

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 379 FASE NACIONAL INCLUIDO CAPITULO II
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 5
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JUAN PABLO CADENA SARMIENTO

Asunto: Radicación: 11-114807- -7
Trámite: 11
Evento: 379
Actuación: 519
Oficio: 10402
Folios: 5

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I		Capítulo II	X
No. Solicitud Internacional	PCT/US2010/09670				
Fecha de Presentación Internacional	01/04/2010				

1. Documentos estudiados

Descripción:	Radicado N° 11-114807-00000-0000	06/09/2011
Reivindicaciones:	Radicado N° 11-114807-00000-0000	06/09/2011
	Radicado N° 11-114807-00006-0000	15/04/2013
Prioridad:	US 61/165,685	01/04/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Título de la solicitud: “EFECTOS ANTI- PÉRDIDA DE HUESO Y PÉRDIDA DE ANTI-

ADHERIMIENTO DE UNA COMPOSICIÓN ORAL”

El problema planteado en esta solicitud consiste en la necesidad de reducir el efecto de las metaloproteinasas de matriz en la manifestación clínica de la periodontitis

Para resolver este problema técnico la solicitud en estudio proporciona un método para identificar un compuesto de utilidad para tratar gingivitis o periodontitis

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): G01N33/569

4. De la Solicitud de Patente

Titulo

El título no corresponde con el objeto de la solicitud porque el titulo “EFECTOS ANTI- PÉRDIDA DE HUESO Y PÉRDIDA DE ANTI-ADHERIMIENTO DE UNA COMPOSICIÓN ORAL no define las características técnicas esenciales del método de identificación reclamado en la solicitud, por cuanto los efectos no corresponden a una invención que pueda ser objeto de patente, por lo cual se sugiere al solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

Las reivindicaciones 1 y 3 no son claras cuando mencionan un control positivo sin especificar que tipo de compuesto es utilizado como control positivo. Se requiere definir dicho control positivo.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, el 01 de abril de 2009.

Consultadas las fuentes de información con que se cuenta, se encontró (encontraron) el (los) siguiente(s) documento(s) que anticipa(n) el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1 ^[1]	WO2005103071	“PEPTIDE INHIBITORS OF METALLOPROTEINASE ACTIVITY”	03/Noviembre/2005
D2	Katzung. B.D. Farmacología Básica y Clínica 10ª edición.	“FARMACOLOGÍA BÁSICA Y CLÍNICA”	2007

El documento D1 divulga compuestos inhibidores de MMP (Pág. 6 línea 20 a Pág. 9 línea 11) y divulga el efecto de MMPs en periodontitis (Pág. 2 línea 32)

El documento D2 divulga la metodología utilizada en los procesos de investigación y selección de fármacos (Págs. 66 y 67)

6. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en un método para identificar un compuesto de utilidad para tratar gingivitis o periodontitis (Ver pág. 7 del radicado N° 11-114807-00006-0000)

1[URL:http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2005103071A1&KC=A1&FT=D&ND=3&date=20051103&DB=worldwide.espacenet.com&locale=en_EP

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D1	D2
Método para identificar un compuesto de utilidad para tratar gingivitis o periodontitis, en donde el método comprende: Contactar una primera muestra gingival, obtenida de un mamífero que sufre de gingivitis o periodontitis, con un compuesto de prueba; Contactar una segunda muestra gingival, obtenida de la cavidad oral de dicho mamífero, con un control positivo, en donde dicho control positivo es un compuesto conocido por regular decrecientemente la expresión de uno o mas metaloproteinasas de matriz; Medir el grado al cual la expresión de una o más de dicha metaloproteinasas de matriz es regulada decrecientemente por dicho compuesto de prueba; Medir el grado al cual la expresión de una o más de dicha metaloproteinasas de matriz es regulada decrecientemente por dicho control positivo; y Comparar el grado al cual la expresión de una o mas de dicha metaloproteinasas de matriz es regulada decrecientemente por dicho compuesto de prueba, con la medida del grado al cual la expresión de una o más de dicha metaloproteinasas de matriz es regulada decrecientemente por dicho control positivo; En donde un compuesto de prueba que regula decrecientemente la expresión de una o mas de dichas metaloproteinasas de matriz a un grado igual o mayor que el del control positivo, es un compuesto útil en tratar la gingivitis o la periodontitis.	Compuesto peptídico inhibidor de metaloproteinasas de matriz involucradas en periodontitis (Pág. 2 línea 32, Pág. 6 línea 20 a Pág. 9 línea 11)	Procesos de investigación de fármacos que incluyen una secuencia de experimentación y caracterización llamada selección de fármacos (Pág. 66; Columna de la izquierda) A nivel molecular, el compuesto se debe probar en búsqueda de la afinidad del receptor a las membranas celulares (Pág. 66; Columna de la derecha) Se deben llevar a cabo estudios sobre enzimas para determinar si el fármaco en cuestión es tal vez un sustrato o inhibidor de esas enzimas o interfiere con el metabolismo de otros fármacos (Pág. 66; Columna de la derecha) Se utilizarían tejidos aislados en especial músculo liso vascular para determinar la actividad farmacológica y la selectividad de los compuestos nuevos en comparación con los de referencia. También se compararía con otros fármacos en otras preparaciones in vitro (Pág. 67; Columna de la izquierda)

El documento D1 divulga el efecto de MMPs en periodontitis además de compuestos inhibidores de MMP (Pág. 2 línea 32; Pág. 6 línea 20 a Pág. 9 línea 11) y

La diferencia entre la invención, definida en la reivindicación 1 y la invención definida en el documento D1 consiste en que no divulga específicamente un método para identificar un compuesto de utilidad para tratar gingivitis o periodontitis consistente en contactar muestras gingivales con un compuesto de prueba y con un control positivo y comparar el grado al cual la expresión de una o más metaloproteinasas de matriz es regulada decrecientemente por dichos compuestos.

El documento D2 divulga la metodología utilizada en los procesos de investigación y selección de fármacos (Págs. 66 y 67)

La diferencia entre la invención, definida en la reivindicación 1 y la invención definida en el documento D2 consiste en que no divulga específicamente un método para identificar un compuesto de utilidad para tratar gingivitis o periodontitis

El efecto que logra el contactar muestras gingivales con un compuesto de prueba y con un control positivo y comparar el grado al cual la expresión de una o más metaloproteinasas de matriz es regulada decrecientemente por dichos compuestos es que permite seleccionar que compuestos presentan utilidad como inhibidores de metaloproteinasas de matriz en el tratamiento de gingivitis

o periodontitis.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar la invención conocida en el documento D1 para obtener un método que permita identificar un compuesto de utilidad para tratar gingivitis o periodontitis”.

Sin embargo, el documento D2 divulga el proceso de selección de un fármaco correspondiente a probar los compuestos sobre los receptores o enzimas de interés y compararlos con respecto a los compuestos de referencia mediante pruebas in vitro y puesto que el documento D1 da a conocer compuestos inhibidores de metaloproteinasas de matriz involucradas en desarrollo de periodontitis sin que en la presente solicitud se sustente que el método reclamado permita llegar a la obtención de compuestos que presenten un efecto técnico sorprendente y por lo tanto sean inventivos con respecto a los compuestos conocidos en el estado de la técnica, la persona del oficio normalmente versada en la materia que conoce dichos procesos de selección de un fármaco según el documento D2, fácilmente realizaría pruebas de selección de compuestos inhibidores de metaloproteinasas de matriz como los divulgados en el documento D1 y demás inhibidores de MMPs conocidos en el estado de la técnica^{2,3} por contacto con muestras gingivales y comparación con respecto a compuestos de control positivo como el triclosan de acción en periodontitis ampliamente conocida^{4,5} para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

En conclusión la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 2 – 11 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

En consecuencia las reivindicaciones 1 – 11 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2005103071	1 – 11	Nivel Inventivo
D2	Katzung. B.D. Farmacología Básica y Clínica 10ª edición.	1 – 11	Nivel Inventivo

2 Yoshioka M. et al. Journal of periodontology. 2003; 74 (8) 1219-1224. “Effect of hidroxicamic Acid-Based Matrix Metalloproteinase Inhibitors on Human Gingival Cells and Porphyromonas gingivalis”.

3 Ruwanpura S.M.P.M et al. Journal of Dental Research. 2004; 83 (3) 259 -265. “Prostaglandin E2 regulates Interleukin -1? - induced Matrix Metalloproteinase-3 Production in Human Gingival Fibroblasts.

