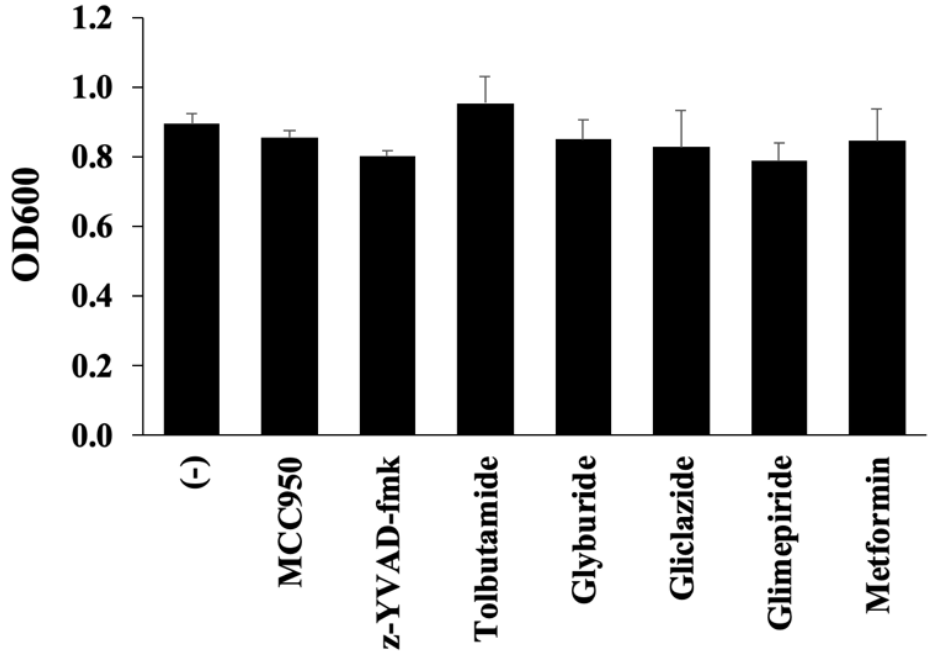
Effects of Sulfonylureas on Periodontopathic Bacteria-Induced Inflammation

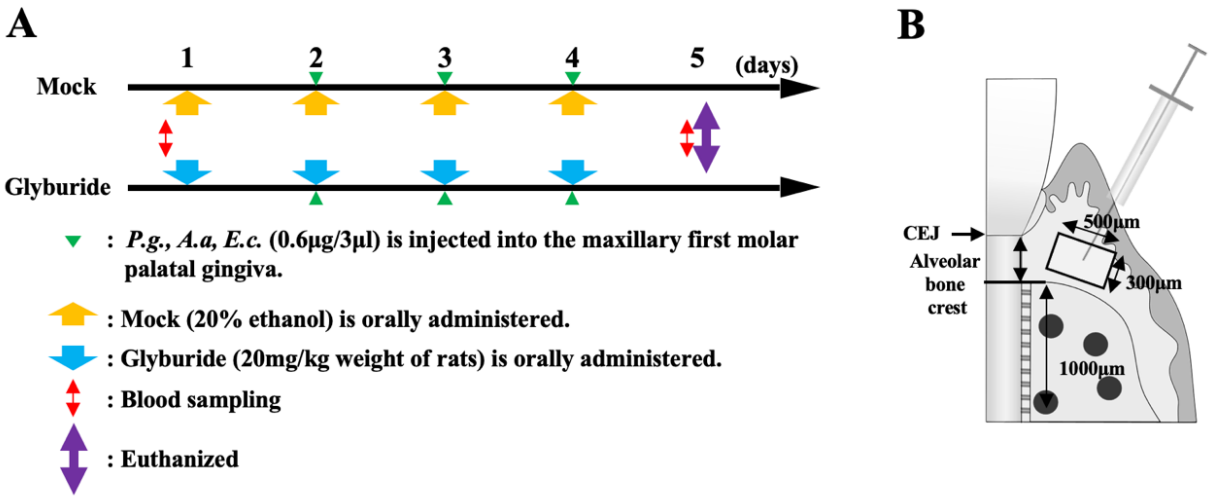
Y. Kawahara, T. Kaneko, Y. Yoshinaga, Y. Arita, K. Nakamura, C. Koga, A. Yoshimura, and R. Sakagami

