121-125 (in Japanese).

Kusaka Y, Kondou H, Morimoto K (1992). Healthy lifestyles are associated with higher natural killer cell activity. *Prev Med* 21:602-615.

Pitiphat W, Merchant AT, Rimm EB, Joshipura KJ (2003). Alcohol consumption increases periodontitis risk. *J Dent Res* 82:509-513.

Sakki TK, Knuuttila ML, Vimpari SS, Hartikainen MS (1995). Association of lifestyle with periodontal health. *Community Dent Oral Epidemiol* 23:155-158.

Shibuya A, Yoshida A (1988). Genotypes of alcohol-metabolizing enzymes in Japanese with alcohol liver diseases: a strong association of the usual Caucasian-type aldehyde dehydrogenase gene [ALDH1(2)] with the disease. *Am J Hum Genet* 43:744-748.

Shizukuishi S, Hayashi N, Tamagawa H, Hanioka T, Maruyama S, Takeshita T, *et al.* (1998). Lifestyle and periodontal health status of Japanese factory workers. *Ann Periodontol* 3:303-311.

Takada A, Tsutsumi M, Kobayashi Y (1994). Genotypes of ALDH2 related to liver and pulmonary diseases and other genetic factors related to alcoholic liver disease. *Alcohol Alcohol* 29:719-727.

Takeshita T, Morimoto K (1996). Effects of genetic polymorphisms in alcohol-metabolizing enzymes on alcohol hypersensitivity and alcohol-related health problems in Orientals. *Environ Hlth Prev Med* 1:1-8.

Takeshita T, Morimoto K, Mao XQ, Hashimoto T, Furuyama J, Furyuama J (1993). Phenotypic differences in low Km aldehyde dehydrogenase in Japanese workers. *Lancet* 341:837-838.

Takeshita T, Morimoto K, Mao X, Hashimoto T, Furuyama J (1994). Characterization of the three genotypes of low Km aldehyde dehydrogenase in a Japanese population. *Hum Genet* 94:217-223.

Takeshita T, Yang X, Inoue Y, Sato S, Morimoto K (2000). Relationship between alcohol drinking, ADH2 and ALDH2 genotypes, and risk for hepatocellular carcinoma in Japanese. *Cancer Lett* 149:69-76.

Tezal M, Grossi SG, Ho AW, Genco RJ (2001). The effect of alcohol consumption on periodontal disease. *J Periodontol* 72:183-189.

Wickramasinghe SN, Gardner B, Barden G (1986). Cytotoxic protein molecules generated as a consequence of ethanol metabolism in vitro and in vivo. *Lancet* II:823-826.

Xu YL, Carr LG, Bosron WF, Li TK, Edenberg HJ (1988). Genotyping of human alcohol dehydrogenases at the ADH2 and ADH3 loci following DNA sequence amplification. *Genomics* 2:209-214.

歯肉メラニン色素沈着と喫煙の関係

埴岡　隆　Takashi Hanioka

口腔が禁煙推進で注目を集めている

カナダではタバコの箱に，画像による健康警告表示が用いられている（図1）．この警告表示についての意識調査がカナダ対癌協会によって行われ，昨年，速報がCNNから流れた．筆者は，その最後の一文「タバコをやめるのに口腔と肺癌の警告画像が効果的だ，と指摘する者が最も多かった」に注目した．その後，口腔画像が印刷されたタバコの箱は，NHKサイエンスアイ，タバコ白書，「医師とたばこ」をはじめ，日本でもさまざまな機会に紹介されている．

最近，調査結果の詳細が公開された．カナダ人2,031名（喫煙者633名）が，「最もタバコをやめるのに効果的」として選んだ人の割合は，口腔と肺癌が飛びぬけて高く，喫煙者，女性，若年者では，肺癌よりも口腔を選んだ者が多かった（表）[1]．カナダ人の評価とはいえ，若年者と女性の喫煙者が急増しているわが国では，タバコ対策に肺癌だけでなく，口腔の有用性が高いことが想像できる．

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歯肉メラニン色素沈着の疫学

歯肉のメラニン色素沈着（図2）は，さまざまな民族・人種で報告され，有所見者率は5～100％と幅広く，遺伝子の関与が示唆される．Addison病，Albright病，Peutz-Jeghers症候群，von Recklinghousen病，HIV感染，抗マラリヤ薬や経口避妊薬投与との関連も指摘されている．

歯肉メラニン色素沈着は喫煙者に多いというだけでなく，量-反応関係も示されている[2]．非喫煙者にも色素沈着が多いマレーシア人やインド系人種では，喫煙により沈着範囲が広がる[3]．禁煙後2年で有所見者は半減し，3年以上で非喫煙者のレベルまで低下する[4]．色素沈着の程度分類（図3）では，側切歯と犬歯の付着歯肉部に沈着が始まり，範囲が広がって連続するようになる[5]．

2つの企業男性従業員317名の調査では，現在喫煙者の有所見者率は82％と最も高く，

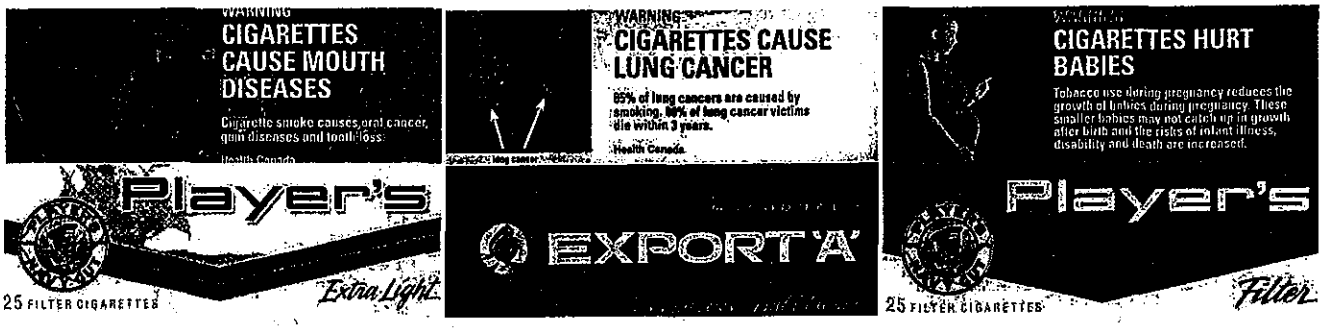

図 1　カナダのタバコの箱の画像警告表示例．左から口腔，肺癌，喫煙する妊婦．このほか合計16種類の警告画像が印刷されている

表　カナダのタバコ箱警告画像のうちで「タバコをやめるのに最も効果的」として選んだ人の割合（%）

上位3種	全　体	喫　煙		性		年　齢			
		喫　煙	非喫煙	男	女	18-29	30-44	45-59	60-
1．口腔画像	17	19	16	16	19	24	17	15	6
2．肺　癌	16	14	17	15	16	16	15	17	14
3．妊婦喫煙	5	5	6	3	7	9	5	3	2

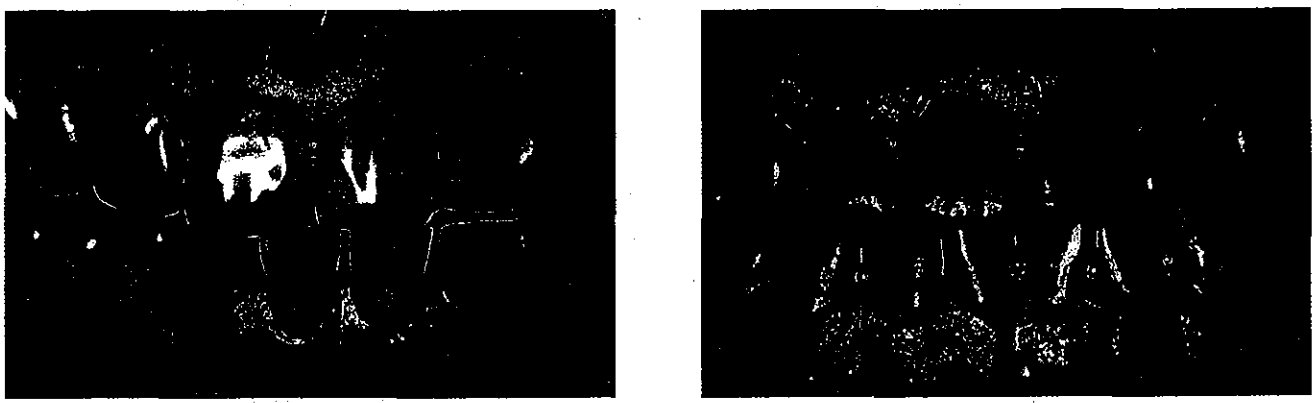

図 2　成人の歯肉メラニン色素沈着の程度，広がりはさまざまである

元喫煙者は51%，非喫煙者は29%であった（図4）[6]．喫煙者の5人に4人は有所見者であると推測され，その場に手鏡ひとつあれば，喫煙者の色素沈着を指摘することで，自分自身への影響を認識させて教育する機会が生まれる．しかし，非喫煙者や元喫煙者にも色素沈着が認められるため，メラニン色素沈着からすぐに喫煙者であるとは断定できない．非喫煙者では軽度沈着の者が多く，反対に喫煙者では中等度以上が多い（図5）．色素沈着が広範囲の場合は喫煙者の可能性が高い．

1日喫煙本数が少ない場合でも，また喫煙開始後1年以内の早い段階でも色素沈着が始まり，やがてピークに達する（図6）ことから，歯肉メラニン色素産生細胞はタバコ煙に敏感だということが推測される．事業所別にみた場合では，現在喫煙者の有症者はともに80%以上だったが，元喫煙者と非喫煙者では事業所間に大きな差が認められた（図7）．A社従業員は事務職，H社従業員は工場勤務が大部分であったことから，10年前の状況では受動喫煙が理由として推定される．

