J Periodont Res 2005; 40; 43–47

JOURNAL OF PERIODONTAL RESEARCH
doi: 10.1111/j.1600-0765.2004.00767.x

Chronic glycine treatment inhibits ligature-induced periodontal disease in Wistar rats

Torbjørn Breivik[1,2], Yngvar Gundersen[2], Frode Fonnum[2], Per Vaagenes[2], Per Kristian Opstad[2]

[1]Department of Periodontology, Faculty of Dentistry, University of Oslo and [2]Norwegian Defence Research Establishment, Division for Protection and Material, Kjeller, Norway

Breivik T, Gundersen Y, Fonnum F, Vaagenes P, Opstad PK. Chronic glycine treatment inhibits ligature-induced periodontal disease in Wistar rats. J Periodont Res 2005; 40: 43–47. © Blackwell Munksgaard 2004

Objective: Dysregulation of immune and stress responses plays a significant role for the development and progression of inflammatory diseases, including periodontal disease. The non-essential amino acid glycine modulates immune and central nervous system (CNS) responses, and has been shown to beneficially affect tissue destructive inflammatory conditions. The purpose of this study was to test the ability of orally administered glycine to influence periodontal disease progression, as well as immune and hypothalamic–pituitary–adrenal (HPA) responses following lipopolysaccharide stimulation.

Methods: Glycine was supplied in the drinking water during the whole experiment to male Wistar rats, starting 3 days before the induction of experimental ligature-induced periodontal disease. Control rats were given tap water only. The periodontal breakdown was assessed after the ligatures had been in place for 34 days. Following intraperitonal lipopolysaccharide stimulation, concentrations of the proximal cytokines tumour necrosis factor-α (TNF-α) and interleukin-10, as well as the HPA axis-derived hormone corticosterone, were measured in blood serum.

Results: Orally administered glycine significantly reduced periodontal bone loss as measured by digital X-rays ($p = 0.007$). Bone loss was negatively correlated with increased serum glycine, whereas no significant relationship was found with TNF-α, interleukin-10, or corticosterone.

Conclusion: Chronic ingestion of glycine supplied in the drinking water significantly reduced periodontal bone loss. No effect of glycine on immune and HPA-axis responses was revealed. Further studies are needed to clarify the mechanisms of action.

Torbjørn Breivik, Department of Periodontology, PO Box 1109 Blindern, N-0317 Oslo, Norway
Tel: +47 22 85 23 56
Fax: +47 22 85 23 51
e-mail: tbreivik@odont.uio.no

Key words: cytokines; glycine; hypothalamo–pituitary–adrenal axis; immune cells

Accepted for publication July 21, 2004

Periodontal disease is a pathological destructive inflammatory condition affecting the supporting structures of teeth. It is characterized by increased colonization of pathogenic microorganisms (periodontal pathogens) in subgingival dental plaques, periodontal attachment fibre destruction, and alveolar bone loss. In the most severe cases the disease may lead to periodontal pocket formation, increased tooth mobility and tooth loss (1).

The increased colonization of periodontal pathogens, including some anaerobic Gram-negative bacteria species, mobile rods, spirochaetes, as well as viruses (2–4), may be due to altered immune responses (5–8). The periodontal fibre destruction may be caused by reactive oxygen species (ROS) and matrix metalloproteinases. Ample amounts of ROS and matrix metalloproteinases are released from activated immune system cells belonging to the innate immune system, including polymorphonuclear phagocytes (PMNs) (9–11). Thus, in patients with periodontal disease, the immune response may be biased so that periodontal pathogens are allowed to grow in

subgingival dental plaques. The subsequent increased accumulation of inflammatory cells belonging to the innate immune system may protect the host from being infected by the periodontal pathogens, but may also be responsible for the destruction of the tooth supporting tissues (12).