Appendix



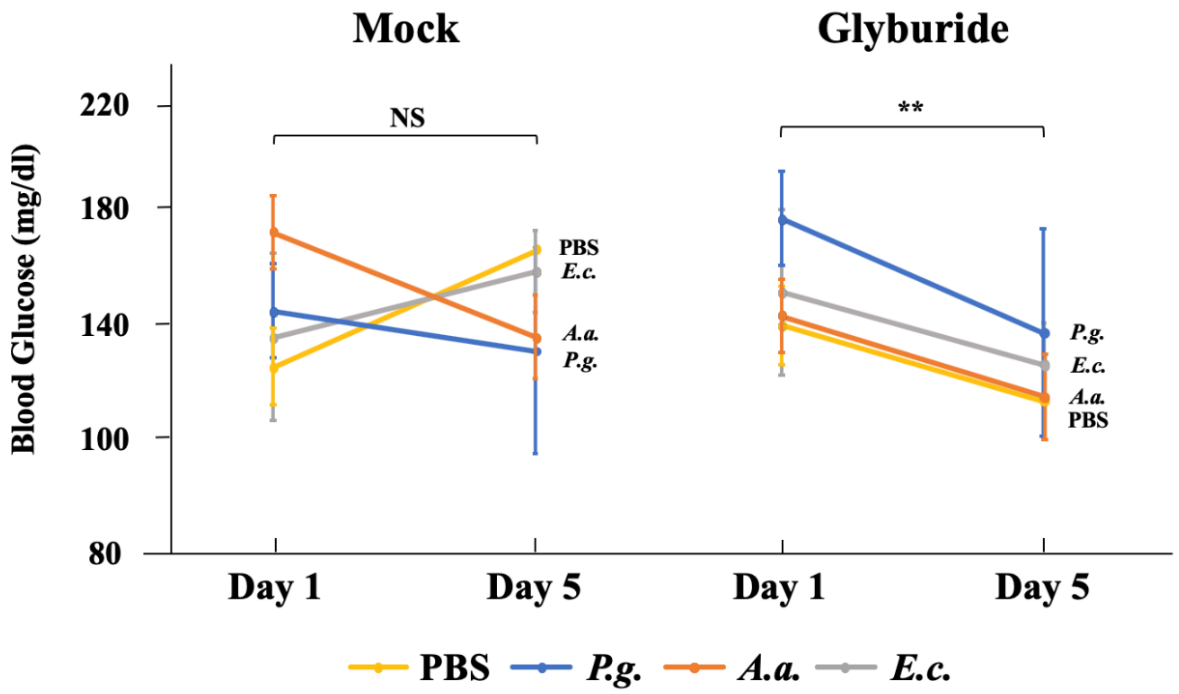
Appendix Figure 1

Effects of inhibitors and hypoglycemic drugs on growth of THP-1 macrophage-like cells. THP-1 macrophage-like cells (1×10^5 cells/100 μL/well in 96-well plates) were stimulated with MCC950 (10 μM), z-YVAD-fmk (2 μM), tolbutamide (100 μM), glyburide (100 μM), gliclazide (100 μM), glimepiride (100 μM), or metformin (5 μM). Twenty-four hours after incubation, 10 μL of the MTT reagent (5 mg/mL, Sigma-Aldrich) was added into the well and incubated for an additional 4 h. After adding 100 μL of the solubilizing solution (10% SDS in 10 mM HCl) overnight, optical density (OD) was measured colorimetrically at 600 nm. There were no statistically significant differences between samples and DMSO by one-factor ANOVA, followed by the Tukey's test.



Appendix Figure 2

(A) Schedule of *in vivo* experiments. Periodontitis in rats was generated by injection of *P. gingivalis*, *A. actinomycetemcomitans,* or *E. coli* into the mesial gingiva in the upper first molar. Rats were divided into two groups: glyburide group (20 mg/kg weight of rats via oral administration with a tube every 24 h) and the mock group (20% Ethanol). Rats were euthanized 24 h after the third bacterial injection, and histological specimens of the periodontal tissues were stained by hematoxylin and eosin (H&E). Tartrate-resistant acid phosphatase (TRAP) was visualized enzymatically to identify osteoclasts. Whole blood was collected from rats on days 1 and 5. (B) Schema of histological analyses. The number of inflammatory cells in 500 μm × 300 μm of connective tissue above the bone crest. The distance between the cement-enamel junction (CEJ) to the alveolar bone crest. TRAP-positive cells on the bone surface of the periodontal ligament between the bone crest and the point 1,000 μm away from the bone crest.

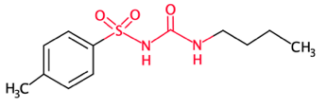
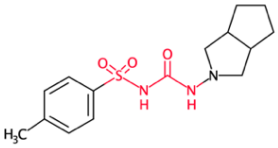
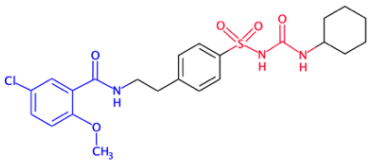


Appendix Figure 3

Changes in blood glucose levels of glyburide-administered rats. Glyburide in 20% ethanol (20 mg/kg) or 20% ethanol (mock) was orally administered to each group (8 rats / group) every 24 h using a tube. Each bacterial sample was injected into the gingiva of rats as described in the Materials & Methods. Blood samples were collected from the retro-orbital venous plexus of the rats at baseline (Day 1) and before sacrifice (Day 5). Serum was prepared from the collected blood. Using 10 μL of the serum, the blood glucose level was measured using a Glutestace Ace R (GlucocardTM G Black, Arkray, Kyoto, Japan). Statistical analysis was performed using the Wilcoxon-Mann-Whitney sign-ranked test. *P. g.*, *P. gingivalis*; *A. a.*, *A. actinomycetemcomitans*; *E. c.*, *E. coli*. $^{**}P < 0.01$. NS: not significant.

Appendix Table

Chemical structures of sulfonylureas used in this study. Glyburide and glimepiride, but neither tolbutamide nor gliclazide, could suppress IL-1β release from THP-1 macrophage-like cells stimulated with periodontal bacteria. Sulfonylurea, which is an essential structure for binding ATP-sensitive potassium channels (resulting in insulin release), is shown in red. Benzamide and pyrrolamide groups in glyburide and glimepiride are shown in blue.

Generation	Sulfonylureas	Inhibition of IL-1 β release	Chemical structure
1	Tolbutamide	(-)	
2	Gliclazide	(-)	
	Glyburide	(+)	
3	Glimepiride	(+)