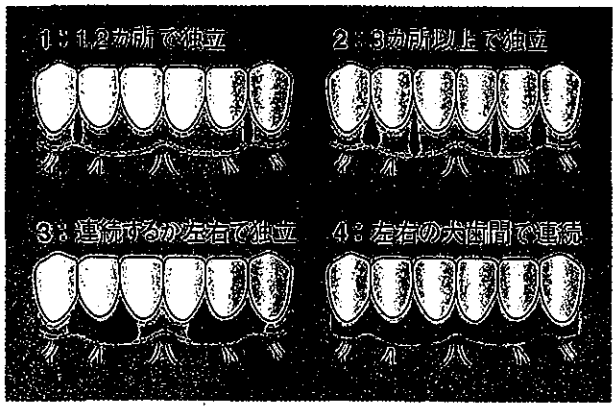

図 3　メラニン色素沈着指数（0：色素沈着を認めない）

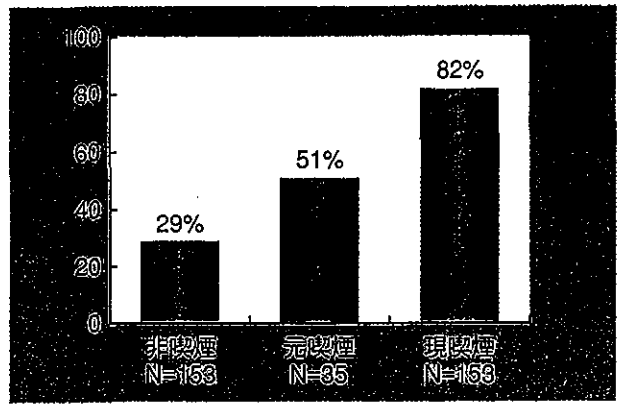

図 4　喫煙とメラニン色素沈着有症者の関係

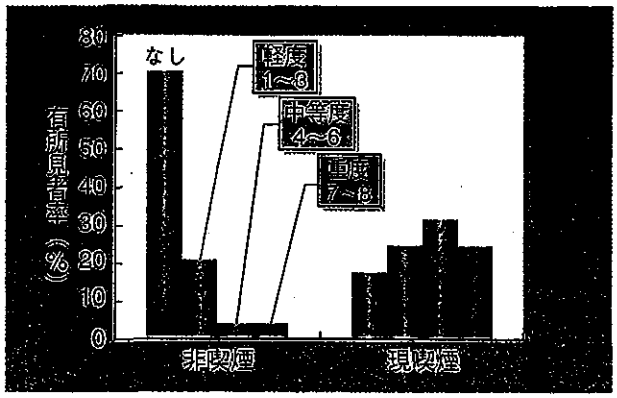

図 5　喫煙の有無別のメラニン色素沈着程度（上下顎合計指数）の分布

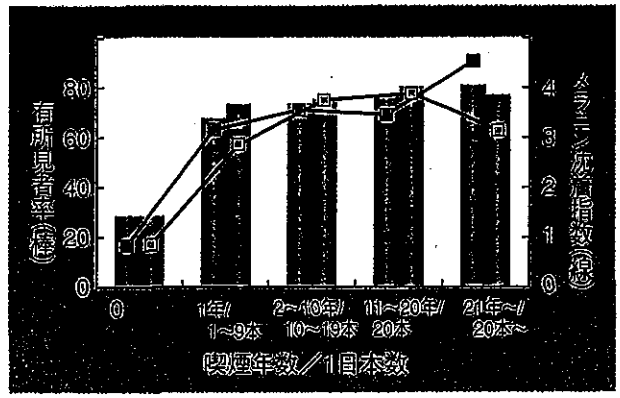

図 6　喫煙本数および喫煙年数とメラニン色素沈着指数との関係

歯肉メラニン色素沈着の知識

メラニン色素沈着の口腔分布は，前歯部頬側で最も多く（下顎53%，上顎43%），白歯部唇側では約30%だったが，舌・口蓋側は5%以下と少なかった[6]．これはスウェーデン人[5]と一致しているが，マレーシア人，インド系人種では口蓋側にも多く認められるという[3]．

メラニンを産生するのは樹状細胞であるメラニン産生細胞（メラノサイト）で，主に付着歯肉の基底細胞層を構成する．メラニンはメラノゾーム（メラニン顆粒）内に蓄えられ，

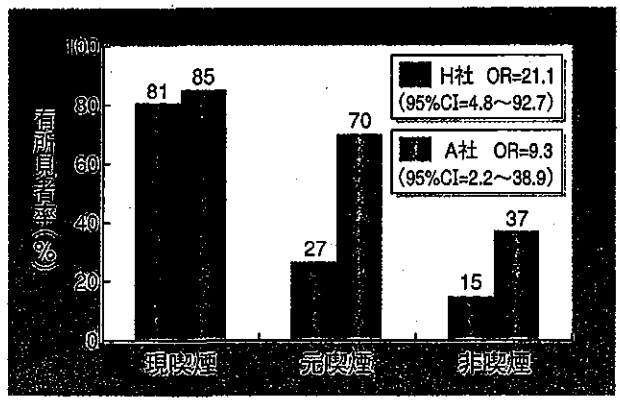

図 7　2つの事業所のメラニン有症者率の比較

上皮層に伸展する樹状突起先端部分から，ケラチノサイト（ケラチン産生細胞）の貪食能によって上皮層に取り込まれる．そして，上皮の代謝により体外に放出されたり，酵素に

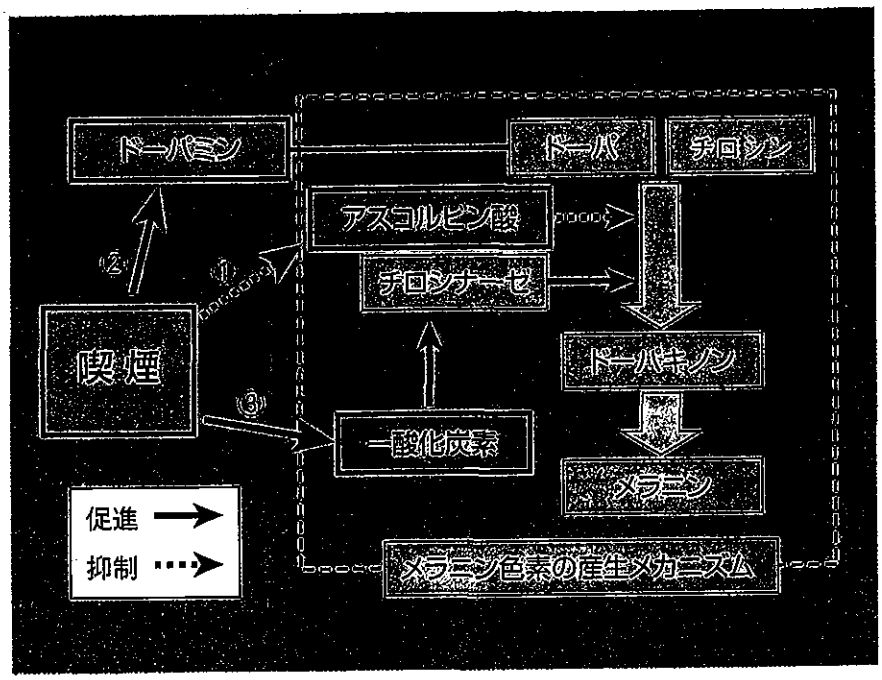

図 8　メラニン色素の生成プロセスへの喫煙の影響の推定経路

より分解されたりして消失する．この過程で酸化により発色したものがメラニン色素である[7]．

メラニンは，チロシナーゼにより，チロシンおよびドーパから，ドーパキノンやユーメラニン等の中間物質を経て合成される（図8）．この過程で，喫煙が関与すると推定されるのは，① ドーパキノン産生を抑制するアスコルビン酸（ビタミンC）が喫煙により分解され，ドーパキノンが増加する，② ニコチン刺激によるドーパミン産生時の副産物としてドーパが産生される，③ タバコ煙に含まれる一酸化炭素がチロシナーゼを活性化し，ドーパキノンが増加する，などがある．このほか，ニコチン[8]，ベンツピレン[9]はメラニンと親和性が高く，メラニンの生合成を促進する．

メラニン色素の生物学的機能の説明として，有害物質からの防衛機能が推定される．まず，紫外線の場合と同様に，有害物質からの生体保護の意味が考えられるが，タバコ煙と接する口蓋側に沈着が見られないことと矛盾する．しかし，ニコチン，ベンツピレンなどの有害物質を体外に排除する意味では，上皮での溶解作用や体外に開く頬側犬歯間の分布と一致する．また，メラニン色素沈着が見えやすく喫煙への反応が早いことから，タバコ煙に限らず異物摂取への警告の意味も考えられる．

メラニン色素沈着は正常状態であるとの認識もある．しかし，審美的理由からメラニン色素の除去あるいは脱色を希望する者に対して，さまざまなメラニン色素除去法が考案されている．フェノール等による脱色術，歯肉掻把術，自家歯肉移植術，レーザー照射術などがある．喫煙者での再発は，施術症例の初期の報告で多い．CO_2レーザー照射術では，4週以後に歯肉色彩に喫煙の影響が出始める[10]．また，メラニン色素が少ない口蓋歯肉を移植することで再発が予防されている[11]．

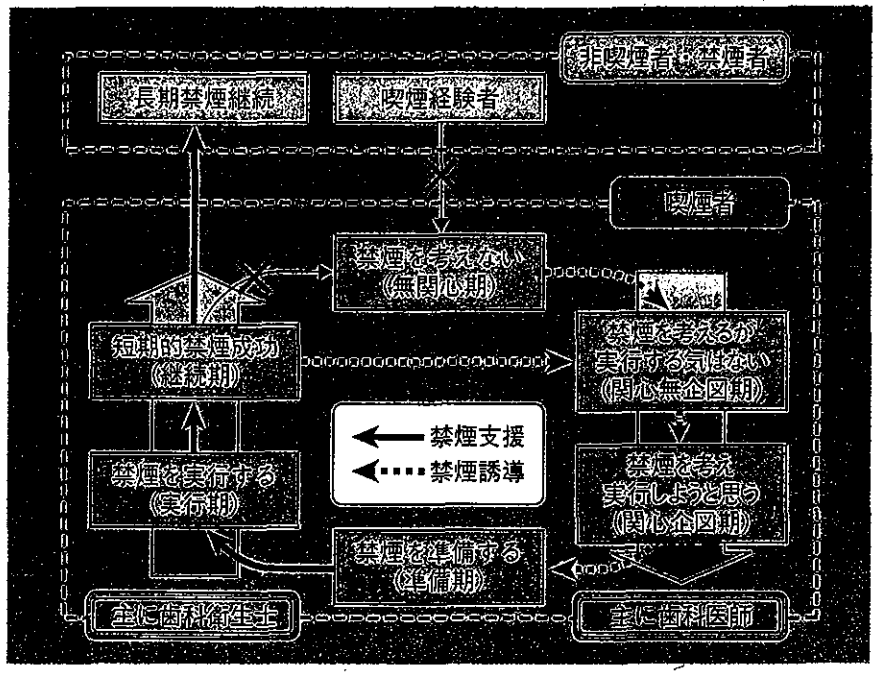

図 9　喫煙から禁煙へのプロセスと禁煙誘導・禁煙支援

しかし，近年の報告では，喫煙に言及したものは少ない．

歯肉メラニン色素沈着と禁煙誘導

歯肉メラニン色素沈着と喫煙との強い関連性は，さまざまな対象集団で報告され，量-反応関係が存在し，禁煙するとメラニン色素沈着が軽減される．喫煙によりメラニン色素の産生が高まることは，生物医学的にも説明がつきそうであり，喫煙とメラニン色素沈着の因果関係が明確になってきた．カナダのタバコの箱の警告表示に描かれた口腔の画像は，喫煙をやめることに強いインパクトがあるが，メラニン色素沈着は描写されていない．

今，保健医療の現場では，禁煙希望者に対する禁煙サポートが動き出した一方で，もう一つの問題解決を求める意識が高まっている．喫煙者が禁煙にいたるまでには，いくつかのプロセスがあり（図9），そのプロセスに対応した指導，支援を行うことが大切とされている．禁煙支援は，行動科学療法およびその補助としてニコチン代替療法を併用したカウンセリング手法が中心であるため，ある程度の時間と経験を要することで臨床現場には馴染みにくいという指摘がある．

そこで，名古屋大学の浜島信之教授は，「強い被指示性を伴わない手法で，かつ簡便に多数の喫煙者に提供できる方法」として，禁煙誘導を提案している．診療施設や検診施設への受診は，喫煙者を健康不安や健康確認という日常生活と異なる心理状況に置くことから，禁煙誘導のよい機会となる．

喫煙の口腔への影響は，メラニン色素沈着や口臭などの身近な影響，歯周病や歯の喪失といった歯科主要疾患，直接生命にかかわる口腔癌，さらに，抜歯後の治癒，インプラント，歯周処置などの治療効果と，多様で幅広い．タバコ対策では，口腔保健医療機関にも