Severe periodontal disease has been found to be associated with genetics, ageing, and environmental factors such as smoking, poorly regulated diabetes, and poorly developed coping strategies to traumatic life events, such as the loss of a spouse by death (12, 13). A positive correlation has also been established with increased activation of the hypothalamic–pituitary–adrenal (HPA) axis (12). In fact, the HPA axis is one of the major overarching immune-regulatory mechanisms controlled by the brain (14–16). In the rats it has been demonstrated that a relationship exists between HPA axis responsiveness and periodontal disease susceptibility and resistance (17–23).

Glycine is a simple, non-essential amino acid with well-known modulatory effects on neurotransmission in the central nervous system (CNS) (24, 25). In addition, glycine has been found to have beneficial effects on tissue destructive inflammatory diseases, mainly due to its modulatory effects on immune cells (26–28). These cells are known to be critical for intracellular signalling involved in the production of immune mediators (27). The present study was accomplished to examine whether orally administered glycine could influence ligature-induced periodontal tissue destruction in a well-established experimental periodontal disease model in the rat. To elucidate possible mechanisms of action working through the immune system or the HPA axis, both systems were powerfully stimulated with lipopolysaccharide just before decapitation.

Materials and methods

Animals

Nineteen male Wistar rats, weighing 310–330 g, were obtained from Møllegaard Breeding Centre (Ejby, Denmark), and used after 2 weeks of acclimatization. Standard rat chow pellets and tap water were available *ad libitum*. The animals were housed in groups of four to five under a 12–24 h light/dark cycle (light from 07.00 h to 19.00 h) with temperature and humidity at 22°C and 40–60%, respectively, and grouped in two by random. Eleven rats were given glycine. Eight served as controls and were given tap water only. The experiments were registered and approved by the Norwegian Experimental Animal Board (NEAB).

Glycine and treatment of rats

Glycine (amino acetic acid) was provided by Sigma (St. Louis, MO, USA) and 75 g of the dry substance was dissolved in 1500 ml water and stored at 4°C. Rats in the treatment group could freely drink the solution, starting 3 days before application of the ligatures. Based on measured amount of consumed water, the average daily intake of glycine was calculated to be close to 1 g. Control rats received tap water only.

Experimental periodontal disease

Three days after glycine induction, all animals were anaesthetized by a subcutaneous injection in the neck with Hypnorm-Dormicum (fentanyl/fluanizone, midazolam), 0.2 ml/100 g body weight. A sterile silk ligature (Ethicon Perma-hand® size 3/0, Norderstedt, Germany) was tied around the neck of the maxillary right 2nd molar tooth in the gingival sulcus of all animals. The ligatures served as a retention device for oral microorganisms. Thirty-four days after application of the ligatures, all animals were killed by decapitation. The maxillae were excised and fixed in 4% formaldehyde.

Radiographic examination

The specimens were stabilized with dental wax on a Sidexis digital X-ray sensor, and orientated with the axis of the teeth parallel to the sensor surface by using 4 × magnification loupe glasses (Zeiss, Oberkochen, Germany). The distances between the cemento-enamel junction and bone on the mesial surface of the 2nd molars were displayed digitally. The evaluations were performed blinded. Each X-ray was read five times, and the mean of three readings calculated. The examiner was unaware of whether the specimens came from experimental or control animals. The reliability of the method has been tested earlier (5). The average percentage difference between individual readings and the mean of the respective triplicate was 3.48 ± 5.12%.

Lipopolysaccharide challenge

Lipopolysaccharide (*Escherichia coli* serotype 0111:B4, Sigma) was injected intraperitoneally (250 μg/kg) 2 h before decapitation. Serum levels of proximal cytokines and corticosterone (the predominant glucocorticoid hormone in rodents released during HPA axis activation) were determined. After decapitation of the rats, the blood samples were collected (6–10 ml from each animal) in vacutainer tubes (10 ml without additives) and allowed to clot on ice for 1 h. Thereafter, the samples were centrifuged for 20 min at 2000 *g*, the serum was removed and stored at −20°C until analysis.

