflect the concentration present in 0.4 mg/ml of *Rikkosan*. *Saishin* and *Shoma*, which stimulated TNF-α production to a comparable extent with that attained by *Rikkosan*, and inhibited LPS-stimulated TNF-α production to almost the same level with *Rikkosan* (Figure 2B). On the other hand, *Bofu*, *Ryutan* and *Kanzo* that stimulated the TNF-α production much less, did not affect LPS-stimulated TNF-α production (Figure 2B).

Effect on IL-1β production in activated macrophages. LPS (0.1-10 μg/ml) dose-dependently stimulated the IL-1β production in RAW264.7 cells (Figure 3A). Unstimulated RAW264.7 cells did not produce the detectable amount of IL-1β, regardless of the concentration of *Rikkosan* (data not shown). *Rikkosan* dose-dependently inhibited the production of IL-1β in LPS (10 μg/ml)-stimulated RAW264.7 cells (CC_{50}=3.915 mg/ml, IC_{50}=0.518 mg/ml, SI=7.6) (Figure 3B, Table II). Among the five constituent herb extracts of *Rikkosan*, *Kanzo* caused the greatest inhibition, followed by *Shoma*, *Saishin*, *Bofu* and *Ryutan* (Figure 3C). Among combinations of the four constitutive herb extracts, that of *Saishin*, *Shoma*, *Bofu* and *Kanzo* inhibited IL-1β production most potently, followed by that of Saishan, *Bofu*, *Kanzo* and *Ryutan*, then *Saishin*, *Shoma*, *Kanzo* and *Ryutan*; *Shoma*, *Bofu*, *Kanzo* and *Ryutan*; then *Saishin*, *Shoma*, *Bofu* and *Ryutan* (Figure 3C). The inhibitory potency of the combination of *Kanzo* with *Shoma* and *Saishin* was comparable with that of *Rikkosan*, whereas that of *Bofu* with *Ryutan* was much less (Figure 4).

Discussion

Recent reports have suggested the possible efficacy of natural products including herbal medicines for periodontal disease (8, 19). Chitosan, cranberry, *Scutellaria baicalensis*, *shosaikoto*, *orento* and *Sasa senanensis* Rehder have been investigated for their anti-inflammatory activity against HGF (15, 20-24). The present study demonstrated that relatively low concentrations (1.0-2.0 mg/ml) of *Rikkosan* inhibited IL-1β-stimulated PGE_2 production in HGFs and HPLFs significantly, and more potently than at 4 mg/ml, suggesting the presence of both stimulator(s) and inhibitor(s) of PGE_2 production in *Rikkosan*. The concentrations of *Rikkosan* needed for maximum inhibition of PGE_2 production were different between HGFs and HPLFs (1 and 2 mg/ml, respectively), possibly reflecting the differences in fibroblast cell types (25). The ability of *Rikkosan* to inhibit PGE_2 production in IL-1β-stimulated HGFs and HPLFs further supports its therapeutic potential against oral diseases such as periodontitis and oral mucositis (20, 26). Since the SIs of RKS for both HGFs and HPLFs, however, were relatively low, it is important to determine the most appropriate concentrations of *Rikkosan* for its clinical application.

The present study demonstrated that *Rikkosan* alone did not induce production of IL-1β in RAW264.7 cells, and inhibited IL-1β production in LPS-stimulated RAW264.7 cells. On the other hand, *Rikkosan* stimulated the production of TNF-α to a level comparable to that attained by LPS in RAW264.7 cells, and inhibited the stimulation of TNF-α production only at the highest concentration (4 mg/ml). This finding suggests that the mechanism of induction of IL-1β and that of TNF-α differ. Yucel-Lindberg *et al*. reported that both IL-1β and TNF-α stimulated HGFs to produce the PGE_2, but that HGFs stimulated with IL-1β produced higher amounts of PGE_2 than those stimulated with TNF-α (14). The inhibition of IL-1β in HGFs and HPLFs may thus be a crucial part of the anti-inflammatory effect of *Rikkosan*.

