The stimulation of macrophage prostaglandin E_2 and thromboxane B_2 secretion by *Streptococcus mutans* insoluble glucans

Yoshimitsu Abiko, Yasuko Shibata, Kazuo Fukushima+, Seidai Murai* and Hisashi Takiguchi

Departments of Biochemistry and +*Microbiology, Nihon University School of Dentistry at Matsudo, 2-870-1, Sakaecho-Nishi, Matsudo, Chiba 271* and **Department of Conservative Dentistry, Nihon University School of Dentistry, 1-8-13, Kanda-Surugadai, Chiyoda-Ku, Tokoyo 101, Japan*

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The effect of insoluble glucan synthesized by *Streptococcus mutans* on [^{3}H]arachidonate metabolites secretion from peritoneal macrophages was studied. Insoluble glucans stimulated [^{3}H]arachidonate release and secretion of prostaglandin E_2 and thromboxane B_2 from macrophages. In contrast, commercial soluble glucan (dextran) did not induce [^{3}H]arachidonate release.

Macrophage *Prostaglandin E_2* *Thromboxane B_2* *Streptococcus mutans* *Insoluble glucans* *Periodontal disease*

1. INTRODUCTION

Dental plaque microorganisms and their metabolic products such as toxins, enzymes and polysaccharides have been shown to induce gingivitis and periodontitis [1]. Bacterial polysaccharides are known to be present in significant quantities in dental plaque [2]. A prime determinant of the ability of *S. mutans* to form dental plaque is the capacity of the organism to synthesize high M_r glucans from sucrose, including highly branched polymer glucans that have α-1,6 and α-1,3-glucosidic linkages [3]. Ivanyi has reported that branched dextran from dental plaque is, additionally, a B-cell mitogen for human lymphocytes and that the incidence and magnitude of the response to branched dextran increased in patients with gingival periodontal disease when compared to subjects with clinically healthy gingiva [4]. Arachidonic acid metabolites appear to be involved in the regulation of inflammation [5]. PGE_2 has been detected in human gingival tissue with periodontal diseases [6], and PG-like products have been detected in the culture media of gingival culture [7]. Administration of PGE_2 in organ culture [8] or in vivo [9] induces bone resorption. The presence in periodontal disease gingiva of increased levels of TXB_2 may reflect the accumulation of inflammatory cells within the gingiva [10]. TXB_2 is the stable end product of TXA_2, which has been shown to possess potent vascular smooth muscle contraction activity [11] and to induce irreversible platelet aggregation [12]. It is known that macrophages have a key role in mediating inflammatory reactions [13] and macrophages are a presumable source of PGE_2 and TXB_2 release [14].

Here we present evidence that insoluble glucans obtained from *S. mutans* induce PGE_2 and TXB_2 secretion from macrophages in cell culture.

Abbreviations: *S. mutans*, *Streptococcus mutans*, PGE_2, prostaglandin E_2, TXB_2, thromboxane B_2, AA, arachidonic acid

2. MATERIALS AND METHODS

2.1. *Macrophage culture*

Peritoneal exudate cells were induced by the in-

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jection of sterile 0.1% glycogen (0.30 μm filter) into female guinea pigs. After 3 days, animals were anesthesized with ether, rapidly bled from the heart and perfused with saline solution. Then, peritoneal exudate cells were harvested with RPMI 1640 medium containing 5 mM Tris-HCl (pH 7.4), 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate and 0.38% sodium citrate. When red blood cells were present, these were lysed with hypotonic saline (0.2%) for 30 s and diluted with an equal volume of 1.6% NaCl solution. The cells were washed twice with RPMI 1640 medium without sodium citrate and plated at 1×10^6 cells/well (Linbro tissue culture multi-well plate, 2.0 cm^2). After incubation for 2 h at 37°C in 5% CO_2 in air, the non-adherent cells were washed off twice.

2.2. S. mutans *glucans*

Insoluble glucans from various species of *S. mutans* were prepared as in [15]. Estimation of α-1,3 glucosidic linkage contents in glucans was carried out by measuring the hydrolysis with excess dextranase. The reaction mixture contained 100 mM sodium acetate buffer (pH 6.0), 2 units dextranase (from *Penicillium* sp., Sigma Chemical Co.) and 1 mg glucan. Hydrolysis of glucan was carried out at 37°C for 24 h. The hydrolyzed sugars were determined by phenol-sulfuric acid reaction. The suspended insoluble glucans were sterilized in the autoclave. Aggregated insoluble glucans were resuspended by sonication and diluted with RPMI medium for use.