重要な役割があることが，世界規模で認識されており，自分への影響が目で見える器官を対象としている，ということも大きな特徴である．

おわりに

日本では，1920年代に歯肉メラニン色素が報告されたが，喫煙との関係は気づかれなかった[7]．喫煙との関係は，スウェーデン人研究者の功績によるところが大きいが，その発見を禁煙推進に役立てる意図は，論文から汲み取れない．アメリカでは，研究成果を現場で実際にどのように役立てるか，研究成果の橋渡しを考えた研究（translational research）が，今，提案されている．

健康増進法では，第25条の受動喫煙防止が注目されているが，第2条には，喫煙のすぐ後に歯の健康が記載されている．喫煙と歯の健康の両面で，健康増進に歯科専門職の役割が期待される．世界に目を向けると，タバコ消費削減への保健医療従事者の役割が盛り込まれたタバコ枠組み条約がWHOにより制定され，「日本の批准も間近」と伝えられている．ヘルシンキでは，昨夏，枠組み条約制定直後に医・歯の相互理解を深めるため，WMA（世界医師会）とFDIのジョイントシンポジウムが開催された．

自分の目で見ることができて多様である喫煙の口腔への影響，その一つの歯肉メラニン色素沈着は，誰もが禁煙推進に利用できる．世界禁煙デーの厚生労働大臣メッセージには，「受動喫煙の危険性やニコチンの依存性を踏まえると，喫煙習慣は個人の嗜好にとどまらない，健康問題であります」とある．歯科疾患の予防と治療効果低下の防止のために，「健康（ヘルス）」の面から，タバコ対策に歯科がかかわることに疑問をはさむ余地はない．

「隗より始めよ」は，タバコ対策に情熱と信念をもって活動された故五島雄一郎先生（元日本禁煙推進医師歯科医師連盟会長）の残された言葉である．

文　献

1) Canadian Cancer Society：*Evaluation of new warnings on cigarette packages*. http：//129.33.170.32/ccs/internet/standard/0,3182,3172_334419_436437_langId-en,00.html
2) Araki S et al：Dose-response relationship between tobacco consumption and melanin pigmentation in the attached gingiva. *Arch Environ Health*, **38**：375-378, 1983.
3) Hedin CA et al：Oral melanin pigmentation in 467 Thai and Malaysian people with special emphasis on smoker's melanosis. *J Oral Pathol Med*, **20**：8-12, 1991.
4) Hedin CA et al：Disappearance of smoker's melanosis after reducing smoking, *J Oral Pathol Med*, **22**：228-230, 1993.
5) Hedin CA：Smokers'melanosis. Occurrence and localization in the attached gingiva. *Arch Dermatol*, **113**：1533-1538, 1977.
6) 埴岡　隆ら：喫煙習慣が関係する歯肉メラニン色素沈着の疫学的研究．口衛誌，**43**：40-47，1993.
7) 三代幸彦ら：審美歯科と基礎医学　2．歯肉メラニンの生化学．*Dental Diamond*，**14**：296-301，1989.
8) Hedin CA et al：*In vitro* activation of amphibian dermal melanocytes by nicotine. *Scand J Dent Res*, **94**：57-65, 1986.
9) Roberto A et al：Uptake of 7,12-dimethylbenz (a) anthracene and benzo (a) pyrene in melanin-containing tissues. *Pharmacol Toxicol*, **79**：92-99, 1996.
10) 神田昌宏ら：CO_2レーザー照射における歯肉色素沈着（メラニン色素沈着）除去の色彩学的評価．日歯保存誌，**42**：738-743，1999.
11) Tamizi M et al：Treatment of severe physiologic pigmentation with free gingival autograft. *Quintessence Int*, **27**：555-558, 1996.

【喫煙と歯周病，禁煙治療】

Smoking, periodontal disease and smoking cessation practice

埴岡　隆

Hanioka　Takashi

Key words
smoking, periodontal disease,
smoking cessation,
translational research

はじめに

口腔と全身の関係の主な着目点は，口腔状態が全身の健康に影響を及ぼす場合と全身状態が口腔の健康に影響を及ぼす相互関係である。これ以外に，口腔と全身の健康に共通に影響を及ぼす要因の視点も，個人の健康や研究の発展を考える上で興味深い。歯周病に関しては喫煙がその共通項に当たる。医療従事者と研究者は，口腔と全身の健康の最大のリスク因子である喫煙に関して，今何が必要なのか，これから何が求められるかを知ることが重要である。

喫煙に関しては，環境対策，価格対策，未成年喫煙防止といった社会科学のアプローチも見逃せない。社会政策と診療や研究活動との関係も重要な視点である。本年は，この視点がとりわけ重要である。なぜなら，昨年WHOで合意されたタバコ規制枠組み条約を各国が批准する年にあたる。4月現在で日本を含め100を越える国が条約に署名を完了した。外務省は，2004年度の通常国会での成立を目指している。この条約は，社会政策をはじめ禁煙治療や研究にもその影響が及ぶ。

最近，喫煙者への介入に際して，様々の用語が使われだした。そして，これらの用語への批判もある。動機を高めるための禁煙教育は，健康教育に対応する。禁煙指導は最も一般的であるが，禁煙への指示性が強く，押し付け的な介入を想起させる場合がある。これに対して，禁煙を希望する者に，その禁煙を手助けするという意味から，禁煙支援という言葉が用いられている。一方，禁煙支援は，カウンセリング的な要素が強く，日常診療で行うには比較的長い時間を必要とする。そこで，簡便に動機を高めて禁煙に導く方法として，禁煙誘導が使われだした。さらに，ニコチン依存度が高い者への行動科学や薬理学アプローチを重視して行う禁煙治療という言葉も使われはじめた。禁煙治療は，喫煙が習慣であるという認識が，すでに過去のものになりつつあることを如実に反映している。

本稿では喫煙と歯周病の関係を解説するが，喫煙を，習慣として捉える以前の考えと，禁煙を難しくさせる病気であると捉える視点があることに注意する必要がある。

1．喫煙と歯周病の因果関係

1）関連の強固性，一致性

喫煙の歯周組織への影響は，1940年代の急性壊死性潰瘍性歯肉炎の報告に始まる。その後，1980年代の北欧の報告が少しあり，20世紀末になって世界規模で疫学・基礎・臨床研究の展開が始まった。喫煙と歯周病の関係は高いオッズ比で代表される。一般にオッズ比が2～3以上あると，臨床現場で気がつく程の関係の強さがあると言われるが，欧米・日本など喫煙率の高い地域で行われた疫学調査では，喫煙者は3～9倍の歯周病のリスク（オッズ比）があった[1]。さらに，年齢，性，糖尿病，歯周病原性細菌，栄養バランス，ストレス，飲酒などの多くの要因を考慮した場合にも，喫煙と歯周病は独立した関係があった。喫煙は，単独で最大の環境リスク要因と表現される。

スウェーデンの歯科衛生士の調査では，喫煙者は非

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喫煙者に比して歯槽骨の吸収が大きかった[2]。つまり，しっかりと歯を磨いていても，喫煙の代わりにはならない。大阪府の事業所の調査では，非喫煙者は，歯周病有病が45歳以上で顕著だったのに比して，喫煙者は，30歳以上で顕著に増加していた[3]。喫煙者は，比較的若い年齢層から歯周病が進行するものと思われる。

2）量－反応関係と喫煙影響の推計

多要因性疾患で，要因の因果関係を示す重要な性質のひとつに量－反応関係がある。喫煙量と歯周組織の破壊との間には，喫煙本数や年数と歯周破壊程度やオッズ比との関係が示されている。歯周病と喫煙との関連性を決定的にしたのは，世界でも最大規模の全米調査(NHANES Ⅲ)の分析である（図1）[4]。歯周ポケット（歯と歯茎の隙間）の深さが4mm以上でかつアタッチメント（歯と歯槽骨の結合）ロスも4mm以上の部位を有する者を歯周病有病者とした場合，喫煙のオッズ比は4.0倍であり，オッズ比と喫煙本数との間には正の相関性が示された。喫煙本数が9本以下の場合でもオッズ比は2以上であった。先に示した多変量の分析において，ごく少量の喫煙でも歯槽骨が吸収することをあわせると，歯周病においても，全身性疾患と同様に，喫煙に安全域はない。

全米調査の分析では，また，米国の歯周病有病者のうち41%（640万人）が現在の喫煙に，11%（170万人）が以前の喫煙に原因があると推計された。喫煙と元喫煙の影響を併せると，実に52%を占めることになる。歯周病原性細菌はこの調査に含まれなかったが，それにしても大きな数字である。この調査結果を利用して，回答による受動喫煙との関連性も分析された。非喫煙者が受動喫煙により歯周病となるリスクは1.6倍であることが報告された[5]。さらに，血清中のコチニン量による受動喫煙と子どものう蝕との関連性（未処置う蝕のオッズ比2.1）も示された[6]。タバコの煙の口腔への影響は顕著である。

3）生物学的説明性

疫学研究の成果を総合した結果，喫煙者の歯周状態の臨床的な主な特徴は，歯槽骨の吸収やアタッチメントロスが大きいこと，そして歯周病原性細菌が生息する深い歯周ポケットの部位数が多いこと，歯石が多いことがあげられる。では，喫煙者ではどのようなメカニズムで破壊が進展するのだろうか。この質問に答えるのが生物学的説明性である。

ニコチンは，歯槽骨と歯を結びつける歯根膜をつくる繊維芽細胞の配列と接着力を傷害し，日々の咀嚼により傷んだ歯根膜の修復能力を低下させて，歯と歯槽骨の結合を弱める。喫煙は，歯肉の微小循環機能を障害し，歯周ポケットの酸素を少なくする（図2）[7]。さらに，口腔細菌への抵抗力の基盤に

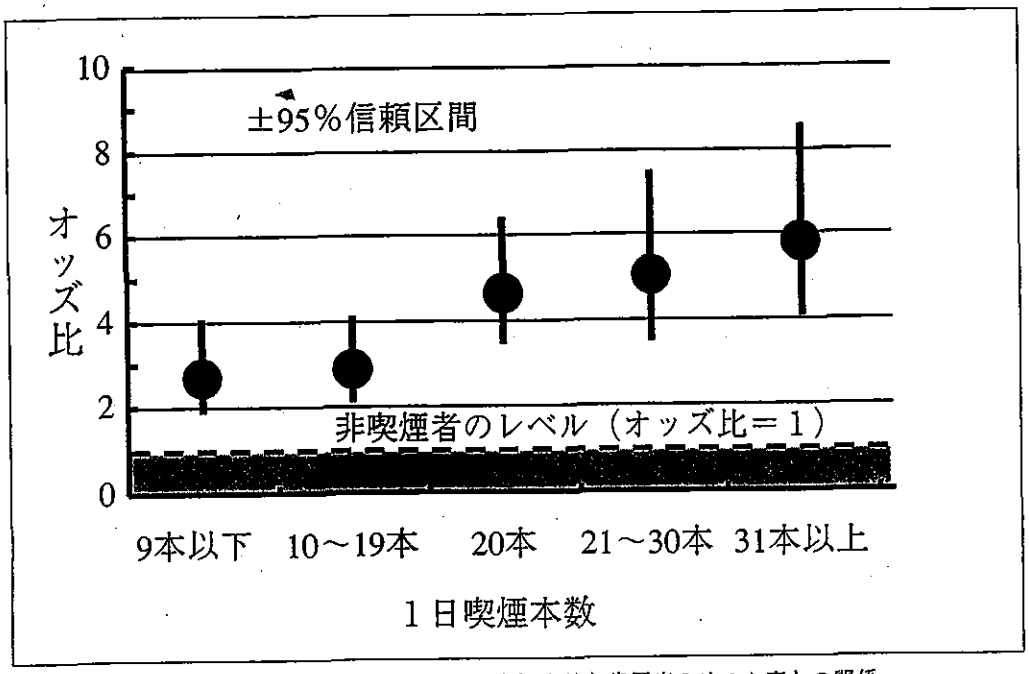

図1　全米12329人の調査による喫煙本数と歯周病のリスク度との関係

なる免疫機能も低下させる。その結果，歯周ポケットが深くなり，ますます酸素不足と自浄力の低下が進んで，歯周病原性細菌が増加し，やがて歯が脱落するようになる。

また，喫煙が歯槽骨の破骨細胞を活性化し，喫煙者の浅い歯周ポケットでも，歯周病原性細菌の割合が高い。サイトカインレベルでは，喫煙者の歯周組織破壊にTNF-αが関与しており，歯周ポケットの酸素分圧の低下や白血球機能亢進とともに発生するオキシダント種による歯周組織の細胞傷害による破壊メカニズムが示唆されている。