4 Osaki f. et al Brazilian Oral Research 2006;20(2):172-7 “Efficacy of a herbal toothpaste on patients with established gingivitis – a randomized controlled trial”

5 Davies R.M. Clin Microbiol Infect 2007; 13 (Suppl. 4): 25–29. “The clinical efficacy of triclosan /copolymer and other common therapeutic approaches to periodontal health”

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-06-18

Elaboró: Rodolfo Cadena Amaya
Revisó: Gloria Jacqueline Alfonso Roza

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
CONSULTA DE DOCUMENTOS

Prostaglandin E₂ Regulates Interleukin-1 β -induced Matrix Metalloproteinase-3 Production in Human Gingival Fibroblasts

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J DENT RES 2004 83: 260
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J Dent Res 83(3):260-265, 2004

ABSTRACT

Prostaglandin E₂ (PGE₂) exerts its biological actions *via* EP receptors (EP₁, EP₂, EP₃, and EP₄). In the present study, we investigated whether PGE₂ regulated interleukin (IL)-1 β -induced matrix metalloproteinase (MMP)-3 production in human gingival fibroblasts (HGF) derived from periodontally healthy subjects and diseased patients. In HGF from healthy gingiva, PGE₂ down-regulated IL-1 β -induced MMP-3 production, whereas in HGF from periodontitis patients, PGE₂ enhanced it. Butaprost (an EP₂ agonist) and ONO-AE1-329 (an EP₄ agonist) suppressed IL-1 β -induced MMP-3 production, and 17-phenyl- ω -trinor PGE₂ (an EP₁ agonist) mimicked the PGE₂ effect in HGF from healthy and periodontally diseased tissues, respectively. Analysis of these data suggests that, in HGF from healthy tissue, IL-1 β -induced MMP-3 production is down-regulated by PGE₂ *via* EP₂ and EP₄ receptors, whereas in cells from periodontally diseased tissue, IL-1 β -induced MMP-3 production is up-regulated *via* EP₁ receptors. Different regulation of IL-1 β -induced MMP-3 production by PGE₂ between healthy and periodontally diseased tissues may be involved in the pathogenesis of periodontal disease.

KEY WORDS: human gingival fibroblasts, interleukin-1 β , matrix metalloproteinase-3, prostaglandin E₂, EP receptors.

Prostaglandin E₂ Regulates Interleukin-1 β -induced Matrix Metalloproteinase-3 Production in Human Gingival Fibroblasts

INTRODUCTION

Prostaglandins (PGs), including PGE₂, play numerous roles under physiological and pathological conditions. PGE₂ is thought to be involved in the pathogenesis of periodontal disease, due to its role as a potent stimulator of bone resorption and association with attachment loss (Offenbacher *et al.*, 1993). PGE₂ exerts its biological actions *via* specific PGE receptors on target cells, and 4 distinct subtypes of PGE receptors—designated EP₁, EP₂, EP₃, and EP₄—have been identified and cloned, each with unique signal transduction mechanisms as a result of coupling to different G proteins (Negishi *et al.*, 1995; Narumiya *et al.*, 1999). EP₁ receptor activation induces elevation of intracellular calcium levels *via* a poorly characterized mechanism involving G proteins. EP₂ and EP₄ receptors activate adenylate cyclase *via* stimulatory G protein, and result in elevation of intracellular cAMP levels. Multiple isoforms of EP₃ receptors with different C-terminal tails mediate several signaling pathways, including inhibition and stimulation of adenylate cyclases, activation of phospholipase C, and mobilization of intracellular calcium.

Matrix metalloproteinases (MMPs), including MMP-3 (also called stromelysin-1), are a family of Zn endopeptidases that play a key role in extracellular matrix (ECM) turnover. MMP-3 is known to utilize various ECM proteins as its substrate, including proteoglycan core protein, fibronectin, elastin, laminin, gelatin, and collagen types II, III, IV, V, IX, X, and XI (Birkedal-Hansen, 1993). MMP-3 can activate procollagenase, including proMMP-1 (Murphy *et al.*, 1987). Expression of MMPs, including MMP-3, in periodontal lesions has been demonstrated (Birkedal-Hansen, 1993; Ingman *et al.*, 1994). Cross-sectional study of gingival crevicular fluid (GCF) analysis has revealed that GCF MMP-3 and tissue inhibitor of metalloproteinase-1 (TIMP-1) levels can differentiate between healthy and diseased periodontal sites (Haerian *et al.*, 1995). Furthermore, it has been shown that periodontal pockets with high GCF levels of MMP-3 and TIMP-1 are at significantly higher risk for periodontal disease progression (Alpagot *et al.*, 2001). Human gingival fibroblasts (HGF) are known to secrete MMP-3 in response to stimuli, including IL-1 (Tewari *et al.*, 1994).

However, the regulation of MMP-3 production in periodontal lesions is poorly understood. The present study was undertaken to investigate the effect of PGE₂ on IL-1 β -induced MMP-3 secretion in HGF derived from periodontally healthy subjects and diseased patients. Furthermore, we examined which subtype(s) of PGE₂ receptors were involved in PGE₂ regulation of IL-1 β -induced MMP-3 secretion.

MATERIALS & METHODS

Culture of HGF

With informed consent, we obtained gingival biopsies from three periodontally healthy subjects (aged 20, 22, and 37 yrs), who had no sites with clinical attachment loss of greater than 2 mm and exhibited no radiographic bone loss, and from three patients (aged 45, 47, and 61 yrs) affected with severe periodontitis, with clinical

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probing depth sites of greater than 5 mm and clinical attachment loss of greater than 5 mm, and radiographic evidence of severe bone loss. Our study protocol, including biopsy of gingival tissue, satisfied the ethical standards of Tokyo Medical and Dental University. Fibroblasts which extended from pieces of gingiva were cultured in α -minimum essential medium (α -MEM) containing 10% fetal bovine serum (FBS) (Bioserum, Victoria, Australia), 100 U/mL penicillin (Sigma Chemical Co., St. Louis, MO, USA), and 100 U/mL streptomycin (Sigma). During their successive passages, cells were cultured at 37°C in a humidified atmosphere with 5% CO₂ in the air. Cells between the fourth and fifteenth passages were used in the experiments.

Cell Stimulation

HGF were seeded in 96-well plates. After confluence, they were serum-starved in α -MEM containing 0.5% FBS to reduce the effect of serum on MMP-3 production. After 24 hrs, the cells were treated with IL-1 β (Sigma), PGE₂ (Cascade Biochem LTD, Berkshire, UK), 17-phenyl- ω -trinor PGE₂ (Cayman Chemicals, Ann Arbor, MI, USA), butaprost (a gift from ONO Pharmaceuticals Co. Ltd, Tokyo, Japan), ONO-AE1-329 (a gift from ONO Pharmaceuticals Co. Ltd.), ONO-AP-324 (a gift from ONO Pharmaceuticals Co. Ltd.), indomethacin, dibutyl cAMP, and forskolin (Wako, Tokyo, Japan), in the combinations and concentrations indicated. Indomethacin was added 30 min prior to stimulation with IL-1 β . Cells were pre-treated with SC51322, an EP₁ receptor antagonist, when indicated. Conditioned medium was collected after 72 hrs of incubation, and stored at -80°C until analysis.

Zymography for Caseinolytic Activity Assay

To determine caseinolytic activity in the culture media, we performed casein zymography. One mg/mL of β -casein was copolymerized in the 10% polyacrylamide gel. The conditioned media samples were diluted in loading buffer containing 2% SDS under non-reducing conditions. Diluted samples were subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) without being boiled, at a constant voltage of 200 V. When the bromophenol blue indicator front reached the bottom of the gel (in approximately 1 hr), electrophoresis was stopped. The gels were subsequently washed in 2.5% Triton-X at room temperature to remove SDS. The gels were then incubated in incubation buffer containing 50 mM Tris HCl, 5 mM CaCl₂, 0.02% NaN₃, 200 mM NaCl, and 0.02% Brij35 (Polyoxyethylene 23 lauryl ether) at 37°C overnight and stained with Coomassie Brilliant Blue R-250.

ELISA for PGE₂ and MMP-3

PGE₂ and MMP-3 levels in the conditioning media collected from IL-1 β -treated HGF were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (PGE₂, Amersham Pharmacia Biotech, Buckinghamshire, UK; MMP-3, Dai-ichi Fine Chemicals, Toyama, Japan) according to manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean $\pm$ standard deviation (SD). Data were subjected to one-way analysis of variance (ANOVA), with the StatView program. Fisher's protected least-significant difference test was used in the *post hoc* comparison of specific groups.