Assay of corticosterone

Corticosterone was measured with a ^{125}I radioimmunoassay (RIA) coat-A-count kit from Diagnostic Products Corporation (Los Angeles, CA, USA, catalogue number TKRC1). The detection limit was 5.7 ng/ml.

Assay of serum interleukin-10 and tumour necrosis factor-α (TNF-α)

The levels of interleukin-10 and TNF-α in the serum samples were measured by means of enzyme-linked immunosorbent assay (ELISA) kits (catalogue numbers R1000 and RTA00, R & D Systems, Inc., Minneapolis, MN, USA). The minimum detectable dose for interleukin-10 is less than 31.2 pg/ml, and less than 12.5 pg/ml for TNF-α.

Assay of serum amino acids

Amino acids were analysed using high performance liquid chromatography.

Statistics

Values are expressed as mean ± standard deviation. Differences between the two groups were examined with Student's *t*-test or Mann–Whitney test as appropriate.

Results

The animals were killed 34 days after application of the ligatures. The mean bone loss measured as the distance between the cemento-enamel junction and the most coronal bone was 0.75 ± 0.11 mm in the glycine-treated animals, compared to 0.97 ± 0.20 mm in the controls ($p = 0.007$ between groups) (Fig. 1). For all animals, there was a significant negative correlation between their serum glycine levels and bone loss ($r = 0.47$, $p < 0.05$, $n = 19$).

The serum concentrations of TNF-α collected 2 h after intraperitoneal injection of lipopolysaccharide (250 μg/kg) tended to be lower (2356 ± 2841 pg/ml) in the glycine-treated rats, compared to 3189 ± 3667 pg/ml in the controls, whereas the serum concentrations of interleukin-10 tended to be higher (55.6 ± 17.9 pg/ml vs. 49.7 ± 11.8 pg/ml) in glycine-treated and control rats, respectively. The differences were, however, not significant.

Because TNF-α and downstream pro-inflammatory cytokines such as interleukin-6 can activate the HPA axis and drive corticosterone secretion, this steroid was measured in the same samples. The glycine-treated rats showed a tendency to a lower serum level of corticosterone (1048 ± 181 nm/l) compared to controls (1172.9 ± 154 nm/l; $p = 0.1$).

Glycine-treated rats had enhanced serum levels of glycine (1866 ± 410 vs. 603 ± 96 μmol/l, $p < 0.0001$) as well as serine (358 ± 44 vs. 224 ± 42 μmol/l, $p < 0.0001$) (Table 1). Serum levels of glycine and serine correlated positively ($r = 0.72$, $p < 0.01$, $n = 19$). Insignificant tendencies towards reduced levels of glutamine (656 ± 81 vs. 725 ± 79 μmol/l, p = 0.08), glutamate (161 ± 23 vs. 182 ± 44 μmol/l, p = 0.20), and alanine (407 ± 61 vs. 453 ± 71 μmol/l, p = 0.15) were noted in the glycine-treated animals. Serum aspartate and taurine did not change (Table 1).