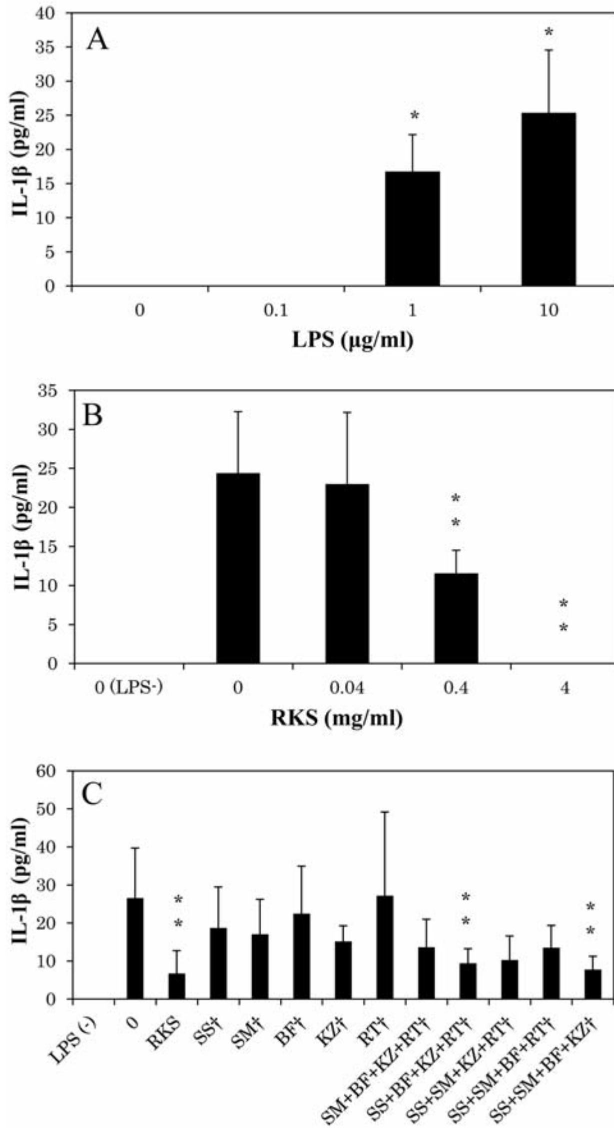

Figure 3. *Effect on interleukin-1β (IL-1β) production by lipopolysaccharide (LPS)-stimulated and unstimulated RAW264.7 cells. RAW264.7 cells were incubated for 24 h without (control) or with the indicated concentrations of LPS (A), Rikkosan (RKS) with 10 μg/ml LPS (B), or each constituent herb extract of RKS† or their combination† (C), and the IL-1β concentration in the medium was determined. Each value represents the mean±SD from three independent experiments. Statistically significant at a probability of $p<0.05$ (*versus without LPS, **versus LPS+ without RKS). †Adjusted to the concentration present in 0.4 mg/ml of RKS.*

The studies of five constituent herb extracts of *Rikkosan* demonstrated that the inhibition of LPS-stimulated IL-1β production in RAW264.7 cells varied considerably between the herb samples. The combination of *Kanzo*, *Shoma* and *Saishin* inhibited LPS-stimulated IL-1β production to an extent comparable to that of *Rikkosan*

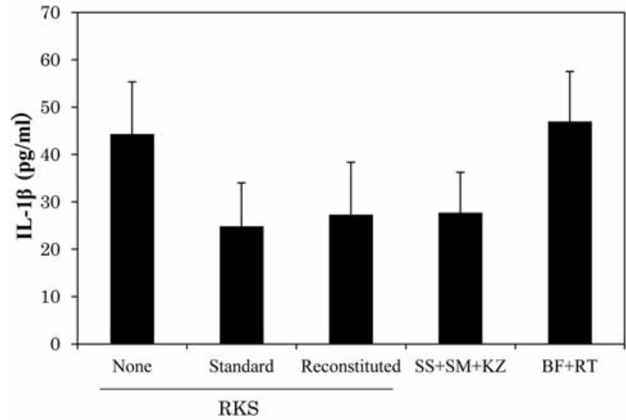

Figure 4. *Effect of Rikkosan (RKS) and the combination of components of RKS on interleukin-1β (IL-1β) production by lipopolysaccharide (LPS)-stimulated and unstimulated RAW264.7 cells. RAW264.7 cells were incubated for 24 h without or with 10 μg/ml LPS in the presence of the indicated concentrations of RKS or the combination of its consituent herb extracts, and the IL-1β concentration in the medium was determined. Each value represents the mean±SD from three independent experiments. RKS (Standard): 0.4 mg/ml RKS; RKS (Reconstituted): 0.4 mg/ml RKS reconstituted by mixing each of the five constituent herb extracts at their concentration present in 0.4 mg/ml of RKS.*

and there was no inhibitory effect of *Bofu* and *Ryutan*. As for TNF-α production, the combination of *Saishin* and *Shoma* led to a relatively strong inhibition of LPS-stimulated TNF-α production but interestingly, *Saishin* and *Shoma* also induced the production of the highest amount of TNF-α. *Saishin* and *Shoma* may have both maximum inhibitory and inducing effects on TNF-α production. These results suggest the presence of both stimulator(s) and inhibitor(s) of cytokine production in the samples of *Saishin* and *Shoma*.