RESULTS

Effect of Indomethacin on IL-1 β -induced MMP-3 and PGE₂ Production in HGF Derived from Healthy Gingiva

To examine the involvement of endogenous prostaglandins on

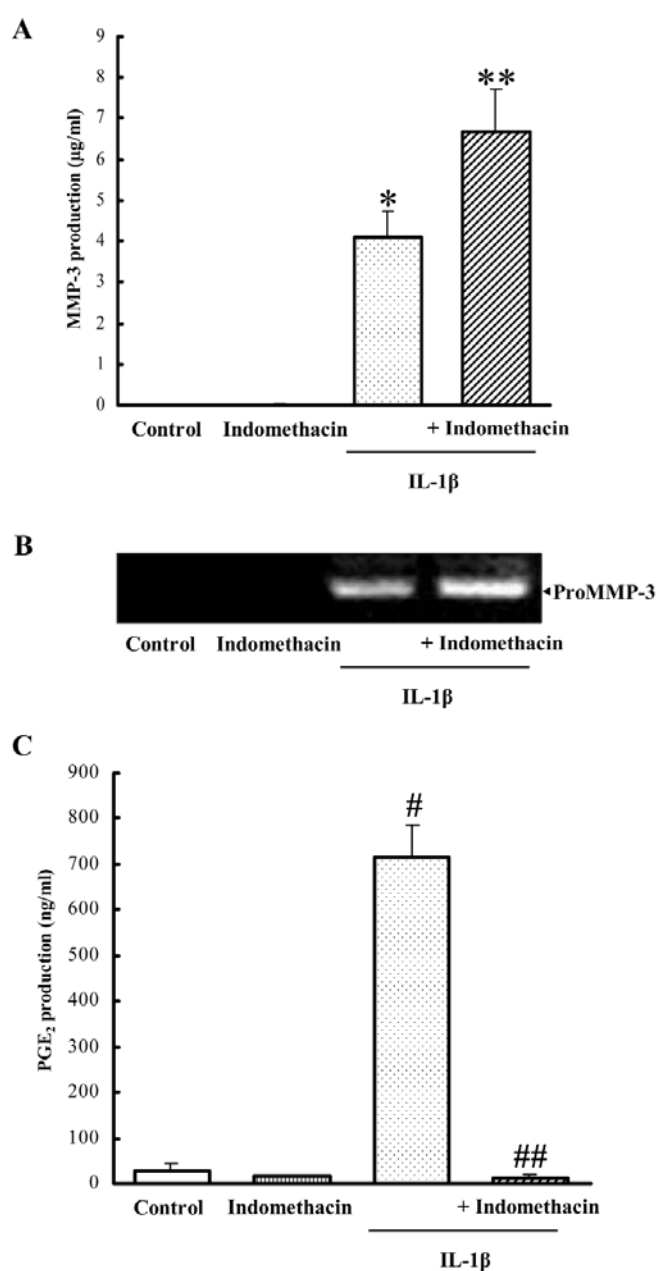


Figure 1. Effect of indomethacin on MMP-3 (A,B) and PGE₂ (C) production in IL-1 β -stimulated HGF derived from healthy gingiva. These cells were stimulated with vehicle or 2 ng/mL IL-1 β in the presence or absence of 1 μ M of indomethacin for 72 hrs. After incubation, MMP-3 (A) and PGE₂ levels (C) and caseinolytic activities (B) in the culture media were evaluated by enzyme-linked immunosorbent assay and casein zymography, as described in MATERIALS & METHODS. Values are mean $\pm$ SD (n = 4). Data are representative of 3 separate experiments. *Significantly different from control (p < 0.0001). **Significantly different from IL-1 β alone (p < 0.001). #Significantly different from control (p < 0.0001). ##Significantly different from IL-1 β alone (p < 0.001).

MMP-3 secretion in HGF derived from healthy gingiva stimulated with IL-1 β , we investigated the effect of indomethacin, a cyclooxygenase inhibitor, on MMP-3 production by the cells. IL-1 β alone induced MMP-3 production in a dose- and time-dependent manner (data not shown). It was

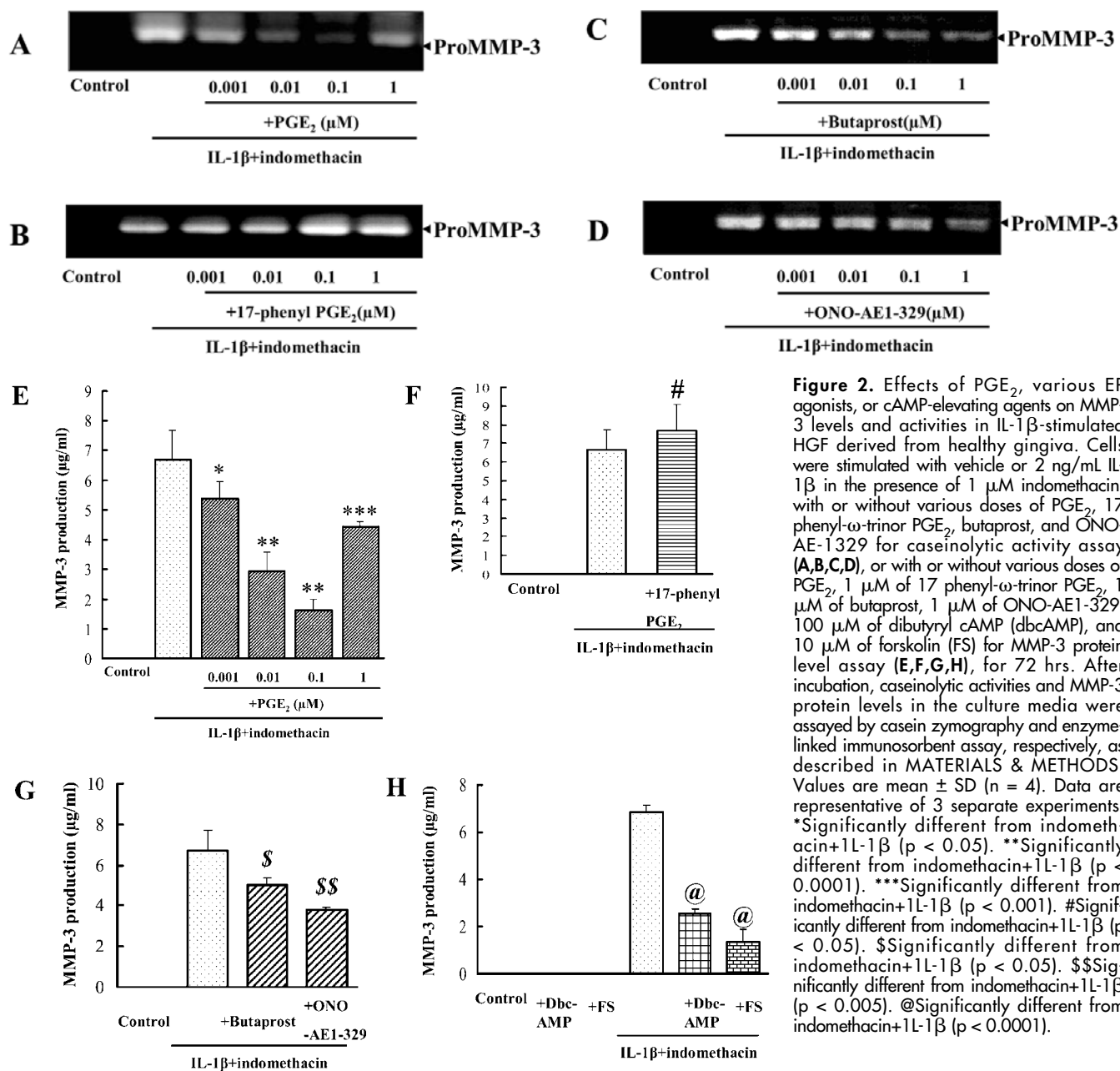


Figure 2. Effects of PGE₂, various EP agonists, or cAMP-elevating agents on MMP-3 levels and activities in IL-1 β -stimulated HGF derived from healthy gingiva. Cells were stimulated with vehicle or 2 ng/mL IL-1 β in the presence of 1 μ M indomethacin, with or without various doses of PGE₂, 17 phenyl- ω -trilor PGE₂, butaprost, and ONO-AE-1329 for caseinolytic activity assay (A,B,C,D), or with or without various doses of PGE₂, 1 μ M of 17 phenyl- ω -trilor PGE₂, 1 μ M of butaprost, 1 μ M of ONO-AE-1329, 100 μ M of dibutyryl cAMP (dbcAMP), and 10 μ M of forskolin (FS) for MMP-3 protein level assay (E,F,G,H), for 72 hrs. After incubation, caseinolytic activities and MMP-3 protein levels in the culture media were assayed by casein zymography and enzyme-linked immunosorbent assay, respectively, as described in MATERIALS & METHODS. Values are mean $\pm$ SD (n = 4). Data are representative of 3 separate experiments. *Significantly different from indomethacin+IL-1 β (p < 0.05). **Significantly different from indomethacin+IL-1 β (p < 0.001). ***Significantly different from indomethacin+IL-1 β (p < 0.0001). #Significantly different from indomethacin+IL-1 β (p < 0.05). \$Significantly different from indomethacin+IL-1 β (p < 0.05). \$\$Significantly different from indomethacin+IL-1 β (p < 0.005). @Significantly different from indomethacin+IL-1 β (p < 0.0001).

shown by ELISA analysis that, in these cells, indomethacin significantly increased IL-1 β -induced MMP-3 secretion, although indomethacin completely inhibited IL-1 β -induced PGE₂ secretion (Figs. 1A, 1C). Casein zymography analysis with culture media showed that caseinolytic activities appeared as clear bands at the Mr size of approximately 55 kDa in the samples derived from healthy tissue cells treated with IL-1 β , although no band was detected in the samples from non-stimulated cells. Expression of the bands was completely inhibited by the addition of EGTA and o-phenanthroline, a general metalloproteinase inhibitor (Stix *et al.*, 2001). The 55-kDa proteinase has been shown to be proMMP-3 (Lark *et al.*, 1990; Kapila *et al.*, 1996). Furthermore, we confirmed, by Western blot analysis, that MMP-3 protein was detected at the Mr size of approximately 55 kDa (data not shown). As shown in Fig. 1B, treatment of IL-1 β -stimulated HGF with indomethacin

enhanced caseinolytic activities. In casein zymography, the active 45-kDa form of MMP-3 was not observed in any samples.