2.3. *Assay for [^{3}H]arachidonate release*

Fresh RPMI 1640 medium (0.5 ml) containing 0.1% bovine serum albumin (BSA) and 0.15 μCi [5,6,8,9,11,12,14,15-^{3}H] arachidonic acid; 127 Ci/mmol (Amersham) was added to adherent macrophage cells in the tissue culture multi-well plate. After 16 h incubation, the radiolabeled macrophages were washed twice with Hank's balanced salt solution; and fresh medium containing 0.1% BSA with or without glucans was added. The radioactivity released into the medium was determined after incubation for varying times.

2.4. *Chromatography of [^{3}H]PGE$_2$ and [^{3}H]TXB$_2$*

Radioactive products in the medium were extracted and determined as in [16]. As standards, 360 nmol PGE_2 and TXB_2 were also mixed with

Table 1.

Effect of various insoluble glucans on arachidonate release

Inducer added		[^{3}H]arachidonate released (dpm)	Relative activity
Controls			
None		9562 ± 315	1.00
Dextran		9258 ± 941	0.97
Insoluble glucans from *S. mutans* strain (serotype)			
6715	*(g)*	25986 ± 1876[a]	2.72
OMZ-176	*(d)*	13746 ± 2330[b]	1.44
PS-14	*(f)*	11425 ± 982[b]	1.20
QP-50	*(c)*	10652 ± 660[b]	1.11

[a] $P \leqslant 0.001$, [b] $P \leqslant 0.05$ compared with zero.

Macrophage culture (1.0×10^6 cells/well) were prelabeled with [^{3}H]arachidonic acid for 16 h. The cells were incubated with or without each inducer for 4 h at 37°C in 5% CO_2 in air. The concentrations of insoluble glucans and dextran were 500 μg/ml. All results are expressed as the mean ± SD ($n = 4$)

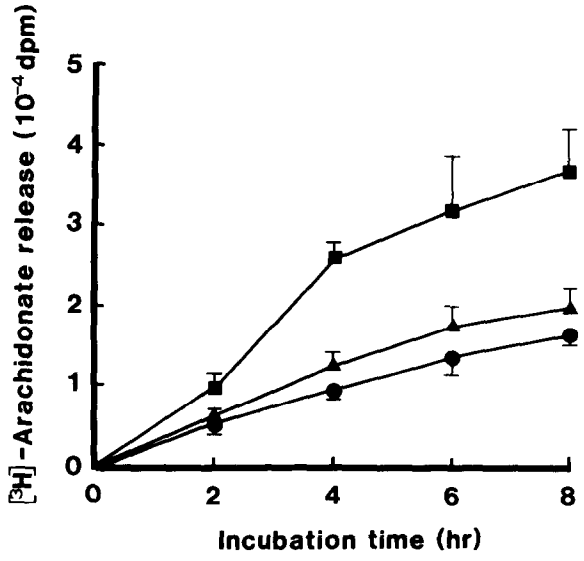

Fig. 1. Time-dependent release of [^{3}H]arachidonate. Macrophage cultures (1×10^6 cells/well) were prelabeled with [^{3}H]arachidonic acid for 16 h. The cells were incubated with insoluble glucan obtained from *S. mutans* 6715 (—▲— 250 μg/ml) or without (—●—). Results show the mean ± SEM of 4 different exp.

Table 2

[^{3}H]arachidonate metabolites elicited by insoluble glucan

Incubation time	Insoluble glucan added (6715)	PGE_2		TXB_2	
		^{3}H-released (dpm)	Rel. act. (%)	^{3}H-released (dpm)	Relative activity (%)
4 h	None	33 ± 15	100.0	67 ± 8	100.0
	500 μg/ml	359 ± 52	1087.8[a]	938 ± 200	1400.0[a]
8 h	None	116 ± 16	100.0	136 ± 18	100.0
	500 μg/ml	1257 ± 199	1083.6[a]	1506 ± 303	1107.4[a]

[a] $P \leqslant 0.01$ compared with none

[^{3}H]Arachidonate metabolites released from macrophages after addition of 0.5 ml RPMI 1640 medium containing 500 μg/ml insoluble glucan suspension were extracted and chromatographed on silical-gel G plates, using the solvent system described in the text. Spots of chromatographed authentic samples were detected by iodine vapor and radioactivity in the areas was counted. The values are expressed as mean ± SD ($n = 4$).

the extracts. The plates (silica gel-G) were developed with a solvent system [benzene:1,4-dioxane:acetic acid (60:30:1)] chosen to separate PGE_2 and TXB_2 and then visualized by exposure to iodine vapor. The areas corresponding to authentic PGE_2 and TXB_2 on the chromatograms were cut out and the radioactivity was determined using a liquid scintillation counter.