歯肉炎では，プラークの蓄積に伴い発赤・腫脹などの歯肉炎症が肉眼で観察されたり，歯肉出血となって自覚されたりするが，喫煙者ではプラークによる歯肉炎症反応が抑制され，機械的刺激による歯肉出血が少ない。歯肉にみられる炎症反応の抑制は，喫煙が歯肉の微小循環機能を障害していることと整合性がある。したがって，喫煙が生体の防御反応としての正常な炎症修復メカニズムを妨害し，組織破壊へと導くことが推測される。また，炎症反応の抑制は，破壊程度に比して，自覚症状が少なくなることを意味しており，早期発見という点で問題である。

2．歯周治療・禁煙の効果と全身性疾患

ニコチンの繊維芽細胞への影響は，創傷治癒にも影響し，抜歯後の治癒やインプラント治療など治療効果への影響は大きい。歯周治療の効果も，喫煙者は非喫煙者と比べて40～80%低下することが，非外科的処置，歯周組織再生誘導法，数年にわたる支援的ケアで示されており，喫煙は歯周病の予後の決定因子，再発のリスク要因でもある[8]。

禁煙すると歯周病のリスクは低減するのだろうか。全米調査では，禁煙後の経過年数とオッズ比との関係が示されている[11]。禁煙すると確実にリスクは低減していき，11年以上経過すると非喫煙者と同等のレベルに回復する（図3）。異なる年代におけるオッズ比の差から，禁煙による歯周病の発症率の低下を推計した報告がある[9]。1955年の歯周病発症を100とすると，米国では，喫煙率の低下により，2000年には31%，2020年には43%の歯周病の発症率が低下すると推計される。禁煙の効果が絶大であることが推測される。しかし，これは発症率の予測であり，喫煙者の歯周状態の回復は遅れることから，歯周病有病者が減少することには直結しない。日本の場合はどうか。日本のタバコ消費の増加は，米国に比べて30年の遅れがある。今，わが国では肺がんが急増期であるが，歯周病の発症も同様に急増期であることが予測される。

歯周病と全身性疾患との関係が明らかになってきた。歯周病と骨粗鬆症，心臓血管疾患および脳卒中，妊娠異常，糖尿病，呼吸器疾患の5つの疾患・症状

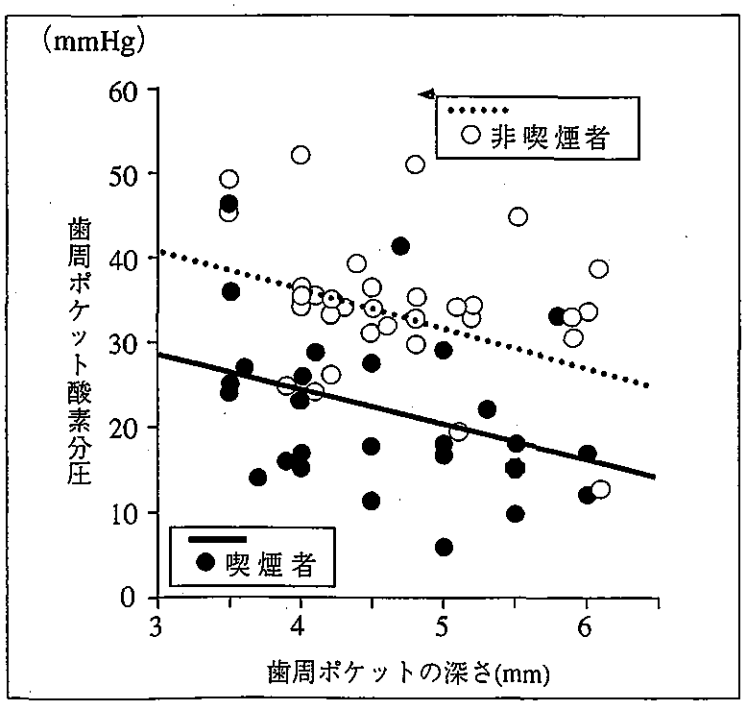

図2　歯周ポケットの酸素分圧の喫煙者と非喫煙者の比較

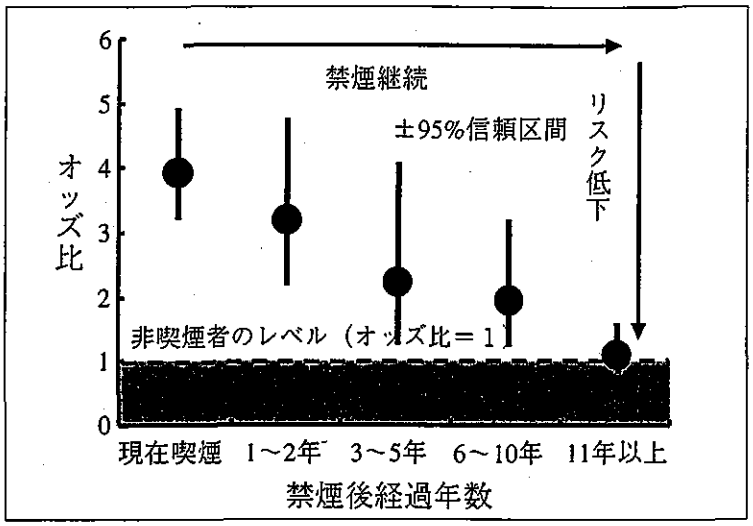

図3　禁煙後経過年数と歯周病リスクとの関係

との独立した関連性が示唆されている[10]。このうち糖尿病などいくつかの疾患については，メカニズムの解明といったところまで究明が進んでいる。一方，歯周病と関連するこれらの疾患は，全て，喫煙関連疾患であることも見逃せない。この点は，3つの点で重要である。ひとつは，喫煙が歯周病と5つの全身性疾患にどのような共通のメカニズムをもつかという生物学的関心である。次に，喫煙以外の歯周病の影響が，どの程度あるかという関心である。純粋にこの疑問を解決するためには，すでに米国では始まっているが，日本でも増加しつつある非喫煙者を対象とした研究の着手が期待される。3つ目は，全身性疾患と歯周病の予防へのアプローチの視点である。タバコ消費が減らない日本の現状を勘案すると，禁煙と歯周病の予防と治療は少なからず全身性疾患の減少に貢献できる。

まとめ

歯周病と喫煙の関係で最も期待されることは禁煙である。次に，禁煙の機会を妨げずに，そして，禁煙を遅らせないことを前提として，禁煙者の歯周治療の効果を高めたり，歯周病の再発のリスクを低減したりする方法の開発が期待される。このことに関しては，それぞれ，診療報酬や研究資金の供給といった問題も表れてくる。タバコ対策の先進諸外国の例として，前者については公的資金の提供が英国ではじまり，後者については，タバコ産業側からの研究資金の利用に関しては厳しい条件が設けられている。

今，日本人の身体に蓄積された喫煙の疾病リスクをいち早く低減させることが，臨床・研究・社会政策上で求められる。タバコ規制枠組み条約の批准を終え，その後に続く法改正や予算措置といった効果的な政策が早急に実現されることを期待したい。

文　献

1) 埴岡　隆, 歯周病のリスク因子, 健康寿命を延ばす歯周病医療. ザ・クイッテンセンス. 22: 52-58, 2003.
2) Bergström J, Eliasson S, Preber H, Cigarette smoking and periodontal bone loss. J Periodontol. 62: 242-246, 1991.
3) 埴岡　隆, 田中宗雄, 玉川裕夫ほか. CPITNを指標とした歯周組織の健康状態と喫煙習慣との関連性について. 日本歯周病学会誌. 35: 347-352, 1993.
4) Tomar SL, Asma S, Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. J Periodontol. 71: 743-751, 2000.
5) Arbes SJ, Agustsdottir H, Slade D, Environmental tobacco smoke and periodontal disease in the United States. Am J Public Health. 91: 253-257, 2001.
6) Aligne CA, Moss ME, Auinger P, *et al.*: Association of pediatric dental caries with passive smoking. JAMA. 289: 1258-1264, 2003.
7) Hanioka T, Tanaka M, Takaya K, *et al.*: Pocket oxygen tension in smokers and non-smokers with periodontal disease. J Periodontol. 71: 550-554, 2000.
8) Goodman SF, Novak MJ, Determination of prognosis, Carranza's clinical Periodontology, 9th ed. (eds. by Newman MG, Takei HH, Carranza FA). Saunders Co, Philadelphia. pp475-486, 2002.
9) Hujoel PP, del Aguila MA, DeRouen TA, *et al.*: A hidden periodontitis epidemic during the 20th century? Community Dent. Oral Epidemiol. 31: 1-6, 2003.
10) The American Academy of Periodontology. Proceedings of the periodontal-systemic connection: A state-of-the-science symposium. Ann. Periodontol. 6: 1-217, 2001.

COLUMN

特別企画

最新たばこの医学

無煙たばこ

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2003年10月，小田急沿線キオスクなどでガムたばこが試験販売された。日本では，大衆向けの無煙たばこ販売は経験が少なく，ガムの「たばこ」は世界初である。現在も，専門店やインターネットで他の無煙たばことともに購入できる。『アエラ』2004年3月29日号には「なぜか嗅ぎタバコ人気」の記事が掲載され，昨年の無煙たばこ輸入量は前年比42倍だったという。

無煙たばこの急増は，たばこ規制枠組み条約と関係がある。健康増進法第25条の受動喫煙防止は，条約批准の条件を整えるために制定され，喫煙場所が制限された。吸えない場所でニコチン補給ができるガムたばこは，条約の副作用ともいえる。

無煙たばこは，世界で用いられている。米国では早くから大衆に拡大し，口腔がんが急増した。大リーガーが使用して，テレビを通じて子どもに広がった。キャンプ場をめぐって選手に使用しないように説得した歯科医師の活躍で，今では映らないが，いったん若者に広がったものは，なかなか減らない。アフリカ，アジアの使用も多く，インド，スリランカでは，口腔がん多発と医療設備の不十分さが重なって問題は深刻である。インド系移民の多い英国でも使用がつづく。一方，スウェーデンでは有害物質の自主基準を設けて，害の少ないたばこを謳い文句に，若者を中心に増加している。

日本禁煙推進医師歯科医師連盟（大島明会長，会員1400人）は，2004年4月に緊急シンポジウム「無煙タバコか健康か」を開催し，無煙たばこの健康と喫煙対策への影響を危惧し，専門家と市民の視点から警告を発した。内容を整理すると，①禁煙のために使うニコチンガムである，②煙が出ないので安全である，③未成年者に販売しないので子どもは使わない，といった基本的な誤解や，④ガムたばこは食品衛生法の規制は受けない，⑤無煙たばこにより肺がんが減少したといった，法律解釈や疫学事象の問題点が指摘された。