The mechanism of inhibition of IL-1β-stimulated PGE_2 production may be due to the inactivation of NF-κB (27). However, a possible association between the stimulatory effect of IL-1β and the components of *Rikkosan* has not been studied. Glycyrrhizin, one of the major components of *Kanzo*, attenuated TNF-α and IL-1β production by the inhibition of NF-κB, and signal transducer and activator transcription-3 activation, in the lung injury mouse model (28). *Bofu* inhibited TNF-α and IL-1β expression in LPS-stimulated RAW264.7 cells *via* suppression of NF-κB- and mitogen-activated protein kinases-dependent pathways (29). β-Sitosterol, one of the major components of *Shoma*, inhibited the expression of IL-1β and TNF-α by inhibiting the NF-κB pathway in a mouse model of 2,4,6-trinitrobenzene sulfonic acid-induced colitis (30). Further studies are required to elucidate the precise molecular anti-inflammatory mechanisms of *Rikkosan* and its ingredients.

In conclusion, the present study provides evidence for the anti-inflammatory potential of *Rikkosan* against HGFs and HPLFs. *Rikkosan* is known to exert an analgesic activity against oral pain. The therapeutic or preventive effects of *Rikkosan* on oral diseases such as periodontitis and oral mucositis may not be exerted by a simple mechanism, but rather by more complicated mechanisms including analgesic and anti-inflammatory effects. Taken together, anti-inflammatory effects of *Rikkosan* may partially be generated by the inhibition of these pro-inflammatory substances *via* the IL-1β network, through macrophage to oral tissue cells.

References

1 Horie N, Satoh K, Hashimoto K, Shimoyama T, Kaneko T, Kusama K and Sakagami H: Inhibition by *Rikko-san* and its major ingredients of LPS-stimulated nitric oxide production by mouse macrophage-like cells. In Vivo *19*: 165-172, 2005.

2 Horie N, Hashimoto K, Kato T, Shimoyama T, Kaneko T, Kusama K and Sakagami H: Concentration-dependent effect of *Rikko-san* on the prostaglandin E_2 production by mouse macrophage-like cells. In Vivo *20*: 491-497, 2006.

3 Horie N, Hashimoto K, Kato T, Shimoyama T, Kaneko T, Kusama K and Sakagami H: COX-2 as possible target for the inhibition of PGE_2 production by *Rikko-san* in activated macrophage. In Vivo *22*: 333-336, 2008.

4 Odau S, Gabler C, Holder C and Einspanier R: Differential expression of cyclooxygenase 1 and cyclooxygenase 2 in the ovine oviduct. J Endocrinol *191*: 263-274, 2006.

5 Saini R, Marawar P, Shete S, Saini S and Mani A: Dental expression and role in palliative treatment. Ind J Palliat Care *15*: 26-29, 2009.

6 Van Dyke TE and Dave S: Risk factors for periodontitis. J Int Acad Periodontol *7*: 3-7, 2005.

7 Varga J, Domokos A, Barna I, Jankord R, Bagdy G and Zelena D: Lack of vasopressin does not prevent the behavioural and endocrine changes induced by chronic unpredictable stress. Brain Res Bull *84*: 45-52, 2011.

8 Chen D, Nie M, Fan MW and Bian Z: Anti-inflammatory activity of curcumin in macrophages stimulated by lipopolysaccharides from *Porphyromonas gingivalis*. Pharmacology *82*: 264-269, 2008.

9 Sugawara S, Sugiyama A, Nemoto E, Rikiishi H and Takada H: Heterogeneous expression and release of CD14 by human gingival fibroblasts: characterization and CD14-mediated interleukin-8 secretion in response to lipopolysaccharide. Infect Immun *10*: 3043-3049, 1998.

10 Kim J and Amar S: Periodontal disease and systemic conditions: a bidirectional relationship. Odontology *94*: 10-21, 2006.

11 Båge T, Kats A, Lopez BS, Morgan G, Nilsson G, Burt I, Korotkova M, Corbett L, Knox AJ, Pino L, Jakobsson PJ, Modeer T and Yucel-Lindberg T: Expression of prostaglandin E synthases in periodontitis immunolocalization and cellular regulation. Am J Pathol *178*: 1676-1688, 2011.

12 Liu CM, Hou LT, Wong MY and Rossomando EF: Relationships between clinical parameters, interleukin-1β and histopathologic findings of gingival tissue in periodontitis patients. Cytokine *8*: 161-167, 1996.

13 Baldini A, Nota A, Fanti E, Martelli FS, Ottomano C and Lippi G: Association between periodontal disease and interleukin-1β +3953 and vitamin D receptor Taq1 genetic polymorphisms in an Italian caucasian population. Ann Stomatol (Roma) *4*: 191-195, 2013.