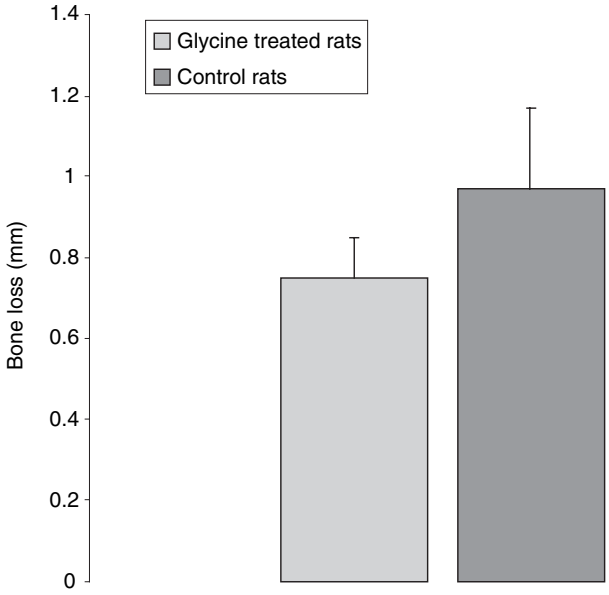

Fig. 1. Bone loss, measured as the distance between the cemento–enamel junction and bone on mesial surface of the 2nd molars. Each point is the mean of five readings for an individual rat. The average percentage difference between individual readings and the mean of the respective triplicate has been found to be 3.48 ± 5.12%. Bone loss was reduced in the animals treated with glycine ($p = 0.007$, Student's *t*-test).

Discussion

The study shows that orally administered glycine significantly inhibits ligature-induced periodontal disease in male Wistar rats. The glycine-treated rats had increased serum levels of glycine, thus confirming that it is readily absorbed by the gut. The amino acid serine, which exists in equilibrium with glycine, was also significantly increased.

Glycine has long been a well-characterized modulator of neurotransmission in the CNS. During the last few years, numerous investigations have also revealed powerful effects on the activation of cells belonging to the innate as well as the adaptive immune system, including macrophages, PMNs, as well as lymphocytes (reviewed in [26]). These effects are largely mediated by specific glycine-gated chloride channels. Upon ligand binding, a short-lived inward flux of chloride ions transiently hyperpolarizes the membrane. The enhancement of intracellular Ca^{2+} usually following

Table 1. Serum amino acid values (mean ± standard deviation) (μmol/l)

Group	Glycine	Tap water	*p*-value
Aspartate	24 ± 12	24 ± 6	0.95
Glutamate	161 ± 23	182 ± 44	0.20
Serine	358 ± 44	224 ± 42	< 0.0001
Glutamine	656 ± 81	725 ± 79	0.08
Glycine	1866 ± 410	603 ± 96	< 0.0001
Taurine	242 ± 40	257 ± 34	0.34
Alanine	407 ± 61	453 ± 71	0.15

external stimuli is thereby blocked, as are the transcription pathways governed by nuclear factor kappa B (27, 29). Interaction of glycine with the membrane-bound glycine gated chloride channels may thus alter important pro-inflammatory signalling pathways.

Beneficial effects on various tissue destructive inflammatory conditions have been attributed this property. For example, intraperitoneal injection of glycine to lipopolysaccharide-treated mice has been shown to reduce serum levels of the pro-inflammatory cytokine TNF-α, to alleviate inflammatory hepatic tissue damage, and to increase significantly the anti-inflammatory and T regulatory cytokine interleukin-10 (30). In our study, these results could not be substantiated, although a weak and insignificant tendency towards reduced TNF-α and enhanced interleukin-10 in response to a lipopolysaccharide challenge was noted. However, circulating cytokine concentrations do not necessarily reflect local synthesis, and it can therefore not be ruled out that the favourable effects observed in our study may partly result from a direct inhibition of local immune cell activation. Likewise, glycine may inhibit the release of effector substances such as ROS from macrophages and PMNs stimulated with lipopolysaccharide (27, 31), and peptidoglycan polysaccharide-induced reactive arthritis in rats (29). In several rat models, glycine protects against sepsis and sepsis-related mortality, inflammatory hepatic tissue injury induced by ischaemia-reperfusion and transplantation, as well as other diseases involving inflammation-induced tissue destruction (27). The inhibition of ROS production and secretion may thus represent one possible explanation for the protective effect of glycine against periodontal breakdown as found in this experiment. In this experiment the production of ROS by immune cells was, however, not measured.