いま判断を誤れば，将来への悪影響はきわめて重大である。まず，子どもへの使用拡大がもっとも懸念される。喫煙の行為が周囲から見えなくなり，喫煙を継続しやすくなる。子どもは，たばこをさまざまな経路で手に入れる能力がある。害が少ないという謳い文句のたばこは，じつは真に有害であった。疫学事象の解釈も慎重でなければならない。個人レベルでは，喫煙場所制限と禁煙教育で高まった禁煙動機は低下する。外国では，飴たばこ，歯磨きたばこも発売されており，ガムたばこは次世代たばこ製品の一つにすぎない。

わが国では「喫煙対策」であるが，諸外国では「たばこ対策」が用いられる。手ぬるい喫煙対策により喫煙率がなかなか下がらない欧米では，一部に無煙たばこ容認の声がある。喫煙者には，禁煙，ニコチン置換の次に，無煙たばこを勧める意見すらある。喫煙対策がほとんど進まなかったわが国には，まず，「喫煙対策」を徹底することが求められよう。

［はにおか・たかし／喫煙対策］

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2004, p. 1313–1315
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.3.1313–1315.2004

Vol. 42, No. 3

Correlation between Detection Rates of Periodontopathic Bacterial DNA in Carotid Coronary Stenotic Artery Plaque and in Dental Plaque Samples

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Received 25 April 2003/Returned for modification 11 August 2003/Accepted 29 November 2003

Utilizing PCR, the 16S rRNA detection rates for *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Bacteroides forsythus*, *Treponema denticola*, and *Campylobacter rectus* in samples of stenotic coronary artery plaques were determined to be 21.6, 23.3, 5.9, 23.5, and 15.7%, respectively. The detection rates for *P. gingivalis* and *C. rectus* correlated with their presence in subgingival plaque.

It has been estimated that several hundred different species of bacteria inhabit the oral cavity. Among these, periodontal disease-associated bacteria adhere to and colonize the subgingival pocket, forming a biofilm (dental plaque). Once these bacteria are incorporated into biofilms, attenuation of the immune response against them may take place as a result of the reduction of phagocytosis, and the effectiveness of antibiotics is diminished (4, 15). These effects of biofilms may induce persistent infection in periodontal lesions. It has been suggested that periodontal disease-associated bacteria can penetrate gingival tissues and enter the bloodstream (8, 19). Microorganisms in the periodontal pocket may also induce a continuous benign bacteremia (5, 22, 32). Ross (30) reported that chronic infection can be one of the contributing factors involved in atherosclerosis. Several epidemiological studies have shown a positive correlation between periodontal disease and ischemic heart disease (2, 9, 27). However, Hujoel et al. (12) reported that their epidemiologic study did not find any evidence of an association between periodontal disease and heart disease. Nevertheless, members of our group and others have demonstrated that periodontal bacterial DNA can be detected in atherosclerotic lesions of aortic tissue (3, 11, 25, 34).

In this study, we sought to detect periodontal disease-associated bacterial DNA from stenotic coronary artery plaques recovered from 51 patients who were scheduled to receive surgical procedures to eliminate the plaque. We obtained informed consent from each subject in the present study. One week prior to surgery, we examined the periodontal status of each subject using a periodontal pocket probe. Thirty-four (30 males and 4 females; mean age, 64.3) of the subjects exhibited four or more periodontal lesions (probing depth, 4 mm and more). Seventeen (13 males and 4 females; average age, 63.5) demonstrated fewer than four periodontal lesions. Teeth were initially gently dried with sterile cotton swabs. After the removal of supragingival plaque with sterile cotton swabs, subgingival plaque samples were collected with sterilized scalers and transferred to 100 μl of sterilized phosphate-buffered saline (pH 7.4). The samples obtained from two periodontitis sites, which represented the deepest periodontal pockets, were pooled for analysis.

To eliminate blood contamination, the vascular endothelial samples were placed in sterilized phosphate-buffered saline and mixed gently, and tissue samples were transferred to fresh tubes. DNA was extracted by using a Puregene kit (Gentra Systems, Minneapolis, Minn.) according to the manufacturer's instructions. Briefly, samples (approximately 100 mg) were dissociated with a spatula, and 6 ml of cell lysis solution was added. Samples were then homogenized thoroughly with a tube pestle. Lysates were incubated at 65°C for 60 min, and further incubation was performed for 30 min after addition of RNase. After addition of protein precipitation solution, lysates were centrifuged for 10 min at 2,000 × *g*. DNA was concentrated by addition of 6 ml of 100% isopropanol to the supernatant and subsequent centrifugation. The DNA pellet obtained was then processed for PCRs. Samples from two young male patients (average age, 11.5) with Kawasaki disease who had healthy periodontal tissue were also examined in this study. One week prior to the cardiac surgical procedures, the periodontal disease status of the 53 patients was assessed and dental plaque samples from the periodontal sites were collected. Standard precautions were taken in handling reagents and samples as well as double blinding the analysts.

To detect *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Bacteroides forsythus*, *Treponema denticola*, and *Campylobacter rectus*, PCR was performed by the method described by Ashimoto et al. (1). Amplified fragments were confirmed following nucleotide sequencing by the dideoxy-chain termination method (31), using a 310A DNA sequencer (Applied Biosystems, Foster, Calif.).

In 51 adult patients, detection rates for 16S rRNA from *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in the coronary artery plaque samples were 21.6, 23.3, 5.9, 23.5, and 15.7%, respectively. The detection frequencies for *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in subgingival plaque were 54.9, 33.3,

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TABLE 1. Comparison of detection rates of 16S rRNA of *P. gingivalis*, *A. actinomycetemcomitans*, *B. forsythus*, *T. denticola*, and *C. rectus* in samples of stenotic coronary artery plaque and subgingival dental plaque from patients possessing fewer than four periodontal lesions and those from patients with four or more periodontal lesions

Species	Detection rate (%)			
	Patients possessing <4 periodontal lesions (n = 17)		Patients possessing 4 or more periodontal lesions (n = 34)	
	Subgingival	Coronary artery	Subgingival	Coronary artery
P. gingivalis	47.1	5.8	58.8	29.4
A. actinomycetemcomitans	41.2	17.6	29.4	26.5
B. forsythus	41.2	5.8	41.2	5.9
T. denticola	58.8	11.8	67.7	29.4
C. rectus	29.4	17.6	41.2	14.7

41.2, 64.7, and 37.3%, respectively. *A. actinomycetemcomitans* and *C. rectus* were detected in a coronary artery sample from one of the two young patients with Kawasaki disease. No defined gingival inflammation, dental plaque accumulation, or dental calculus was found in these patients. Kawasaki disease often accompanies coronary aneurysms; however, this disease is not normally associated with periodontitis. In our previous studies, no periodontopathic bacterial DNA was detected in healthy arterial walls. Recently, Lalla et al. (18) reported that oral infection by *P. gingivalis* accelerates early atherosclerosis and that two of nine infected mice tested demonstrated the presence of DNA for this organism. These reports suggested that there is a relationship between the detection of periodontopathic bacterial DNA in atherosclerotic lesions and periodontal disease.

Table 1 shows a comparison of the detection rates for periodontal pathogens from subgingival plaque and stenotic coronary artery plaque in patients possessing four or more periodontal lesions with those for patients with fewer than four lesions. The detection rates for *P. gingivalis*, *A. actinomycetemcomitans*, and *T. denticola* in patients possessing four or more periodontal lesions were higher than those for patients with fewer than four lesions. The *P. gingivalis* 16S rRNA locus was detected from the coronary artery in 10 of 34 patients possessing four or more periodontal lesions and in 1 of 17 patients possessing fewer than four lesions. We detected *P. gingivalis* in both coronary artery and subgingival plaque samples from 10 of 11 patients. *A. actinomycetemcomitans* in coronary artery samples was detected for 9 of 34 patients possessing four or more periodontal lesions and 3 of 17 patients with fewer than four lesions. Unexpectedly, we detected *A. actinomycetemcomitans* in both coronary artery and subgingival plaque samples for only 2 of 12 patients. The sampling sites for subgingival plaque in the present study were the deepest periodontal pockets. Normally anaerobic conditions might be expected to produce these results, because *A. actinomycetemcomitans* is a facultative anaerobic bacterial species. *B. forsythus* in coronary artery samples was the least frequent of the periodontal pathogens examined. We detected this organism in only 1 of 17 patients possessing fewer than four periodontal lesions and 2 of 34 patients possessing four or more periodontal lesions. We detected the microorganisms in samples of both coronary artery plaque and subgingival plaque from two of the three patients. *T. denticola* was detected in coronary artery samples from 2 of 17 patients possessing fewer than four periodontal lesions and 10 of 34 patients possessing four or more lesions. We detected *T. denticola* in subgingival plaques from 7 of 10 patients whose coronary artery samples were positive for these microorganisms. Detection of *C. rectus* in coronary artery samples was positive for 3 of 17 patients possessing fewer than four periodontal lesions and 5 of 34 patients possessing four or more lesions. We detected the microorganisms in samples of both coronary artery plaque and subgingival plaque from four of these five patients. Statistical analysis using a chi-square test showed that detection of 16S rRNA of *P. gingivalis* and *C. rectus* in coronary artery plaque samples significantly correlated with colonization by these organisms in subgingival sites ($P < 0.01$).

In this study, sampling from periodontal pockets was performed 1 week in advance of sampling from coronary arteries. It is possible to cause bacteremia by sampling the periodontal pockets. Roberts et al. (28) reported that the highest yield of microorganisms from blood samples occurs at approximately 30 s after the onset of dentally induced bacteremia. The peak of bacteremia after injection of human oral microorganisms into the bloodstream was within a minute in animal experiments (33). The reduction of bacteremia by host defense systems occurs over several minutes after dental instrumentation (26). In addition, daily tooth brushing and mastication were also reported to induce bacteremia (10, 28). Moreover, the detection rate for *T. denticola* in our previous study is similar to that of the present study. Taken together, these results suggest that probing and sampling of subgingival plaque 1 week in advance of sampling from the heart should not affect the detection rates in arterial plaque.

A relationship between chronic inflammation and atherosclerosis has been reported (21, 29). Previously, we detected the 316-bp 16S rRNA of *T. denticola* in plaque samples from aneurysmal sites in 6 of 26 patients (23.1%), along with antigens of *T. denticola* in and around foam cells in the lesions (25). However, no other periodontal disease-associated bacterial DNA was detected. Presumably, *T. denticola* reaches aneurysmal sites by means of its high motility. Recently periodontal pathogens were detected from samples of carotid endarterectomy (3, 11). It has also been demonstrated that subgingival bacteria, such as *P. gingivalis* and *A. actinomycetemcomitans*, are able to invade both epithelial and endothelial cells (6, 7, 19, 24, 36). In addition, Madianos et al. (23) showed that *P. gingivalis* can persist and multiply within epithelial cells. In the present study, the detection rates for *P. gingivalis* and *C. rectus* in coronary artery plaques correlated with detection from subgingival plaque. The increased detection rates for *P. gingivalis* and *T. denticola* in coronary artery samples are also paralleled by their detection in periodontal pockets. These results suggest that these microorganisms in periodontal pockets may penetrate subgingival epithelial cells and invade the bloodstream.