Effect of Exogenous PGE₂ on IL-1 β -induced MMP-3 Secretion in HGF Derived from Healthy Gingiva

Since IL-1 β is a potent stimulator of PGE₂ production in HGF, we examined the effect of exogenous PGE₂ on IL-1 β -induced expression of MMP-3 by these cells. As seen in Figs. 2A and 2E, exogenous PGE₂ inhibited IL-1 β -stimulated MMP-3 secretion between 0.001 μ M and 1 μ M. At the dose of 0.1 μ M, PGE₂ showed the maximum inhibitory effect.

Effects of EP Receptor Agonists and cAMP-elevating Agents on IL-1 β -induced MMP-3 Production in HGF Derived from Healthy Gingiva

To evaluate which subtypes of EP receptors were involved in PGE₂ regulation of IL-1 β -induced MMP-3 production in these

cells, we investigated the effects of various selective EP agonists on IL-1 β -induced MMP-3 production. In HGF derived from healthy tissue, treatment of EP₁ selective agonist, 17-phenyl- ω -trinor PGE₂, enhanced IL-1 β -stimulated MMP-3 secretion in a concentration-dependent manner (Figs. 2B, 2F). As shown in Figs. 2C, 2D, and 2G, butaprost, a specific EP₂ agonist, and ONO-AE1-329, a selective EP₄ agonist, depressed IL-1 β -stimulated MMP-3 production. Furthermore, we evaluated the effects of dibutyryl cAMP, a cAMP analogue, and forskolin, an adenylate cyclase stimulator, on IL-1 β -induced MMP-3 generation. As demonstrated in Fig. 2H, dibutyryl cAMP and forskolin significantly inhibited IL-1 β -induced MMP-3 secretion.

Effect of Indomethacin on IL-1 β -induced MMP-3 and PGE₂ Production in HGF Derived from Periodontally Affected Gingiva

Contrary to the result obtained in HGF derived from healthy tissue, in HGF from periodontally affected tissue, indomethacin significantly inhibited IL-1 β -induced MMP-3 secretion (Fig. 3A). Similarly, casein zymography analysis showed that indomethacin caused an inhibitory effect on IL-1 β -induced caseinolytic activities (Fig. 3B). Indomethacin completely decreased IL-1 β -induced PGE₂ production (Fig. 3C).

Effects of PGE₂ and EP Receptor Agonists on IL-1 β -induced MMP-3 Production in HGF Derived from Periodontally Affected Gingiva

In these cells, exogenous PGE₂ dose-dependently stimulated IL-1 β -induced MMP-3 secretion (Fig. 4A). Analysis of ELISA and zymography data from the use of EP agonists showed that 17-phenyl- ω -trinor PGE₂ dose-dependently stimulated IL-1 β -induced MMP-3 secretion, whereas butaprost and ONO-AE1-329 seemed to have no effect (Fig. 4). We further examined the effect of SC51322, an EP₁ receptor antagonist, on IL-1 β -induced MMP-3 secretion. SC51322 dose-dependently inhibited IL-1 β -induced MMP-3 secretion in these cells (data not shown).

DISCUSSION

In the present study, we demonstrated that PGE₂ differently regulated IL-1 β -induced MMP-3 production in HGF derived from healthy and periodontally affected gingival tissues. Treatment of these cells with indomethacin increased and decreased IL-1 β -induced MMP-3 production, respectively, although IL-1 β -induced PGE₂ production was completely depressed in both HGF (Figs. 1, 3). Furthermore, exogenous PGE₂ suppressed and enhanced IL-1 β -induced MMP-3 production in HGF from healthy and periodontally affected gingivae, respectively (Figs. 2A, 2E; 4A, 4E). From these data, we suggest that IL-1 β -induced MMP-3 production is down-regulated by PGE₂ endogenously produced in the former, whereas IL-1 β -induced MMP-3 production is up-regulated by PGE₂ endogenously produced in the latter. It has been reported that PGE₂ inhibits IL-1-induced MMP-3 transcription in human rheumatoid synovial fibroblasts, although, in human dermal fibroblasts, PGE₂ does not affect MMP-3 induction by IL-1 (Case *et al.*, 1990; Mauviel *et al.*, 1994). Thus, PGE₂ regulation of MMP-3 induction by IL-1 may be dependent on cell types. It has been shown that cultures of HGF contain a heterogeneous population of cells in growth, morphology, collagen synthesis,

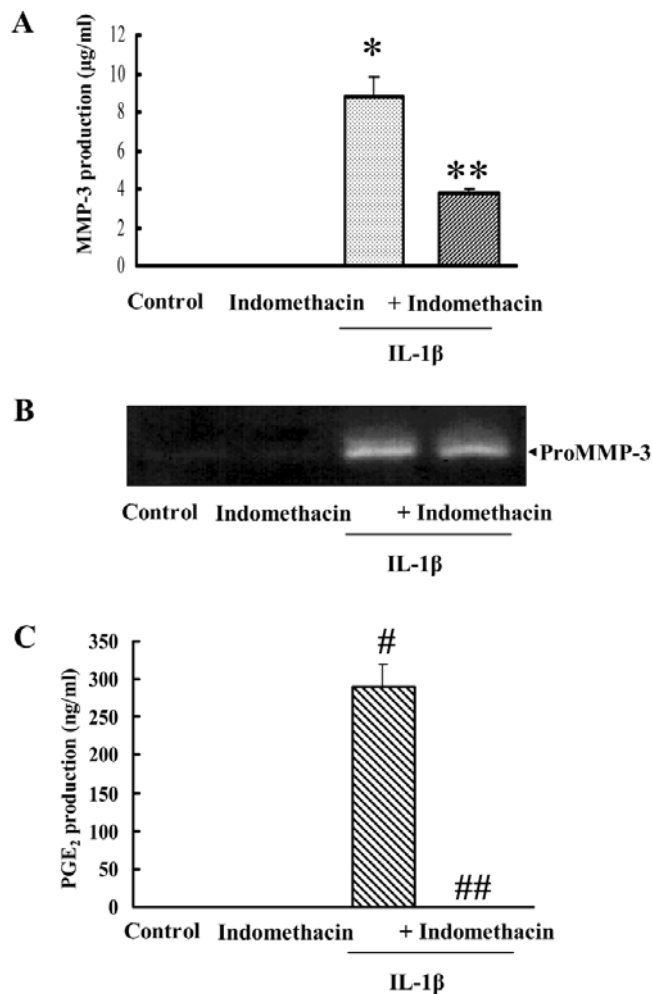


Figure 3. Effect of indomethacin on MMP-3 (A,B) and PGE₂ (C) production in IL-1 β -stimulated HGF derived from periodontally affected tissue. Cells were stimulated with vehicle or 2 ng/mL IL-1 β in the presence or absence of 1 μ M of indomethacin for 72 hrs. After incubation, MMP-3 (A) and PGE₂ (C) levels and caseinolytic activities (B) in the culture media were evaluated by enzyme-linked immunosorbent assay and casein zymography, as described in MATERIALS & METHODS. Values are mean $\pm$ SD (n = 4). Data are representative of 3 separate experiments. *Significantly different from control (p < 0.0001). **Significantly different from IL-1 β alone (p < 0.001). #Significantly different from control (p < 0.0001). ##Significantly different from IL-1 β alone (p < 0.001).

and CD14 expression (Narayanan and Page, 1976; Hurum *et al.*, 1982; Sugawara *et al.*, 1998). The mechanisms underlying the differentiation of such subpopulations remain unclear. It has been suggested that pro-inflammatory substances, including lymphokines and PGs in the chronic inflamed tissue, may modulate fibroblast growth and populations (Narayanan and Page, 1976). Furthermore, it has been reported that PGE₂ differently regulates IL-1 β -induced IL-6 production in healthy and periodontally affected tissue-derived cells (Takigawa *et al.*, 1994; Czuszek *et al.*, 1996; Noguchi *et al.*, 2002).