Influence on the shift of the activity of T-helper (Th) lymphocytes from Th1 towards Th2 may also contribute to a favourable outcome. In the gingival tissues from patients with untreated advanced periodontal disease, there seems to be an altered Th1/Th2 balance compared to that found in gingival tissues of patients with gingivitis. For example, the cytokine pattern in gingival tissue from cases of periodontal disease reveals a relative increase in Th2 cytokines (interleukin-4, interleukin-5 and interleukin-13) (32). Moreover, vaccination with a Th2 to Th1 skewing vaccine developed from heat-killed *Mycobacterium vaccae* significantly inhibits periodontal disease in rats (5–7). The ability to generate a Th1 or Th2 dominance has turned out to depend not only on the cytokine profile pattern and the microbial peptide presentation by antigen presenting cells, but also on the ROS release from macrophages and PMNs (33). Thus, a reduced ROS secretion by macrophages and PMNs, induced by glycine, may skew the Th1/Th2 balance towards Th1 dominance, which in turn may reduce periodontal breakdown. Indeed, the T regulatory cytokine interleukin-10 tended to be higher in the glycine-treated rats, without reaching statistical significance. The shift towards increased interleukin-10 by dietary glycine treatment is well documented by others (30), and this may suggest that increased interleukin-10 production may be favourable in periodontal disease.

The HPA axis response (as assessed by corticosterone production after lipopolysaccharide challenge) did not differ significantly between the groups. This has previously been shown to be a major factor in determining periodontal breakdown (17, 20). The present work on glycine opens up a novel way of modulating the pathological effect. Alternatively, glycine (and serine) has been found to bind to the glycine sites on *N*-methyl-D-aspartate (NMDA) receptors in brain cells (29), and to stimulate glutamatergic neurotransmission (34, 35). Glutamate, which tended in this experiment to be reduced in the glycine-treated rats, is also highly involved in HPA axis regulation, and glucocorticoid secretion in response to lipopolysaccharide stimulates glutamate in the brain by binding to NMDA receptors (36). Interestingly, we have newly found that treatment with the glutamate receptor antagonist MK-801, which binds to the NMDA receptor and stimulates the HPA axis at high doses, dramatically increases ligature-induced periodontal disease in rats (37).

NMDA receptors have recently been found even on osteoblasts and osteoclasts (38). Both cell types are highly involved in periodontal bone production and breakdown, and a direct and/or indirect inhibiting effect of glycine on osteoclast function may contribute to the observed protective effects. However, a recent study indicate that glutamate, the neurotransmitter that binds to NMDA receptors, does not play a major role in controlling bone formation and resorption (39). This makes the effect of glycine modulating the bone resorption through NMDA receptors in this case less likely.

Addition of glycine to the drinking water led to a more than threefold increase of serum levels, and the ample amounts of glycine (and serine) thus readily available may have a direct impact upon metabolism. Moreover, as glycine occupies every third position in the amino acid sequence of collagen, a positive effect on periodontal connective tissue synthesis and repair seems plausible.

In conclusion, dietary glycine had a powerful inhibiting effect on the ligature-induced periodontal inflammatory breakdown process. Diverse biological mechanisms may be involved. We did not find compelling evidence of effects on the HPA axis. Neither did the results point to a significant modulating effect of glycine on the activation on the immune system, although other studies have shown that chronic glycine treatment may alter immune and inflammatory responses. The precise mechanisms of action for the favourable effects of glycine on periodontal tissue breakdown thus remain conjectural and can only be clarified by further experimental studies.

Acknowledgements

We are grateful to the Norwegian Defence Research Establishment, Division of Protection and Material, Kjeller, Norway for supporting this

work. The authors thank Ingjerd Thrane and Trine Reistad for their skilful technical assistance and help with cytokine and corticosterone analysis.