Outer membrane vesicles of *P. gingivalis* in macrophages appear capable of inducing foam cell formation in these cells (16, 17). Likewise, it has been reported that when $ApoE^{+/-}$ atherosclerosis-prone mice were fed a high-fat diet and in-

fected with *P. gingivalis*, atherosclerosis was further accelerated (20). In addition, induction of atherosclerosis by oral infection with *P. gingivalis* in ApoE$^{-/-}$ mice was reported (18). Furthermore Jain et al. (13) reported that rabbits with experimentally induced periodontitis from *P. gingivalis* had more extensive accumulations of lipids in the aorta than did control animals, and there was a positive correlation between the severity of periodontal disease and the extent of lipid deposition. Poor oral hygiene was also found to be a high risk factor for infective endocarditis (35). Kiechl et al. (14) demonstrated that polymorphism of the toll-like receptor 4, which attenuates receptor signaling and diminishes the inflammatory response to gram-negative bacteria, is associated with a decreased risk of atherosclerosis. Thus, asymptomatic bacteremia due to periodontopathic gram-negative bacteria may accelerate stenotic coronary artery plaque progression. Taken together, the present study supports the hypothesis that periodontal disease-associated bacteria could enter the bloodstream and play a direct or indirect role in the progression of stenotic coronary artery plaque lesions.

This investigation was supported in part by a grant of the Waksman Foundation of Japan, Inc., and grant 5A01 from the Oral Health Science Center of Tokyo Dental College.

REFERENCES

1. **Ashimoto, A., C. Chen, I. Bakker, and J. Slots.** 1996. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. Oral Microbiol. Immunol. 11:266–273.
2. **Beck, J. D., J. Pankow, H. A. Tyroler, and S. Offenbacher.** 1999. Dental infections and atherosclerosis. Am. Heart J. 138:S528–S533.
3. **Chiu, B.** 1999. Multiple infections in carotid atherosclerotic plaques. Am. Heart J. 138:S534–S536.
4. **Costerton, J. W., P. S. Stewart, and E. P. Greenberg.** 1999. Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322.
5. **Daly, C. G., D. H. Mitchell, J. E. Highfield, D. E. Grossberg, and D. Stewart.** 2001. Bacteremia due to periodontal probing: a clinical and microbiological investigation. J. Periodontol. 72:210–214.
6. **Deshpande, R. G., M. B. Khan, and C. A. Genco.** 1998. Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. Infect. Immun. 66:5337–5343.
7. **Dorn, B. R., W. A. Dunn, Jr., and A. Progulske-Fox.** 1999. Invasion of human coronary artery cells by periodontal pathogens. Infect. Immun. 67:5792–5798.
8. **Fives-Taylor, P., D. Meyer, and K. Mintz.** 1995. Characteristics of *Actinobacillus actinomycetemcomitans* invasion of and adhesion to cultured epithelial cells. Adv. Dent. Res. 9:55–62.
9. **Genco, R., S. Offenbacher, and J. Beck.** 2002. Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. J. Am. Dent. Assoc. 133(Suppl.):14S–22S.
10. **Guntheroth, W. G.** 1984. How important are dental procedures as a cause of infective endocarditis? Am. J. Cardiol. 54:797–801.
11. **Haraszthy, V. I., J. J. Zambon, M. Trevisan, M. Zeid, and R. J. Genco.** 2000. Identification of periodontal pathogens in atheromatous plaques. J. Periodontol. 71:1554–1560.
12. **Hujoel, P. P., M. Drangsholt, C. Spiekerman, and T. A. DeRouen.** 2000. Periodontal disease and coronary heart disease risk. JAMA 284:1406–1410.
13. **Jain, A., E. L. Batista, Jr., C. Serhan, G. L. Stahl, and T. E. Van Dyke.** 2003. Role for periodontitis in the progression of lipid deposition in an animal model. Infect. Immun. 71:6012–6018.
14. **Kiechl, S., E. Lorenz, M. Reindl, C. J. Wiedermann, F. Oberhollenzer, E. Bonora, J. Willeit, and D. A. Schwartz.** 2002. Toll-like receptor 4 polymorphisms and atherogenesis. N. Engl. J. Med. 347:185–192.
15. **Kolenbrander, P. E.** 2000. Oral microbial communities: biofilms, interactions, and genetic systems. Annu. Rev. Microbiol. 54:413–437.
16. **Kuramitsu, H. K., I. C. Kang, and M. Qi.** 2003. Interactions of *Porphyromonas gingivalis* with host cells: implications for cardiovascular diseases. J. Periodontol. 74:85–89.
17. **Kuramitsu, H. K., M. Qi, I. C. Kang, and W. Chen.** 2001. Role for periodontal bacteria in cardiovascular diseases. Ann. Periodontol. 6:41–47.
18. **Lalla, E., I. B. Lamster, M. A. Hofmann, L. Bucciarelli, A. P. Jerud, S. Tucker, Y. Lu, P. N. Papapanou, and A. M. Schmidt.** 2003. Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein e-null mice. Arterioscler. Thromb. Vasc. Biol. 23:1405–1411.
19. **Lamont, R. J., A. Chan, C. M. Belton, K. T. Izutsu, D. Vasel, and A. Weinberg.** 1995. *Porphyromonas gingivalis* invasion of gingival epithelial cells. Infect. Immun. 63:3878–3885.
20. **Li, L., E. Messas, E. L. Batista, Jr., R. A. Levine, and S. Amar.** 2002. *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. Circulation 105:861–867.
21. **Libby, P.** 2002. Inflammation in atherosclerosis. Nature 420:868–874.
22. **Lofthus, J. E., M. Y. Waki, D. L. Jolkovsky, J. Otomo-Corgel, M. G. Newman, T. Flemmig, and S. Nachnani.** 1991. Bacteremia following subgingival irrigation and scaling and root planing. J. Periodontol. 62:602–607.
23. **Madianos, P. N., P. N. Papapanou, U. Nannmark, G. Dahlen, and J. Sandros.** 1996. *Porphyromonas gingivalis* FDC381 multiplies and persists within human oral epithelial cells in vitro. Infect. Immun. 64:660–664.
24. **Meyer, D. H., J. E. Lippmann, and P. M. Fives-Taylor.** 1996. Invasion of epithelial cells by *Actinobacillus actinomycetemcomitans*: a dynamic, multistep process. Infect. Immun. 64:2988–2997.
25. **Okuda, K., K. Ishihara, T. Nakagawa, A. Hirayama, Y. Inayama, and K. Okuda.** 2001. Detection of *Treponema denticola* in atherosclerotic lesions. J. Clin. Microbiol. 39:1114–1117.
26. **Pallasch, T. J., and J. Slots.** 1996. Antibiotic prophylaxis and the medically compromised patient. Periodontol. 2000 10:107–138.
27. **Persson, R. E., L. G. Hollender, V. L. Powell, M. MacEntee, C. C. Wyatt, H. A. Kiyak, and G. R. Persson.** 2002. Assessment of periodontal conditions and systemic disease in older subjects. II. Focus on cardiovascular diseases. J. Clin. Periodontol. 29:803–810.
28. **Roberts, G. J., P. Gardner, and N. A. Simmons.** 1992. Optimum sampling time for detection of dental bacteraemia in children. Int. J. Cardiol. 35:311–315.
29. **Ross, R.** 1999. Atherosclerosis is an inflammatory disease. Am. Heart J. 138:S419–S420.
30. **Ross, R.** 1999. Atherosclerosis—an inflammatory disease. N. Engl. J. Med. 340:115–126.
31. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
32. **Sconyers, J. R., J. J. Crawford, and J. D. Moriarty.** 1973. Relationship of bacteremia to toothbrushing in patients with periodontitis. J. Am. Dent. Assoc. 87:616–622.
33. **Silver, J. G., L. Martin, and B. C. McBride.** 1975. Recovery and clearance rates of oral microorganisms following experimental bacteraemias in dogs. Arch. Oral Biol. 20:675–679.
34. **Stelzel, M., G. Conrads, S. Pankuweit, B. Maisch, S. Vogt, R. Moosdorf, and L. Flores-de-Jacoby.** 2002. Detection of *Porphyromonas gingivalis* DNA in aortic tissue by PCR. J. Periodontol. 73:868–870.
35. **Strom, B. L., E. Abrutyn, J. A. Berlin, J. L. Kinman, R. S. Feldman, P. D. Stolley, M. E. Levison, O. M. Korzeniowski, and D. Kaye.** 2000. Risk factors for infective endocarditis: oral hygiene and nondental exposures. Circulation 102:2842–2848.
36. **Weinberg, A., C. M. Belton, Y. Park, and R. J. Lamont.** 1997. Role of fimbriae in *Porphyromonas gingivalis* invasion of gingival epithelial cells. Infect. Immun. 65:313–316.

Oral Microbiology Immunology 2004: 19: 61–64
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Oral Microbiology and Immunology

Short communication

A comparison of the antibacterial efficacies of essential oils against oral pathogens

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Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol Immunol 2004: 19: 61–64 © Blackwell Munksgaard, 2004.

Cariogenic bacteria and periodontopathic bacteria are present in dental plaque as biofilms. In this study, we investigated the antibacterial effects of essential oils on the following oral bacteria: *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Streptococcus mutans*, and *Streptococcus sobrinus*. We tested manuka oil, tea tree oil, eucalyptus oil, lavandula oil, and romarinus oil and determined their minimum inhibitory concentration and minimum bactericidal concentration. The essential oils inhibited the growth of the bacteria tested, manuka oil being the most effective. Minimum bactericidal concentration values showed that lavandula oil acts bacteriostatically, and the remaining oils, bactericidally. Periodontopathic bacterial strains tested were killed completely by exposure for 30 s to 0.2% manuka oil, tea tree oil or eucalyptus oil. Tea tree oil and manuka oil showed significant adhesion-inhibiting activity against *P. gingivalis*. All the essential oils tested inhibited the adhesion of *S. mutans*. This study showed that, among the essential oils tested, manuka oil and tea tree oil in particular had strong antibacterial activity against periodontopathic and cariogenic bacteria. From the viewpoint of safety, we also examined the effects of these essential oils on cultured human umbilical vein endothelial cells and found that, at a concentration of 0.2%, they had little effect on cultured cells.

Key words: antibacterial effect; cariogenic bacteria; essential oil; periodontopathic bacteria

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Accepted for publication August 27, 2003

The human oral cavity is inhabited by more than 500 species of bacteria at 10^8–10^9 bacteria per ml saliva or mg dental plaque (19). Caries is a disease caused by the plaque bacteria such as *Streptococcus mutans* and *Streptococcus sobrinus* (5, 10). Gram-negative bacilli such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* have frequently been isolated from periodontal lesions and have been shown to be related to the onset and progression of periodontal disease (12, 23–25, 27, 30). Furthermore, it has been suggested in recent years that oral bacteria are associated with many systemic diseases such as pneumonia and cardiovascular disease (2, 9, 17); therefore, the need for oral care in a systemic health regimen has also been emphasized. Dental plaques that have been deposited firmly as biofilms must be removed mechanically, but antibacterial mouthrinses are effective in decreasing tooth surface plaque. In general, mouthrinses contain fluorides, alcohols, and detergents or antibacterial substances. Ideal antibacterial substances must be effective against more microorganisms, act rapidly, maintain activity at low concentrations, have no side effects, and be usable without causing discomfort. Frequently used antibacterial chemicals include povidone iodine products, chlorhexidine, and cetylpyridinium chloride; in addition, natural antibacterial substances have attracted attention (8, 13, 20). In this study, we compared the antibacterial activities of phytochemical essential oils against oral bacteria.