PGE₂ exerts its biological actions *via* specific PGE receptors on target cells and, based on ligand-binding selectivity and signaling pathway activation, PGE₂ receptors are classified as EP₁, EP₂, EP₃, and EP₄ subtypes (Negishi *et al.*, 1995;

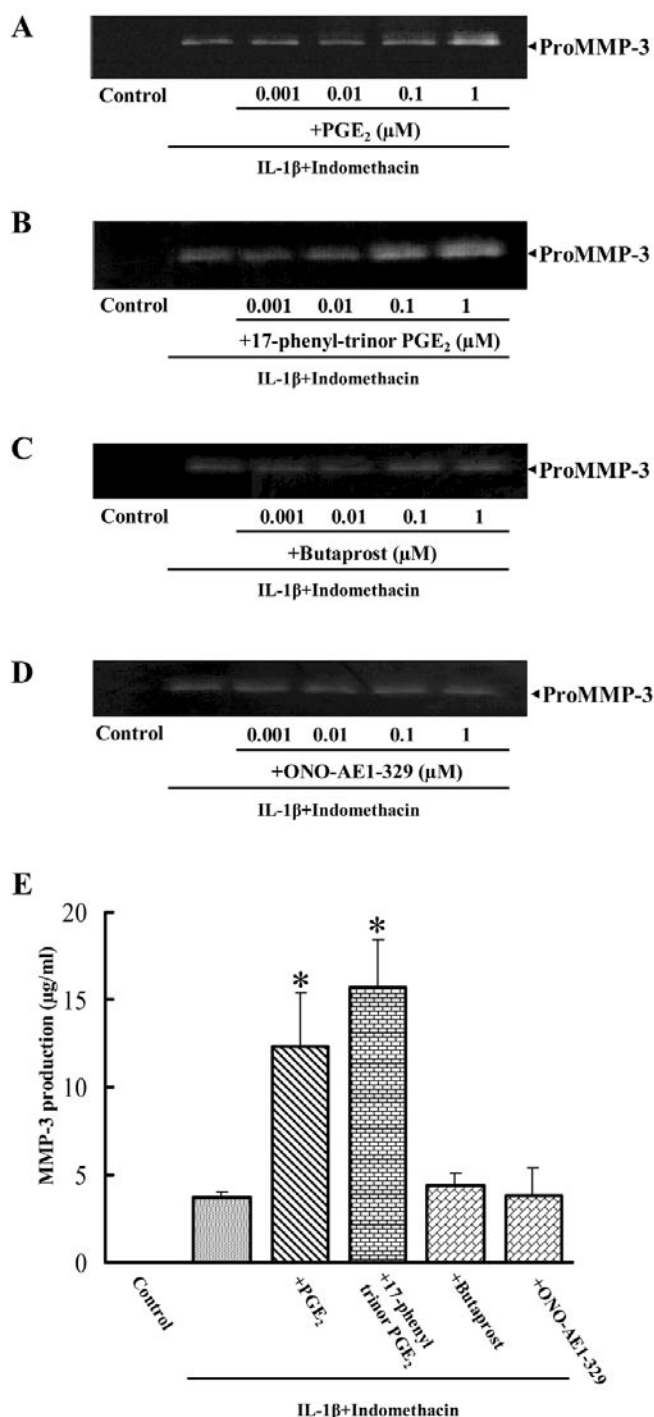


Figure 4. Effects of PGE₂, 17 phenyl- ω -trinor PGE₂, butaprost, and ONO-AE1-329 on production of MMP-3 in IL-1 β -stimulated HGF derived from periodontally affected gingiva. Cells were stimulated with vehicle or 2 ng/mL IL-1 β in the presence of 1 μ M indomethacin, with or without various doses of PGE₂, 17 phenyl- ω -trinor PGE₂, butaprost, and ONO-AE1-329 for caseinolytic activity assay (A,B,C,D), or with or without 1 μ M of PGE₂, 17 phenyl- ω -trinor PGE₂, butaprost, and ONO-AE1-329 for MMP-3 protein level assay (E), for 72 hrs. After incubation, caseinolytic activities and MMP-3 protein levels in the culture media were assayed by casein zymography and enzyme-linked immunosorbent assay, respectively, as described in MATERIALS & METHODS. Values are mean $\pm$ SD (n = 4). Data are representative of 3 separate experiments. *Significantly different from indomethacin+IL-1 β (p < 0.0001).

Narumiya *et al.*, 1999). We have previously demonstrated that functional EP₁, EP₂, and EP₄ subtypes of PGE₂ receptors are expressed in HGF (Noguchi *et al.*, 2000). 17-phenyl- ω -trinor PGE₂, a selective EP₁ agonist, up-regulated IL-1 β -stimulated MMP-3 production in both healthy and periodontally affected tissue-derived cells (Figs. 2B, 2F; 4B, 4E). EP₁ receptor activation is linked to elevation of intracellular Ca²⁺ levels. It has been shown that intracellular Ca²⁺ elevation interacts synergistically with protein kinase C (PKC) to induce MMP-3 gene expression (Rodland *et al.*, 1992). Since IL-1 β can induce PKC activation in HGF, it is likely that the intracellular Ca²⁺ increase mediated by EP₁ receptors and PKC activation by IL-1 β synergistically enhanced MMP-3 production in these cells. However, both butaprost (a selective EP₂ agonist) and ONO-AE1-329, a selective EP₄ agonist, down-regulated IL-1 β -stimulated MMP-3 production in HGF derived from healthy tissue, although the agents had no or little effect on IL-1 β -stimulated MMP-3 production in their periodontally affected counterparts (Figs. 2C, 2D, 2G; 4C, 4D, 4E). It has been demonstrated that cAMP can inhibit MMP-3 induction by IL-1 at the transcriptional level in human rheumatoid synovial fibroblasts and uterine cervical fibroblasts, although not in human dermal fibroblasts (Case *et al.*, 1990; Takahashi *et al.*, 1991; Mauviel *et al.*, 1994). EP₂ and EP₄ receptors are linked to intracellular cAMP elevation. Since cAMP-elevating agents including dibutyl cAMP and forskolin mimicked inhibition of IL-1 β -induced MMP-3 production by PGE₂ in HGF from healthy tissue (Fig. 2H), we suggest that EP₂ and EP₄ receptor activation causes inhibition of IL-1 β -induced MMP-3 production *via* cAMP-dependent pathways in these cells. The levels of intracellular cAMP in both cell types stimulated with PGE₂, butaprost and ONO-AE1-329, were similar (data not shown), which shows that functional EP₂ and EP₄ receptors were expressed in them. In the present study, we did not investigate why EP₂ and EP₄ receptor activation could not suppress IL-1 β -induced MMP-3 production in HGF from periodontally affected tissue, although EP₂ and EP₄ receptor activation resulted in intracellular cAMP elevation. It is possible, however, that the difference of regulation is further downstream in the EP₂- and EP₄-mediated signaling cascade. From these results, we suggest that PGE₂ down-regulates IL-1 β -stimulated MMP-3 production *via* EP₂/EP₄ receptors in HGF from healthy tissue, whereas EP₁ receptors are involved in PGE₂ up-regulation of IL-1 β -stimulated MMP-3 production in HGF from periodontally affected cells. Further studies are needed to clarify the mechanism by which PGE₂ differently regulates IL-1 β -induced MMP-3 production in these cells.

MMP-3 expression in periodontal lesions has been demonstrated (Ingman, 1994). Cross-sectional study of gingival crevicular fluid (GCF) analysis has revealed that GCF MMP-3 and TIMP-1 levels can differentiate between healthy and diseased periodontal sites (Haerian *et al.*, 1995). Furthermore, it has been shown that periodontal pockets with high GCF levels of MMP-3 and TIMP-1 are at significantly higher risk for periodontal disease progression (Alpagot *et al.*, 2001). MMP-3 not only utilizes various ECM proteins as its substrate—including proteoglycan core protein, fibronectin, elastin, laminin, gelatin, and collagen types II, III, IV, V, IX, X, and XI (Birkedal-Hansen, 1993)—but also activate procollagenase, including proMMP-1 (Murphy *et al.*, 1987). MMP-3 activates proforms of cytokines like IL-1. Therefore, it

is likely that PGE₂ enhancement of IL-1 β -induced MMP-3 production in HGF from diseased tissue is involved in periodontal tissue breakdown. Further studies are necessary to confirm this hypothesis.

In conclusion, we suggest that IL-1 β -induced MMP-3 production is down-regulated by PGE₂ via EP₂ and EP₄ receptors in HGF derived from healthy gingiva, whereas IL-1 β -induced MMP-3 production is up-regulated via EP₁ receptors in HGF from periodontally affected tissue. These data may reflect the heterogeneity of inflammatory responses in healthy and diseased gingiva. Differential regulation of IL-1 β -induced MMP-3 production by PGE₂ in HGF may play critical roles in the regulation of extracellular matrix breakdown in periodontal disease.

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Efficacy of a herbal toothpaste on patients with established gingivitis – a randomized controlled trial

Eficácia de um dentifrício fitoterápico em pacientes com gengivite estabelecida – ensaio clínico aleatório

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ABSTRACT: The aim of this randomised, double blind controlled trial was to verify the efficacy of a herbal dentifrice on the reduction of plaque and gingivitis. Forty eight volunteers with established gingivitis were randomly assigned to either a test group (herbal dentifrice) or positive control group (dentifrice with triclosan and fluoride). The dentifrices were distributed in plain white tubes by an independent pharmacy, which revealed the contents of each tube only after the experimental period. Plaque and gingivitis assessments were carried out on baseline and after 28 days of product use. All examinations were conducted by the same calibrated investigator. Subjects were instructed to brush their teeth three times daily using their assigned dentifrice for 28 days. There was a significant reduction in plaque levels in both the test and control groups. However, there was no significant difference between the groups. A significant reduction in gingivitis was observed in both groups, although there was no significant difference between them. No adverse reactions were reported. The authors concluded that both dentifrices were effective in reducing plaque and gingivitis in subjects with established gingivitis.

DESCRIPTORS: Gingivitis; Plant preparations; Phytotherapy; Triclosan.