14 Yucel-Lindberg T, Nilsson S and Modéer T: Signal transduction pathways involved in the synergistic stimulation of prostaglandin production by interleukin-1β and tumor necrosis factor-α in human gingival fibroblasts. J Dent Res *78*: 61-68, 1999.

15 Ono N, Kantoh K, Ueki J, Shimada A, Wakabayashi H, Matsuta T, Sakagami H, Kumada H, Hamada N, Kitajima M, Oizumi H and Oizumi T: Quest for anti-inflammatory substances using IL-1β-stimulated gingival fibroblasts. In Vivo *25*: 763-768, 2011.

16 Kantoh K, Ono M, Nakamura Y, Nakamura Y, Hashimoto K, Sakagami H and Wakabayashi H: Hormetic and anti-radiation effect of tropolone-related compounds. In Vivo *24*: 843-852, 2010.

17 Ueki J, Shimada A, Sakagami H and Wakabayashi H: Hormetic and UV-protective effects of azulene-related compounds. In Vivo *25*: 41-48, 2011.

18 Suga A, Narita T, Zhou L, Sakagami H, Satoh K and Wakabayashi H: Inhibition of NO production in LPS-stimulated mouse macrophage-like cells by benzo[*b*]cyclohept[*e*][1,4]oxazine and 2-aminotropone derivatives. In Vivo *23*: 691-698, 2009.

19 Park SY, Kim YH, Kim EK, Ryu EY and Lee SJ: Heme oxygenase-1 signals are involved in preferential inhibition of pro-inflammatory cytokine release by surfactin in cells activated with *Porphyromonas gingivalis* lipopolysaccharide. Chem Biol Interact *188*: 437-445, 2010.

20 Arancibia R, Maturana C, Silva D, Tobar N, Tapia C, Salazar JC, Martínez J and Smith PC: Effects of chitosan particles in periodontal pathogens and gingival fibroblasts. J Dent Res *92*: 740-745, 2013.

21 Bodet C, Chandad F and Grenier D: Cranberry components inhibit interleukin-6, interleukin-8, and prostaglandin E production by lipopolysaccharide-activated gingival fibroblasts. Eur J Oral Sci *115*: 64-70, 2007.

22 Chung CP, Park JB and Bae KH: Pharmacological effects of methanolic extract from the root of *Scutellaria baicalensis* and its flavonoids on human gingival fibroblast. Planta Med *61*: 150-153, 1995.

23 Ara T, Maeda Y, Fujinami Y, Imamura Y, Hattori T and Wang PL: Preventive effects of a Kampo medicine, Shosaikoto, on inflammatory responses in LPS-treated human gingival fibroblasts. Biol Pharm Bull *31*: 1141-1144, 2008.

24 Ara T, Honjo K, Fujinami Y, Hattori T, Imamura Y and Wang PL: Preventive effects of a Kampo medicine, *orento* on inflammatory responses in lipopolysaccharide treated human gingival fibroblasts. Biol Pharm Bull *33*: 611-616, 2010.

25 Hormdee D, Nagasawa T, Kiji M, Yashiro R, Kobayashi H, Koshy G, Noguchi K, Nitta H and Ishikawa I: Protein kinase-A-dependent osteoprotegerin production on interleukin-1 stimulation in human gingival fibroblasts is distinct from periodontal ligament fibroblasts. Clin Exp Immunol *142*: 490-497, 2005

26 Watanabe S, Suemaru K, Takechi K, Kaji H, Imai K and Araki H: Oral mucosal adhesive films containing royal jelly accelerate recovery from 5-Fluorouracil-induced oral mucositis. J Pharmacol Sci *121*: 110-118, 2013.

27 Tipton DA, Gay DC and DeCoster VA: Effect of a cyclooxygenase-2 inhibitor on interleukin-1β-stimulated activation of the transcription factor nuclear factor-κB in human gingival fibroblasts. J Periodontol *78*: 542-549, 2007.

28 Menegazzi M, Di Paola R, Mazzon E, Genovese T, Crisafulli C, Dal Bosco M, Zou Z, Suzuki H and Cuzzocrea S: Glycyrrhizin attenuates the development of carrageenan-induced lung injury in mice. Pharmacol Res *58*: 22-31, 2008.

29 Yoon T, Cheon MS, Lee AY, Lee do Y, Moon BC, Chun JM, Choo BK and Kim HK: Anti-inflammatory activity of methylene chloride fraction from Glehnia littoralis extract *via* suppression of NF-κB and mitogen-activated protein kinase activity. J Pharmacol Sci *112*: 46-55, 2010.

30 Lee IA, Kim EJ, and Kim DH: Inhibitory effect of β-sitosterol on TNBS-induced colitis in mice. Planta Med *78*: 896-898, 2012.

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