Leptospermum scoparium (manuka) oil, *Melaleuca alternifolia* (tea tree) oil, *Eucalyptus radiata* (eucalyptus) oil, *Lavandula officinalis* (lavandula) oil, and *Romarinus officilalis* (romarinus) oil were obtained from Laboratoire PhytoSun'Aroms (Ance, France), and used in this study. An oil containing ω 3, 6, & 9 fatty acids was used as a control. The cariogenic bacteria *S. mutans* JC-2, and *S. sobrinus* 6715 and B13, and the periodontopathic bacteria *A. actinomycetemcomitans* strains Y4, ATCC 29523, ATCC 29524 and ATCC 33384, *P. gingivalis* strains ATCC 33277, ATCC 53977, Su63, and W50, and *F. nucleatum*

strains ATCC 25586, #2 and #20, were used in this study. These strains were maintained anaerobically on blood agar plates containing trypticase soy agar (Becton Dickinson Microbiology System, Cockeysville, MD) supplemented with 10% defibrinated horse blood, hemin (5 μg/ml; Sigma Chemical Co., St. Louis, MO) and menadione (0.5 μg/ml; Wako Pure Chemical Industries, Osaka, Japan).

The minimum inhibitory concentrations of the respective essential oils against oral bacteria were determined with liquid cultures in 96-well cell culture plates according to a modification of the method described by Shapiro et al. (22). Todd Hewitt broth (Becton Dickinson Microbiology System) was used for mutans streptococci and *F. nucleatum*. For *A. actinomycetemcomitans*, Todd Hewitt broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used. For *P. gingivalis*, trypticase soy broth (Becton Dickinson Microbiology System) containing hemin and menadione was used. Serial dilutions (1.0–0.002%) of each essential oil were prepared in each culture medium. Aliquots (200 μl) of each dilution were dispensed in 96-well cell culture plates (Nunc, Naperville, IL). Subsequently, 10^5–10^6 test bacteria that had been cultured overnight in each culture medium were inoculated into each well, and cultured for 1–2 days under anaerobic conditions. Then the absorbance was measured at 595 nm. The highest dilution at which no growth ($OD_{595} \leq 0.05$) was observed, was defined as the minimum inhibitory concentration. As shown in Table 1, manuka oil effectively inhibited the growth of oral bacteria. The minimum inhibitory concentrations were 0.25% and 0.13% against oral streptococci, and 0.03% against the gram-negative bacteria tested. Tea tree oil and eucalyptus oil had an minimum inhibitory concentration of 1% against oral streptococci, and 0.06–0.5% against the gram-negative bacteria tested. Lavandula oil and romarinus oil inhibited the growth of gram-negative bacteria but did not inhibit the growth of oral streptococci even at 1%. Control oil did not inhibit the growth of any of the bacteria tested in this study at a concentration of 1%. After the measurement of minimum inhibitory concentration, 50-μl aliquots of cultures were taken from wells showing no bacterial growth, inoculated onto blood agar plates, and cultured for 1 week under anaerobic conditions. The concentration at which no bacterial growth was observed was defined as the minimum bactericidal concentration. The minimum bactericidal concentrations of the essential oils except lavandula oil were the same as or 2–4 times the minimum inhibitory concentrations of the respective essential oils (Table 2). However, lavandula oil did not show any bactericidal activity at its minimum inhibitory concentration, suggesting that it acts bacteriostatically. Exposure for 30 s to 0.5% manuka oil, tea tree oil and eucalyptus oil killed *S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* or *F. nucleatum* completely (data not shown). These three essential oils completely killed gram-negative bacterial strains tested, even at 0.2% concentration. Romarinus oil also exhibited bactericidal activity against tested bacteria. Lavandula oil was not effectively bactericidal against any bacterial strain tested.

Table 1. Minimum inhibitory concentration values (%) for essential oils towards oral bacteria

Strains	Essential oils				
	Manuka	Tea tree	Eucalyptus	Lavandula	Romarinus
S. sobrinus					
6715	0.13	1.0	1.0	>1.0	>1.0
B13	0.25	1.0	1.0	>1.0	>1.0
S. mutans					
JC-2	0.25	1.0	1.0	>1.0	>1.0
A. actinomycetemcomitans					
Y4	0.03	0.5	0.5	0.5	0.5
ATCC 29523	0.03	0.5	0.5	0.5	0.5
ATCC 29524	0.03	0.5	0.5	0.5	0.5
ATCC 33384	0.03	0.25	0.5	0.5	0.5
P. gingivalis					
ATCC 33277	0.03	0.13	0.5	0.5	1.0
ATCC 53977	0.03	0.13	0.25	0.5	0.5
W50	0.03	0.25	0.5	1.0	1.0
Su63	0.03	0.13	0.5	0.5	1.0
F. nucleatum					
ATCC 25586*	0.03	0.06	0.13	0.25	0.5
#2*	0.03	0.06	0.25	0.25	0.5
#20*	0.03	0.06	0.25	0.25	0.5

Each essential oil was tested at concentrations of 1.0%, 0.5%, 0.25%, 0.13%, 0.06%, 0.03%, 0.016%, 0.008%, 0.004%, and 0.002%.
Optical density (OD) at 595 nm was measured on the day after inoculation.
*: measured after 2 days.
Each assay was repeated on at least 3 different days.

Dental plaques are understood to be biofilms composed of many species of bacteria, and the adhesive ability of these bacteria seems to be an important pathogenic factor. Therefore, we investigated the inhibitory effect of essential oils on the adhesion of *P. gingivalis* and *S. mutans* to the bottom of cell culture wells. The inhibitory effect of essential oils on the adhesion of *P. gingivalis* ATCC 33277 and *S. mutans* JC-2 to the bottom of cell culture plates

Table 2. Minimum bactericidal concentration values (%) for essential oils towards oral bacteria

Strains	Essential oils				
	Manuka	Tea tree	Eucalyptus	Lavandula	Romarinus
S. sobrinus					
6715	0.25	1.0	1.0	>1.0	>1.0
B13	0.25	1.0	1.0	>1.0	>1.0
S. mutans					
JC-2	0.25	1.0	1.0	>1.0	>1.0
A. actinomycetemcomitans					
Y4	0.13	0.5	0.5	>1.0	1.0
ATCC 29523	0.13	0.5	0.5	>1.0	1.0
ATCC 29524	0.13	0.5	0.5	>1.0	1.0
ATCC 33384	0.13	0.5	0.5	>1.0	1.0
P. gingivalis					
ATCC 33277	0.06	0.5	0.5	>1.0	1.0
ATCC 53977	0.03	0.13	0.25	>1.0	0.5
W50	0.06	0.25	0.5	>1.0	1.0
Su63	0.06	0.25	0.5	>1.0	1.0
F. nucleatum					
ATCC 25586*	0.03	0.25	0.5	>1.0	0.5
#2*	0.03	0.25	0.5	>1.0	0.5
#20*	0.03	0.25	0.5	>1.0	0.5

Each assay was repeated on at least 3 different days.

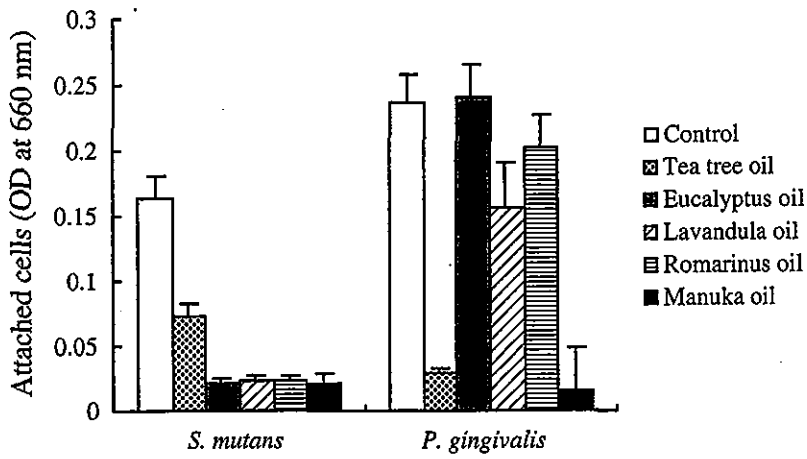

Fig. 1. Inhibitory effect of essential oils on adhesion of *P. gingivalis* and *S. mutans*. Bacteria attached to culture dishes were stripped off with a cell scraper, and cell suspensions were measured for absorbance. All essential oils significantly inhibited adhesion of *S. mutans* (*vs.* control; $P < 0.05$). Tea tree oil and manuka oil showed significant adhesion-inhibiting effects on *P. gingivalis* (vs. control; $P < 0.05$). Data are means ± standard deviation from three duplicate independent assays.

(Nunc) was also examined. Bacterial cells were cultured with 0.1% of each essential oil for 4 days under anaerobic conditions. After culture, the culture medium and the floating bacterial cells were removed, and the wells were rinsed twice with PBS. Subsequently, PBS was added, and the attached bacteria were stripped off with a cell scraper, and the turbidity of the bacterial suspension was measured to examine the adhesion-inhibiting effect. The Mann-Whitney *U*-test was used to identify statistically significant differences. As shown in Fig. 1, all the essential oils had marked adhesion-inhibiting effects on *S. mutans* ($P < 0.05$). Tea tree oil and manuka oil showed a significant adhesion-inhibiting effect on *P. gingivalis* ($P < 0.05$). The inhibitory effect on adhesion found suggests that the phytochemical oils suppress the biofilm formation.

It might be suspected that strongly antibacterial essential oils have side effects on the host; however, a cytotoxicity test on human epithelial cells and fibroblasts showed that tea tree oil had a low toxicity (26). In the present study, the effect of essential oils on the host was examined using human umbilical vein endothelial cells (HUVEC) 8715 (BioWhittaker Inc., Walkersville, MD). Essential oils were added to precultured, confluent HUVEC to final concentrations of 0.2% or 0.5%, and each culture was continued to examine the effect of essential oils on the cells under a microscope or with a Cell Titer 96™ AQ Assay Kit (Promega, Madison, WI). Figure 2 shows the relative activity (%) of HUVEC at each essential oil concentration, defining the activity of HUVEC measured using a Cell Titer 96™ AQ Assay Kit in the absence of essential oils as 100%. Essential oils at 0.5% increased the number of dying cells and decreased the activity of cells, but had little effect on these cells at 0.2%. In particular, lavandula oil had little effect on these cells, which showed virtually no differences from controls. Microscopic examination also showed that at an essential oil concentration of 0.5%, considerable numbers of cells became detached from the bottom of culture wells, but at an essential oil concentration of 0.2%, only a few cells became detached.