RESUMO: O objetivo deste ensaio clínico aleatório duplo-cego foi verificar a eficácia de um dentifrício fitoterápico na redução de placa e gengivite. Quarenta e oito voluntários com gengivite estabelecida foram aleatoriamente alocados aos grupos teste (dentifrício fitoterápico) e controle positivo (dentifrício com triclosan e flúor). Os dentifrícios foram distribuídos em tubos brancos por uma farmácia independente, que revelou o conteúdo de cada tubo apenas após o final do período experimental. A aferição de placa e gengivite foi conduzida por um único examinador, previamente calibrado, no início e após 28 dias de uso do produto. Os sujeitos da pesquisa foram orientados a escovar os dentes com o dentifrício de seu grupo três vezes ao dia, por 28 dias. Houve redução significativa na quantidade de placa nos grupos teste e controle. No entanto, não houve diferença significativa entre os grupos. Os dois grupos experimentais apresentaram redução significativa nos níveis de gengivite, porém não houve diferença significativa entre eles. Não foram observadas reações adversas. Os autores concluíram que os dois dentifrícios foram eficazes na redução de placa e gengivite em indivíduos com gengivite estabelecida.

DESCRIÇÕES: Gengivite; Preparações de plantas; Fitoterapia; Triclosan.

INTRODUCTION

Self-performed mechanical plaque removal is an unquestioned method of controlling plaque and gingivitis^{4,8,14}. However, toothbrushing and flossing are difficult tasks, and most of the patients

are not able to completely remove plaque in all teeth surfaces¹¹. Mechanical plaque control is also time-consuming, and some individuals may lack motivation for these procedures. In an effort to

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improve the efficacy of mechanical tooth-cleaning procedures, antimicrobial agents have been added to dentifrices^{2,17}.

One of the most effective agents for supragingival plaque control is chlorhexidine^{1,7,10}. However, a significant reduction of its antiplaque potential may be observed when it is used in a toothpaste preparation⁵. Triclosan has also been incorporated into dentifrices, and some studies have demonstrated a significant reduction of plaque and gingivitis^{9,15}.

Interest in alternative mouthrinses and toothpastes based on plant extracts has increased recently. Among these herbal products, Parodontax® (GlaxoSmithKline, Middlesex, United Kingdom) has received great attention. It is composed of sodium bicarbonate, sodium fluoride (1,400 ppm) and herbal ingredients: chamomile, which is supposed to have anti-inflammatory properties and to decrease gingival inflammation; echinacea, which is reputed to stimulate the immune response; sage and rhatany, which have anti-hemorrhagic properties; myrrh, claimed to be a natural anti-septic; and peppermint oil, which has analgesic, anti-septic and anti-inflammatory properties.

Some studies have reported that Parodontax® is able to significantly decrease plaque and gingivitis^{24,25}, while other publications showed no effectiveness of the dentifrice when compared to a control^{16,18,20}.

In many trials, study subjects receive dental prophylaxis and oral hygiene instructions prior to the commencement of the experiment. However, for the majority of the population, plaque removal is unsupervised, which leads to high scores of plaque accumulation and gingivitis. For a clearly evident effect of an antimicrobial agent to be proven, it should be demonstrated on established gingivitis subjects, which are representative of the vast majority of dentifrice users.

The aim of this study was to evaluate the efficacy of the Parodontax® dentifrice in the reduction of plaque and gingivitis in subjects with established gingivitis.

MATERIALS AND METHODS

Volunteers for this study were recruited in the Dental Clinic at the Associação Paulista de Cirurgiões Dentistas, in São Caetano (Brazil). They were admitted to the study if they met the following eligibility criteria: age ≥ 18 years, a minimum of 15 teeth, good general health, a baseline

Plaque Score mean > 1.5 (Quigley, Hein¹⁹, 1962 as modified by Turesky *et al.*²³, 1970) and presence of established gingivitis. Established gingivitis was defined as a baseline Löe, Silness¹³ (1963) Gingival Index mean > 1.0 . The plaque score mean and the Gingival index mean were based on inclusion criteria used by Binney *et al.*⁶ (1996) and Owens *et al.*¹⁷ (1997). Exclusion criteria were: presence of advanced periodontal disease, which was defined as presence of CPITN code 4 teeth³, use of orthodontic appliances, use of antibiotics in the previous 3 months, continuous use of mouthrinses containing chemical agents in the previous 3 months, and a history of allergy to toothpastes or Parodontax.

Initially, 48 healthy subjects (20 males and 28 females), ranging in age from 18-69 years (mean age 33.19 ± 13.57) were considered eligible for the study. All subjects were given oral and written information about the study, and before entering the study, they signed a written informed consent. The study protocol was approved by the Ethics Committee of the Ibirapuera University. All procedures in this experiment were performed according to the ethical principles established by the Declaration of Helsinki.

The study was designed as a randomised, double blinded, parallel arm controlled trial. The subjects were randomly assigned to either the test or positive control group. The random allocation sequence was generated by one of the authors (C.M.P.), who used a random-number table. The random allocation sequence was concealed from the main investigator (F.O.) until the dentifrices were assigned to the participants. The main investigator was responsible for enrolling the subjects and assessing the study variables. Blinding and allocation concealment were controlled by the independent Pharmacy "Fórmula e Ação", which distributed the toothpastes in plain white tubes, identified as "group A" and "group B" tubes. All investigators and study subjects were unaware of the contents of each tube. The pharmacy revealed the contents of each tube only after the experimental period was over.

Volunteers in the test group received a toothpaste tube containing 90 g of Parodontax® dentifrice (GlaxoSmithKline, Middlesex, United Kingdom) containing sodium bicarbonate, sodium fluoride 1,400 ppm, chamomile, echinacea, sage, rhatany, myrrh and peppermint oil. Subjects in the control group received a toothpaste tube containing 90 g of Colgate Total (Colgate-Palmolive Company, New York, United States of America), containing

0.3% triclosan, 2% copolymer and 0.243% sodium fluoride. The control dentifrice was modified by “Fórmula e Ação” Pharmacy, to have its colour and taste similar to the Parodontax dentifrice.

No prophylaxis was undertaken prior to commencement of the study, and no attempt was made to modify the volunteers’ oral hygiene habits. All participants used dental floss during the study. At baseline, the amount of plaque and gingival inflammation was measured on all teeth, at the buccal, mesial, distal and lingual aspects, with the exception of the third molars. The participants were stained for plaque using an erythrosine disclosing solution and cotton swabs. The amount of plaque was scored using the Turesky²³ (1970) modification of the Quigley, Hein¹⁹ (1962) index. After that, gingival inflammation was recorded using the Gingival Index (GI)¹³. All measurements were conducted by the main investigator (F.O.), who was previously calibrated. For calibration, two measurements were performed with one-hour interval. Intra-examiner calibration was performed in 5 patients until an 80% agreement was obtained.

After scoring plaque and gingival inflammation, the subjects were instructed to brush with the assigned toothpaste 3 times a day. Rinsing with water after toothbrushing was not allowed, as well as using any other toothpaste or mouthrinse during the experimental period.

After 28 days, the subjects returned for another appointment, in which the amounts of plaque and gingival inflammation were scored again by the same investigator. To check for compliance, the participants were asked to return their assigned tubes, so that the investigators could verify the amount of dentifrice that was used. Volunteers were also asked about adverse events, such as taste disturbance, mucosal sensitivity etc.

Statistical analysis was performed using a statistics package (SPSS 11.0 for Windows). The subject was the unit of analysis. Plaque scores

and Gingival Index scores were averaged on a per-subject basis. Each participant had a whole-mouth average score for baseline and another for the 28-day exam, presented separately for buccal/lingual aspects and mesial/distal aspects. The distribution of the variables was not normal, so group means were compared using the Mann-Whitney U test. Differences between baseline and the 28-day exam were evaluated using the Wilcoxon test. Groups were also compared regarding age by means of the Student’s *t* test, and association between group and sex was verified by means of the chi-square test. All statistical tests employed a level of significance of $\alpha = 0.05$.

RESULTS

Forty-two (16 males and 26 females) of the initial 48 subjects completed the 28-days study period. There were six dropouts throughout the trial. Four individuals were contacted but declared they were “too busy” or “didn’t have time” to come to the 28-day exam. They were examined and received a prophylaxis one or two weeks later, but data pertaining to these patients was not used in the statistical analysis. Two individuals declared they did not have interest in another appointment because they “have already received a dental prophylaxis”.

The mean age in the test group ($n = 20$) was 31.55 ± 14.6 , and in the control group ($n = 22$) was 34.68 ± 12.73 . There was no significant difference between groups according to age ($p = 0.46$), and there was no significant association between group and sex ($p = 0.23$), which means that both groups were homogeneous.

At baseline, there was no significant difference in Plaque Scores between the two groups. After 28 days, the test group presented an average 19.9% reduction in plaque at the buccal and lingual surfaces, whereas the control group showed an 18.3% reduction (Table 1). At the proximal surfaces, a

TABLE 1 - Mean, median and comparison between groups according to the Plaque Index at buccal and lingual aspects.