References

1. Page RC. The aetiology and pathogenesis of periodontitis. *Compend Contin Educ Dent* 2002;**23:**11–14.
2. Socransky SS, Haffajee AD. The bacterial aetiology of destructive periodontal disease. *J Periodont* 1992;**63:**322–331.
3. Sela MN. Role of *Treponema denticola* in periodontal disease. *Crit Rev Oral Biol Med* 2001;**12:**399–413.
4. Kamma JJ, Slots J. Herpes virus–bacterial interaction in aggressive periodontitis. *J Clin Periodont* 2003;**30:**420–426.
5. Breivik T, Rook GA. Prevaccination with SRL172 (heat-killed *Mycobacterium vaccae*) inhibits experimental periodontal disease in Wistar rats. *Clin Exp Immunol* 2000;**120:**463–467.
6. Breivik T, Rook GA. Treatment with SRL172 (heat-killed Mycobacterium vaccae) inhibits progression of established experimental periodontal disease in Wistar rats. *J Periodont Res* 2002;**37:**210–214.
7. Breivik T, Rook GA. Oral treatment with SR299 (killed *Mycobacterium vaccae*) inhibits experimental periodontal disease in Wistar rats. *J Clin Periodont* 2003;**30:** 931–936.
8. Tokoro Y, Matsuki Y, Yamamoto T, Suzuki T, Hara K. Relevance of local Th2-type cytokine mRNA expression in immunocompetent infiltrates in inflamed gingival tissue to periodontal diseases. *Clin Exp Immunol* 1997;**107:**166–174.
9. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodont Res* 1993;**28:**500–510.
10. Ding Y, Haapasalo M, Kerosuo E, Lounatmaa K, Kotiranta A, Sorsa T. Release and activation of human neutrophil matrix metallo- and serine proteinases during phagocytosis of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Treponema denticola*. *J Clin Periodont* 1997;**24:**237–248.
11. Lohinai Z, Benedek P, Feher E *et al.* Protective effects of mercaptoethylguanidine, a selective inhibitor of inducible nitric oxide synthase, in ligature-induced periodontitis in the rat. *Br J Pharmacol* 1998;**123:**353–360.
12. Breivik T, Thrane PS. Psychoneuroimmune interactions in periodontal disease. In: Ader R, Felten DL, Cohen N, eds. *Psychoneuroimmunlogy*, 3rd edition. San Diego, CA: Academic Press, 2001: 627–644.
13. Hugoson A, Ljungquist B, Breivik T. The relationship of some negative events and psychological factors to periodontal disease in an adult Swedish population 50–80 years of age. *J Clin Periodont* 2002;**29:** 247–253.
14. Ader R, Cohen N, Felten D. Psychoneuroimmunology: Interactions between the nervous system and the immune system. *Lancet* 1995;**345:**99–103.
15. Breivik T, Thrane PS, Murison R, Gjermo P. Emotional stress effects on immunity, gingivitis and periodontitis. *Eur J Oral Sci* 1996;**104:**327–334.
16. Chrousos GP. The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995;**332:** 1351–1361.
17. Breivik T, Opstad PK, Gjermo P, Thrane PS. Effects of hypothalamic–pituitary–adrenal axis reactivity on periodontal tissue destruction in rats. *Eur J Oral Sci* 2000;**108:**115–122.
18. Breivik T, Sluyter F, Hof M, Cools A. Differential susceptibility to periodontitis in genetically selected Wistar rat lines that differ in their behavioral and endocrinological response to stressors. *Behav Genet* 2000;**30:**123–130.
19. Breivik T, Thrane PS, Gjermo P, Opstad PK. Glucocorticoid receptor antagonist RU-486 treatment reduces periodontitis in Fischer 344 rats. *J Periodont Res* 2000;**35:**285–290.
20. Breivik T, Thrane PS, Gjermo P, Opstad PK, Pabst R, von Horsten S. Hypothalamic–pituitary–adrenal axis activation by experimental periodontal disease in rats. *J Periodont Res* 2001;**36:**295–300.