At present, chlorhexidine and povidone iodine products are generally used as antibacterial agents for cleaning the oral cavity (11). Natural antibacterial substances have attracted attention as being safer (14–16, 28). Studies of the antibacterial activity of essential oils, mainly tea tree oil, have pointed out their usefulness (1, 3, 4, 6, 7, 18, 22). In this study, we compared several essential oils with regard to their antibacterial activity against cariogenic and periodontopathic bacteria. Among the essential oils used, tea tree oil and manuka oil, in particular the latter, showed strong antibacterial activity. It has been reported that the antimicrobial activity of manuka oil is associated with a fraction containing several triketones including leptospermone (18, 29). Among the constituents of tea tree oil, the main constituents with antibacterial activity are terpinen-4-ol and γ-terpinene. Tea tree oil has been reported to have a several times stronger effect than these constituents alone (3). In addition to these ingredients, tea tree oil is known to contain about 100 different molecules, which presumably synergistically increase the effect on the oral bacteria studied. Although it has been indicated that lipophilic terpenens act on the phospholipid layer of the cell membrane and can destroy its normal structure and function (4), the antibacterial

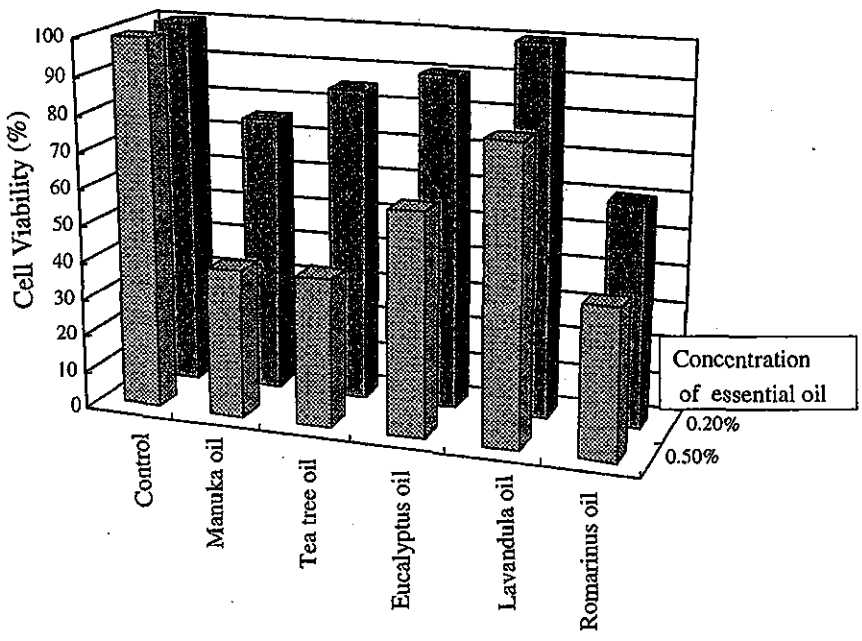

Fig. 2. Effect of essential oils on the viability of HUVEC. Essential oils were added to precultured, confluent HUVEC to final concentrations of 0.2% or 0.5%. Defining the activity of HUVEC measured using a Cell Titer 96™ AQ Assay Kit in the absence of essential oils as 100%, the relative activity of HUVEC at each essential oil concentration was expressed as a percentage. Data are means from three duplicate independent assays with standard deviations of less than 15%.

mechanisms of essential oils still need to be elucidated.

We were thus able to show that essential oils, particularly manuka oil and tea tree oil, exhibited growth-inhibiting and bactericidal effects on periodontopathic and cariogenic bacteria, and also adhesion-inhibiting effects on *P. gingivalis* and *S. mutans*. From the viewpoint of safety, these essential oils seem to be promising antibacterial substances for oral care at concentrations of 0.2% or lower, at which they had little effect on human cells.

Acknowledgment

This study was supported by a grant from the Kanpou Science Foundation.

References

1. Banes-Marshall L, Cawley P, Phillips CA. *In vitro* activity of *Melaleuca alternifolia* (tea tree) oil against bacterial and Candida spp. isolates from clinical specimens. Br J Biomed Sci 2001: **58**: 139–145.
2. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Pariodontal disease and cardiovascular disease. J Periodontol 1996: **67**: 1123–1137.
3. Cox SD, Mann CM, Marham JL. Interactions between components of the essential oil of *Melaleuca alternifolia*. J Appl Microbiol 2001: **91**: 492–497.
4. Cox SD, Mann CM, Markham JL, et al. The mode of antibacterial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol 2000: **88**: 170–175.
5. Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980: **44**: 331–384.
6. Harkenthal M, Reichling J, Geiss HK, Saller R. Comparative study on the *in vitro* antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil, and eucalyptus oil. Pharmazie 1999: **54**: 460–463.
7. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of a variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect Chemother 2001: **7**: 251–254.
8. Kato T, Iijima H, Ishihara K, et al. Antibacterial effects of Listerine on oral bacteria. Bull Tokyo Dent Coll 1990: **31**: 301–307.
9. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev 2000: **13**: 547–558.
10. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986: **50**: 353–380.
11. Maruniak J, Clark WB, Walker CB, et al. The effect of 3 mouth rinses on plaque and gingivitis development. J Clin Periodontol 1992: **19**: 19–23.
12. Mayrand D, Holt SC. Biology of asaccharolytic black-pigmented Bacteroides species. Microbiol Rev 1988: **52**: 134–152.
13. Morgan TD, Beezer AE, Mitchell JC, Bunch AW. A microcalorimertric comparison of the anti-*Streptococcus mutans* efficacy of plant extracts and antimicrobial agents in oral hygiene formulations. J Appl Microbiol 2001: **90**: 53–58.
14. Muzzarelli RAA, Torsi R, Filippini O, Giovanetti E, Biagini G, Varaldo PE. Antimicrobial properties of N-carboxybutyl chitosan. Antimicrob Agents Chem 1990: **34**: 2018–2023.
15. Ohshima T, Minami T, Aono W, et al. Oolong tea polyphenols inhibit experimental dental caries in SPF rats infected with mutans streptococci. Caries Res 1993: **27**: 124–129.
16. Ohshima T, Minami T, Matsumoto M, Fujiwara T, Sobue S, Hamada S. Comparison of the cariostatic effects between regimens to administer oolong tea polyphenols in SPF rats. Caries Res 1998: **32**: 75–80.
17. Offenbacher S, Beck JD, Lieff S, Slade G. Role of periodontitis in systemic health: spontaneous preterm birth. J Dent Educ 1998: **62**: 852–858.
18. Porter NG, Willins AL. Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. Phytochemistry 1999: **50**: 407–415.
19. Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect 2000: **2**: 1599–1607.
20. Saeki Y, Ito Y, Shibata M, Sato Y, Okuda K, Takazoe I. Antimicrobial action of natural substances on oral bacteria. Bull Tokyo Dent Coll 1989: **30**: 129–135.
21. Shapira L, Ayalon S, Brenner T. Effects of *Porphyromonas gingivalis* on the central nervous system: activation of glial cells and exacerbation of experimental autoimmune encephalomyelitis. J Periodontol 2002: **73**: 511–516.
22. Shapiro S, Meier A, Guggenheim B. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. Oral Microbiol Immunol 1994: **9**: 202–208.
23. Slots J. The predominant cultivable microflora of advanced periodontitis. Scand J Dent Res 1977: **85**: 114–121.
24. Slots J, Bragd L, Wilkström M, Dahlén M. The occurrence of *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis*, and *Bacteroides intermedius* in destructive periodontal disease in adults. J Clin Periodontol 1986: **13**: 570–577.
25. Slots J, Listgarten MA. *Bacteroides gingivalis*, *Bacteroides intermedius* and *Actinobacilus actinomycetemcomitans* in human periodontal diseases. J Clin Periodontol 1988: **15**: 85–93.
26. Söderberg TA, Johansson A, Gref R. Toxic effects of some conifer resin acids and tea tree oil on human epithelial and fibroblast cells. Toxicology 1996: **107**: 99–109.
27. Tanner ACR, Haffer C, Bratthall GT, Visconti RA, Socransky SS. A study of the bacteria associated with advancing periodontitis in man. J Clin Periodontol 1979: **6**: 278–307.
28. Torsi R, Corbin B, Pruzzo C, Muzzarelli RAA. Effects of low-molecular-weight chitosans on the adhesive properties of oral streptococci. Oral Microbiol Immunol 1998: **13**: 217–224.
29. van Klink JW, Brophy JJ, Perry NB, Weavers RT. β-triketones from myrtaceae: Isoleptospermone from *Leptospermum scoparium* and papuanone from *Corymbia dallachiana*. J Nat Prod 1999: **62**: 487–489.
30. van Winklehoff AJ, van Steenbergen TJM, DeGraaff J. The role of black-pigmented Bacteroides in human oral infections. J Clin Periodontol 1988: **15**: 145–155.

Oral Microbiology Immunology 2004: 19: 150–154
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Oral Microbiology and Immunology

Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation

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Yamanaka A, Kimizuka R, Kato T, Okuda K. Inhibitory effects of cranberry juice on attachment of oral streptococci and biofilm formation.
Oral Microbiol Immunol 2004: 19: 150–154. © Blackwell Munksgaard, 2004.

Cranberry juice is known to inhibit bacterial adhesion. We examined the inhibitory effect of cranberry juice on the adhesion of oral streptococci strains labeled with [^{3}H]-thymidine to saliva-coated hydroxyapatite beads (s-HA). When the bacterial cells were momentarily exposed to cranberry juice, their adherence to s-HA decreased significantly compared with the control ($P < 0.01$). Their hydrophobicity also decreased dependently with the concentration of cranberry juice. We also evaluated the inhibitory effect of cranberry juice on biofilm formation. By using a microplate system, we found that the high molecular mass constituents of cranberry juice inhibited the biofilm formation of the tested streptococci. The inhibitory activity was related to the reduction of the hydrophobicity. The present findings suggest that cranberry juice component (s) can inhibit colonization by oral streptococci to the tooth surface and can thus slow development of dental plaque.

Key words: adsorption; biofilm; cranberry; oral streptococci

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Accepted for publication November 13, 2003

The existence of microorganisms as the polyspecies consortium known as oral biofilm and called dental plaque has profound implications for the etiology of caries and periodontal diseases (6, 8, 9, 11, 20–22, 25, 26). The adhesion of streptococci to the pellicle on tooth surfaces appears to be the first step in the formation of dental plaque. Mutans streptococci such as *Streptococcus mutans* and *Streptococcus sobrinus* have been strongly implicated as causative organisms of dental caries (8). The adsorption of mutans streptococci to the tooth surface is an essential step in the development of dental caries. It has been noted that the exclusion or reduction of such pathogenic bacteria is beneficial in controlling oral infections such as dental caries (8, 15–17, 24, 31).

The American cranberry is a member of the heath family native to North America. The fruit is widely used in a variety of food products including juices and confectionery. Cranberry juice has been shown to affect the adhesion of uropathogenic microorganisms to uroepithelial cells by interfering with specific receptor-ligand modes of microbial adhesion (13, 23) and to inhibit the sialic acid-specific adhesion of *Helicobacter pylori* to human gastric mucosa and erythrocytes (2, 3). Avorn et al. (1) have demonstrated that drinking cranberry juice decreased the frequency of bacteria with pyuria in elderly women. The high molecular weight nondialyzable material of cranberry juice constituents reversed the coaggregation of the majority of coaggregating bacterial pairs tested (28). In a preliminary clinical trial, nondialyzable material reduced the *S. mutans* count in saliva (29). Inhibition of bacterial colonization is a rational strategy for prevention of chronic oral infectious diseases caused by dental plaque bacteria.

The purpose of this study was to investigate the inhibitory effects of cranberry juice on the adherence of oral streptococci to saliva-coated hydroxyapatite (s-HA) beads and on biofilm formation.

Materials and methods

Microbial strains and culture conditions

The organisms used in this study were *S. sobrinus* 6715 and B13; *S. mutans* MT8148R, JC2, Ingbritt and ATCC 10449; *Streptococcus criceti* E49; *Streptococcus sanguinis* ATCC 10556; *Streptococcus oralis* ATCC 10557; *Streptococcus mitis* ATCC 9811 and *Streptococcus gordonii* Challis. These strains of streptococci were cultured in trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD). The organisms were grown at 37°C for 24–72 h in an anaerobic chamber (N_2 80%, H_2 10%, CO_2 10%). To radiolabel the