		Baseline	28 days	p (W)
Herbal toothpaste N = 20	Mean $\pm$ SD	2.11 ± 0.68	1.69 ± 0.64	0.033*
	Median	2.03	1.69	
Positive control N = 22	Mean $\pm$ SD	2.13 ± 0.60	1.74 ± 0.60	0.001*
	Median	2.08	1.65	
	p (MW)	0.93	0.60	

*Significant at the 5% level; W = Wilcoxon test; MW = Mann-Whitney test.

reduction of 19.6% and 15.4% was observed for test and control groups, respectively (Table 2). Both dentifrices produced a significant reduction in plaque scores. However, the difference between the groups was not statistically significant.

There was no significant difference in the Gingival Index between the groups at baseline. After 28 days, a mean 28.4% and 36.3% reduction in GI was observed in the test and control groups, respectively, at the buccal and lingual surfaces (Table 3). At the proximal surfaces, a decrease of 23.5% was observed in the test group, and a 32.5% reduction occurred in the control group (Table 4). In both groups a significant decrease in mean GI was observed, although there was no significant difference between the groups.

No adverse reactions were observed during the trial. None of the volunteers reported any se-

rious reaction or discomfort related to the toothpastes.

DISCUSSION

Lately, there has been growing interest in natural products. Even though studies in animals and *in vitro* may show the antimicrobial properties of several of these products, there is no other way of knowing their real clinical effect without conducting a randomized clinical trial. Detergents and abrasives may alter the substantivity or the antimicrobial activity of active ingredients. The principal ingredients of Parodontax (chamomile, echinacea, sage, rhatany, myrrh and peppermint oil) have several medicinal properties. However, data pertaining to the substantivity of these products cannot be found in literature. It is important that clinical tri-

TABLE 2 - Mean, median and comparison between groups according to the Plaque Index at mesial and distal aspects.

		Baseline	28 days	p (W)
Herbal toothpaste N = 20	Mean ± SD	2.44 ± 0.69	1.96 ± 0.53	0.018*
	Median	2.56	2.01	
Positive control N = 22	Mean ± SD	2.40 ± 0.59	2.03 ± 0.55	0.002*
	Median	2.47	1.94	
	p (MW)	0.85	0.66	

*Significant at the 5% level; W = Wilcoxon test; MW = Mann-Whitney test.

TABLE 3 - Mean, median and comparison between groups according to the Gingival Index at buccal and lingual aspects.

		Baseline	28 days	p (W)
Herbal toothpaste N = 20	Mean ± SD	1.02 ± 0.25	0.73 ± 0.37	0.012*
	Median	1.01	0.67	
Positive control N = 22	Mean ± SD	1.02 ± 0.28	0.65 ± 0.28	0.001*
	Median	1.03	0.62	
	p (MW)	0.94	0.45	

*Significant at the 5% level; W = Wilcoxon test; MW = Mann-Whitney test.

TABLE 4 - Mean, median and comparison between groups according to the Gingival Index at mesial and distal aspects.

		Baseline	28 days	p (W)
Herbal toothpaste N = 20	Mean ± SD	1.19 ± 0.27	0.91 ± 0.34	0.009*
	Median	1.18	0.89	
Positive control N = 22	Mean ± SD	1.20 ± 0.28	0.81 ± 0.25	0.001*
	Median	1.15	0.83	
	p (MW)	0.92	0.26	

*Significant at the 5% level; W = Wilcoxon test; MW = Mann-Whitney test.

als verify the efficacy of any new product, instead of simply assuming that the product is efficient based on laboratory studies.

In a previous study¹⁸, Parodontax® was unable to promote a significant reduction in PI and GI when compared to a standard dentifrice containing only fluoride. One possible reason for the conflicting results obtained by either study is the population participating in each study. In the first experiment, the sample was composed of Dentistry students, who presented low PI and GI scores at baseline, which would have lessened the impact of any chemical agent on the levels of plaque and gingivitis. Comparatively, in the present study, all volunteers enrolled in the study started out with a large amount of established plaque and gingivitis. Prophylaxis and scaling were not carried out previous to the experimental phase, as in the studies by Lindhe *et al.*¹² (1993), Triratana *et al.*²² (1993) and Triratana *et al.*²¹ (2002). The association of toothbrushing and the test dentifrice promoted reductions of 19% in the Plaque Index and 23-28% in the Gingival Index, while Triratana *et al.*²¹ (2002) observed reductions of 39.9% in PI and 25.7% in GI. Lindhe *et al.*¹² (1993) observed an increase in the number of sites presenting a plaque score of

0 (plaque-free surfaces) from 12% at baseline to 35% at the end of the trial, and a mean reduction in GI from 1.3 to 1.1.

Our results showed that there was no additional benefit of the test dentifrice over the positive control toothpaste (Colgate Total®). Nevertheless, these results don't mean that the test dentifrice has not been efficient. The Parodontax® group showed a significant decrease in Plaque Index and in the Gingival index. The reductions in both indices were significant when compared to baseline values. Moreover, the product was compared with a dentifrice containing triclosan, which is an antimicrobial agent whose safety and efficacy have been well established⁹. It can be inferred, by our results, that the herbal dentifrice was as efficacious as the one with triclosan, and may be an alternative for people interested in natural products.

CONCLUSION

The authors conclude that both dentifrices were able to reduce plaque and gingivitis, although no additional benefit of the test dentifrice over the positive control toothpaste could be observed.

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The clinical efficacy of triclosan/copolymer and other common therapeutic approaches to periodontal health

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ABSTRACT

The maintenance of an effective level of oral hygiene is the cornerstone of all attempts to prevent and control periodontal disease, and yet the widespread prevalence of the disease indicates the inability of most people to maintain a level of plaque control commensurate with periodontal health. The inclusion of antibacterial agents, such as chlorhexidine and triclosan, in oral care products has provided a means to improve oral health. Randomised, controlled clinical trials have demonstrated that the unsupervised use of a dentifrice (toothpaste) containing triclosan/copolymer significantly improves gingival health, prevents the onset of periodontitis and reduces further progression of tissue destruction. The delivery of such benefits has positive implications for the oral health of individuals and populations.

Keywords Oral care products, periodontal disease, triclosan

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INTRODUCTION

The inflammation causing periodontal diseases is initiated by bacterial biofilms (dental plaque) that form on the surfaces of teeth in close proximity to the supporting tissues of the teeth. Initially, the biofilms develop above (Fig. 1a) the gum margin (supragingival) but, in the absence of effective oral hygiene, will inevitably extend below (Fig. 1b) the gum margin (subgingival). The inflammatory and immune responses to these biofilms are primarily responsible for the subsequent destruction of the periodontal tissues. The most common forms of periodontal disease are gingivitis and periodontitis. The term gingivitis denotes a lesion that is confined to the gingiva (gum) and is reversible, provided that the individual maintains an effective level of plaque control. However, gingivitis is the precursor of periodontitis, which is caused by the apical spread of inflammation, and the irreversible loss of tooth support. Gingivitis and periodontitis are a continuum, and

although the susceptibility of an individual to periodontitis is influenced by many factors, such as smoking, diabetes and genetics, the weight of evidence indicates that the prevention of gingival inflammation prevents periodontitis [1].

Periodontal diseases are prevalent in populations around the world [2]. More than 82% of US adolescents have overt gingival inflammation [2], and in Western Europe 36% of those aged 35–44 years have moderate periodontitis and approximately 10% have severe periodontitis [3]. The prevalence and severity of periodontitis increases with age [4] and, as the increasingly numerous elderly population retain more of their teeth into later life, periodontal disease will pose an increasing threat to their natural dentition and, potentially, their general health.

Although many factors influence the susceptibility of an individual to periodontitis, the process of tissue destruction is initiated by plaque bacteria. The cornerstone of any attempt to prevent and control periodontal disease is the maintenance of an effective level of plaque control by the individual at home, and available evidence indicates that most people find it difficult to maintain this level. In the UK, for example, 74% of adults claimed to brush their teeth twice a day, and yet 72% of 25–34-year-olds had visible plaque on 30% of teeth, and those aged 65 years and over

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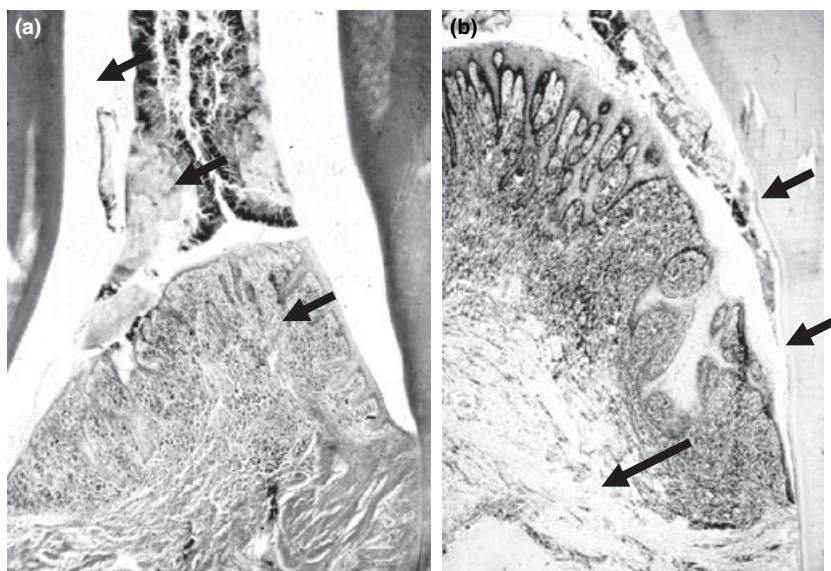


Fig. 1. Supragingival plaque and gingival inflammation (a). Subgingival plaque and irreversible loss of tooth support (b).

had visible plaque on 44% of teeth [4]. It was thus concluded that there is a need to improve the oral cleanliness of the majority of the population if periodontal health is to be improved.