21. Breivik T, Thrane PS, Gjermo P, Fonnum F. Postnatal glutamate-induced central nervous system lesions alter periodontal disease susceptibility in adult Wistar rats. *J Clin Periodont* 2001;**28:**904–909.
22. Breivik T, Thrane PS, Gjermo P, Cools A, Myhrer T. Effects of hippocampal lesioning on experimental periodontal disease in Wistar rats. *J Periodont Res* 2002;**37:**360–365.
23. Breivik T, Stephan M, Brabant GE, Straub RH, Pabst R, von Horsten S. Postnatal lipopolysaccharide-induced illness predisposes to periodontal disease in adulthood. *Brain Behav Immunol* 2002;**16:**421–438.
24. Shoham S, Javitt DC, Heresco-Levy U. High dose glycine nutrition affects glial cell morphology in rat hippocampus and cerebellum. *Int J Neuropharmacol* 1999;**2:** 35–40.
25. Gomeza J, Ohono K, Betz H. Glycine transporter isoforms in the mammalian central nervous system: structures, functions and therapeutic promises. *Curr Opin Drug Discov Devel* 2003;**6:**675–682.
26. Wheeler MD, Ikejema K, Enomota N *et al.* Glycine: a new anti-inflammatory immunonutritient. *Cell Mol Life Sci* 1999;**56:**843–856.
27. Wheeler MD, Stachlewitz RF, Yamashina S, Ikejima K, MorroWAL, Thurnman RG. Glycine-gated chloride channels in neutrophils attenuate calcium influx and superoxide production. *FASEB J* 2000;**14:**476–484.
28. Wheeler MD, Rose ML, Ymashhima S *et al.* Dietary glycine blunts lung inflammatory cell influx following acute endotoxin. *Am J Physiol* 2000;**279:**390–398.
29. Li X, Bradford BU, Wheeler MD *et al.* Dietary glycine prevents peptidoglycan polysaccharide-induced reactive arthritis in the rat: Role for glycine-gated chloride channels. *Infect Immunol* 2001;**69:**5883–5891.
30. Bruck R, Wardi J, Aeed H *et al.* Glycine modulates cytokine secretion, inhibits hepatic damage and improves survival in a model of endoxemia in mice. *Liver Int* 2003;**23:**276–282.
31. Yang S, Koo DJ, Chaudry IH, Wang P. Glycine attenuates hepatocellular depression during early sepsis and reduces sepsis-induced mortality. *Crit Care Med* 2001;**29:** 1201–1206.
32. Yamazaki K, Nakajima T, Hara K. Immunohistological analysis of T cell functional subsets in chronic inflammatory periodontal disease. *Clin Exp Immunol* 1995;**99:**384–391.
33. Murata Y, Yamashita A, Saito T, Sagamura K, Hamuro J. The conversion of redox status of peritoneal macrophages during pathological progression of spontaneous inflammatory bowel disease in Janus family tyrosine kinase 3 –/– and interleukin-2 receptor γ–/– mice. *Int Immunol* 2002;**14:**627–636.
34. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: Actions and mechanisms of action. *Physiol Rev* 1999;**79:**1–71.
35. Ziegler DR, Herman JP. Local integration of glutamate signalling in the hypothalamic paraventricular region: regulation of glucocorticoid stress responses. *Endocrinology* 2000;**141:**4801–4804.
36. De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. *Endocrinol Rev* 1998;**19:**269–301.
37. Breivik T, Gundersen Y, Osmundsen H, Opstad PK, Fonnum F. Chronic treatment with the glutamate receptor antagonist MK-801 alters periodontal disease susceptibility. *J Periodont Res* 2004; doi: 10.1111/j.1600-0765.2004.00765.x
38. Skerry TM, Taylor AF. Glutamate signalling in bone. *Curr Pharmacol Des* 2001;**7:**737–750.
39. Gary C, Marie H, Arora M *et al.* Glutamate does not play a major role in controlling bone growth. *J Bone Miner Res* 2001;**16:**742–749.