3. RESULTS

We have found that the percentage solubilization of glucans differed between various *S. mutans*; 6715 (11%), QP-50 (36%), OMZ-176 (58%) and PS-14 (68%); suggesting that glucan from *S. mutans* 6715 has the highest amount of α-1,3 glucosidic linkage content. The effect of insoluble glucans synthesized by several strains of *S. mutans* on [^{3}H]AA release from macrophages was examined. About 55% of [^{3}H]AA radioactivity added was incorporated into macrophages and 70% of the incorporated radioactivity was distributed into the phospholipid fraction under our incubation conditions (not shown). Insoluble glucan from *S. mutans* strain 6715 caused the highest [^{3}H]AA release, whereas commercial soluble dextran (M_r $5-40 \times 10^6$) did not induce [^{3}H]AA release. The [^{3}H]AA release stimulated by various concentrations of insoluble glucan from *S. mutans* 6715 occurred in a time-dependent manner over 8 h (Fig. 1). The [^{3}H]AA metabolites released in culture medium by stimulation with 500 μg/ml insoluble glucan from *S. mutans* 6715 were identified by thin-layer chromatography. At 500 μg/ml, insoluble glucan from *S. mutans* 6715 caused an 11-fold increase in secretion of [^{3}H]PGE_2 and a 14-fold increase in [^{3}H]TXB_2 secretion at 4 h and an 11-fold increase in secretion of both [^{3}H]PGE_2 and [^{3}H]TXB_2 at 8 h as shown in table 2.

4. DISCUSSION

This study demonstrates that insoluble glucan from *S. mutans* can dramatically induce glycogen-elicited guinea pig peritoneal macrophages to secrete PGE_2 and TXB_2. The magnitude of response is dependent on the duration of incubation time.

Considerable evidence has accumulated regarding the chemical structure of insoluble glucans from various strains of *S. mutans*. The large proportion of α-1,3-glucosidic linkages found in insoluble glucan explains the insoluble nature of this polymer and the consecutive α-1,3-glucosidic linkages from long chains as the backbone of a highly branched insoluble glucan [3]. Since morphology and size of insoluble glucan from *S. mutans* has the fibrillar form (28×5.2 nm) [17], and sonicated insoluble glucan has a high M_r ($> 1.5 \times 10^7$) [18], macrophage phagocytosis of insoluble glucan might be related to their [^{3}H]AA metabolism.

Several serotypes of *S. mutans* may be responsible for dental plaque formation, but it is not clear

whether one serotype is more virulent than another. We have found that glucan from *S. mutans* 6715 had the highest α-1,3 glucosidic linkage content. When compared with our data in table 1, insoluble glucans from *S. mutans* 6715 caused the highest [^{3}H]AA release, it suggests that [^{3}H]AA release from macrophages may depend on the proportion of α-1,3 glucosidic linkage contents of glucan. We have not investigated here the functional mechanisms of α1,3 glucosidec linkage glucan on the stimulation of AA release. In [19], alveolar macrophage phagocytosis of zymosan increased the release of AA and PGs; in contrast, phagocytosis of inert latex particles had no effect [19]. The insoluble polysaccharide β-1,3 glucan is the active component of zymosan for producing hyperplasia and hyperfunction of the reticuloendothelial system [20]. The following possibilities may therefore be considered: membrane changes occurring after interiorization of the cell surface by phagocytosis of α-1,3 glucosidic linkage glucan may play roles of Ca^{2+} efflux, activation of membrane phospholipase A_2 or C, or release of lysosomal phospholipase A_2 related to AA release.

TXB_2 and PGE_2 may contribute to inflammatory processes by causing chemotaxis of leukocytes, vascular permeability and histamine release [21,22]. Prior treatment of rat gingival epithelial surface with *Streptococcus* hyaluronidase allowed penetration of the polysaccharide resulting in destructive changes in rat gingiva [23].

Thus, our data suggest that insoluble glucan from *S. mutans* in dental plaque may contribute to pathophysiological inflammation and bone resorption in periodontal tissues through the induction of TXB_2 and PGE_2 release from macrophages. Experiments to clarify the detailed mechanism of α-1,3 glucosidic linkage insoluble glucan on AA release from macrophages are in progress.

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