Conventional dentifrices (toothpastes) are complex mixtures of abrasives, surfactants and humectants that enhance the physical removal of plaque bacteria from the tooth surfaces. The principle and benefits of adding chemical agents to dentifrices is exemplified by the inclusion of fluoride, which has been a major factor in reducing the prevalence of dental caries worldwide [5]. Given the importance of plaque bacteria in initiating periodontal disease, the incorporation of an antibacterial agent into oral care products would appear to be a logical development. This review will consider clinical studies, of at least 6 months' duration, that have assessed the effectiveness of agents in improving plaque control and periodontal disease.

DENTIFRICES

Stannous fluoride

Although launched initially in the 1950s to deliver the anti-caries benefit of fluoride, dentifrices containing stannous fluoride also exhibit intrinsic antimicrobial effects [6]. Stabilised stannous fluoride dentifrices improve gingival health as compared with a conventional dentifrice, but this has not always been accompanied by a significant reduction in plaque [7,8].

Triclosan

Triclosan is a broad-spectrum antibacterial agent with a good safety record as used in many consumer products, e.g., deodorants and soaps. The fact that triclosan is a non-ionic molecule allows it to be formulated in conventional dentifrices, but it does not bind to the oral surfaces for more than a few hours and therefore does not deliver a sustained level of anti-plaque activity. Two attempts have been made to overcome this problem.

Triclosan/copolymer

The first approach was to add a copolymer, polyvinylmethyl ether maleic acid, which increased the uptake of triclosan and its retention [6] on oral surfaces (plaque, teeth and mucosa). Subsequent studies have demonstrated that triclosan/copolymer dentifrices containing 0.3% triclosan, 2.0% copolymer and fluoride (1100/1450 p.p.m. F) (Colgate Total, Colgate-Palmolive company, NJ, USA) maintain concentrations of triclosan in plaque that exceed the MIC values of many plaque bacteria for up to 12 h [9].

The efficacy of this triclosan/copolymer dentifrice in reducing plaque and improving gingival health has been assessed in numerous randomised, controlled clinical trials, and the results have been summarised in a number of traditional and systematic reviews [10,11]. The participants in all studies were adults with plaque and gingivitis

[11]. Participants were randomly allocated to use either the triclosan/copolymer dentifrice or a conventional fluoride dentifrice for 6 months. They were given no oral hygiene instruction and were simply asked to use their allocated dentifrice twice a day. With the exception of one trial, all dentifrices were supplied in plain white tubes.

The meta-analyses of data from 16 studies demonstrated that the triclosan/copolymer dentifrice was significantly more effective in improving plaque control and gingival health than a fluoride dentifrice. The weighted mean difference (WMD) of -0.48 for the Quigley-Hein Plaque Index corresponds to a 23% reduction in plaque when compared with a fluoride dentifrice. The WMD of -0.15 for heavy plaque deposits corresponds to a 15% reduction. In relative terms, there was a 49% reduction in the proportion of sites that had heavy plaque. The WMD of -0.26 for gingival inflammation corresponds to a 23% reduction. The WMD of -0.34 for gingival bleeding corresponds to a 12% reduction which, in relative terms, corresponds to a 49% reduction in the proportion of sites with bleeding.

The progression of gingivitis to periodontitis is an unpredictable event, but bleeding sites are more likely to progress to periodontitis than non-bleeding sites [12]. Since the triclosan/copolymer dentifrice significantly reduced gingival bleeding, further studies have assessed whether the dentifrice could also have beneficial effects on periodontitis. A 3-year randomised clinical trial involving adolescents who were at risk of developing early periodontitis demonstrated that the triclosan/copolymer dentifrice was indeed significantly more effective than a standard fluoride toothpaste in preventing the onset of periodontitis [13]. After 3 years, the triclosan/copolymer dentifrice reduced the amount of attachment loss (early periodontitis) per subject by 50% and the number of affected sites by 41%. In another 3-year study, which involved patients who already had periodontitis, the triclosan/copolymer dentifrice was significantly more effective than a fluoride dentifrice in reducing pocket depth and further attachment loss [14]. Data from a 5-year clinical trial in Australia demonstrated that the triclosan/copolymer dentifrice could slow the progression of disease in a normal adult population [15]. Finally, the triclosan/copolymer dentifrice significantly improved the gingival health and

periodontal status in smokers when compared with a standard fluoride dentifrice [16].

No adverse events have been reported in any of the studies, and no shifts in the microflora of supragingival plaque favouring the growth of either opportunistic or pathogenic bacterial or yeast species have been observed [17,18].

Initially, the benefits of triclosan/copolymer dentifrice were attributed solely to the antibacterial activity of triclosan, but several studies have demonstrated that triclosan may also exert a significant anti-inflammatory effect. Triclosan reduces skin and mucosal inflammation [19,20], and there is clinical evidence that this may also occur in the gingiva [21]. Further in-vitro studies have demonstrated that triclosan reduces the production of prostaglandin E_2 by cells stimulated with either interleukin- 1β or tumour necrosis factor- α [22], and the production of interleukin- 1β by fibroblasts stimulated by tumour necrosis factor- α [23]. More recently, it has been reported that triclosan reduces microsomal prostaglandin E synthase-1 expression in human gingival fibroblasts [24].

Triclosan/zinc citrate

The second approach to overcoming the lack of substantivity and efficacy of triclosan has been to combine triclosan with zinc citrate, the latter having been shown to be substantive and to inhibit plaque and gingivitis [25]. Several clinical studies of at least 6 months' duration have shown that dentifrices containing zinc citrate and triclosan are significantly more effective than a fluoride dentifrice in controlling plaque and gingivitis [26].

MOUTH RINSES

Chlorhexidine

Chlorhexidine digluconate is the most powerful anti-plaque agent available. Since 1970, numerous clinical trials have demonstrated its effectiveness in improving plaque control and gingival health when used as an adjunct to toothbrushing [27]. Its effectiveness is attributed to the dicationic nature of chlorhexidine, which ensures its reversible binding with the negatively charged molecules in the oral cavity, so that activity persists for several hours after application. However, its

long-term use is precluded because of its propensity to stain the teeth, which is the result of interactions between chlorhexidine on the tooth surfaces and tannins in the diet.

Triclosan/copolymer

Three clinical trials of 6 months' duration have assessed the effectiveness of a mouthrinse containing triclosan/copolymer [28]. Reductions in plaque and gingivitis ranged from 24% to 36% and from 19% to 23%, respectively, when compared with a placebo rinse.

Cetylpyridinium chloride

Mouthrinses containing the cationic surface-active agent cetylpyridinium chloride have been marketed since 1940. When used as an adjunct to toothbrushing, the rinse significantly reduced plaque and gingivitis by 28% and gingival bleeding by 67%, respectively [29].

Essential oils

A combination of thymol and eucalyptol, together with menthol and methylsalicylate in a hydroalcohol vehicle (Listerine, Johnson & Johnson, Raritan, NJ, USA), is also used. Several clinical trials [30,31] have demonstrated that when used as an adjunct, this antiseptic mouthwash provides significant benefits, with up to 56% reductions in plaque and 35% reductions in gingivitis. However, in general, it has been found to be less effective than chlorhexidine.

Amine fluoride/stannous fluoride

Although clinical studies of short duration have generally shown an amine fluoride/stannous fluoride rinse to improve plaque control and gingival health [32], these benefits have not been consistently demonstrated in long-term studies. In a clinical study of 7 months' duration, an amine fluoride/stannous fluoride rinse reduced plaque by 5% and gingival bleeding by 30% when compared with a placebo rinse, but no benefit to gingival health has been observed [33]. In 1996, it was reported that a combination of amine/stannous fluoride dentifrice and amine/stannous fluoride mouthrinse was no more effective than a conventional fluoride dentifrice and

rinse in improving plaque control and gingival health. Subsequent studies have reported that the combination of amine/stannous fluoride dentifrice and rinse significantly reduced plaque by 25% and 16% [33] but failed to deliver gingival benefits when compared with a conventional fluoride dentifrice and mouthrinse.

CONCLUSION

The need for effective control of dental plaque has led to a large number of clinical studies with antibacterial agents in oral hygiene formulations. Antibacterial agents have been shown to vary in their effectiveness in improving plaque control and gingival health when compared to a conventional fluoride dentifrice in studies of at least 6 months' duration. The unsupervised use of a dentifrice containing triclosan/copolymer has been shown to prevent the onset and progression of periodontitis for at least 3 years. Such improvements in periodontal health could have beneficial effects on systemic health.

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