CHAPTER V

DISCUSSION and CONCLUSION

WF10 is a form of tetrachlorodecaoxygen anion complex (TCDO),and as an oxygen-stabilized chlorite solution. To be activated as an oxygen donor, TCDO has to form complex with heme moieties in biomolecules such as hemoglobin or myoglobin. WF10 has been shown to have immmunostimulatory effects on monocytes, NK cells, and cytotoxic T cells (OXO Chemie,1999). Furthermore, WF10 displays wound healing activity as well as antimicrobial activity (Hinz et al,1986; Stoll et al,1993). Because of these features, WF10 is considered to be attractive for using as adjunctive therapy in periodontitis.

In the present study, we re-evaluated the immunostimulatory activity of WF10 on human cells and also investigate its effect on cytokine production especially when combined with periodontopathic bacteria, *P. gingivalis*. Incubation of PBMC cultures with WF10 for 24 h. led to activation of different lymphocyte subsets (CD4+T cells, CD8+ T cells, NK cells, $\gamma\delta$+ T cells and B cells) as monitored by up-regulation of CD69 expression. There is no study so far to demonstrate that WF10 is able to activate different lymphocyte subsets. It is not clear how WF10 up-regulate CD69 expression of these cells. According to manufacturer claim, the major target cells of this compound are monocytes/macrophages. Mediator(s) of activated monocytes such as IFN-α is shown to augment CD69 expression on T cells (Sun et al., 1998). It has been hypothesized that WF10 activate monocytes/macrophages through its interaction with cell membrane-associated heme complexes (OXO Chemie, 1999). The ability

ability of WF10 to enhance function of monocytes/macrophages and other phagocytic cells may be useful in controlling infection. It is of interest to study whether WF10 could enhance phagocytic cell function against periodontopathic bacteria.

The concentrations of WF10 used in the present study were 1:900, 1:300 and, 1:100. These concentrations appear to be non-toxic since cell viability was assessed 24 h. after cultured with WF10 and no sign of acute toxicity at any concentrations was observed. This range is agreed with that being safely administered to patients i.e. approximately 1:200 from the initial formulated TCDO concentration (base on 35 mL of WF10 diluted into 5L of blood in a standard 70-kg individual) (McGrath et al., 1998).

WF10 at non-toxic concentration (1:900-1:100) moderately induce TNF-α, IL-12, and IFN-γ production. The production of TNF-α and IFN-γ in WF10 treated PBMC cultures appeared to be dose dependent and peaked at 1:100 dilution. However, IL-12 production was optimal at 1:300 dilution of WF10. The only study of WF10 related to cytokine production is in AIDS patients. McGrath et al. (1998) illustrated that blood macrophage TNF-α expression, which was elevated in AIDS dementia, was down-regulated *in vitro* after exposure to WF10. This data is not agreed with our finding which suggests that WF10 up-regulated TNF-α production. The ability of WF10 to promote tissue repair or regeneration may also involve different cytokines e.g. growth factors such as transforming growth factors β (TGF-β), platelet-derived growth factor and insulin-like growth factors.

Future study on WF10 induced growth factors should provide useful information, particularly in the area of periodontal regeneration.

P. gingivalis is widely regarded as one of the major causative agents of chronic inflammatory periodontal disease (Slots, 1999). Host response to the pathogen is therefore most relevant in the context of periodontal disease. It is known that host mediates much of the pathogenic effects of these bacteria through immune mediators such as cytokines (Page et al., 1997). *P. gingivalis* has been well recognized for its ability to stimulate host cells to release cytokines. There was a very early report of bone resorbing activity in an outer membrane-associated microcapsule from *Bacteroides gingivalis* (a previous name of *P. gingivalis*) (Holt 1982, cited as an unpublished observation). Reddi et al. (1996) showed that *P. gingivalis* stimulated human peripheral blood mononuclear cells to secrete pro-inflammatory cytokines including IL-1β, TNF-α and IL-6. Furthermore, *P. gingivalis* was shown to stimulate IL-1β and TNF-α secretion but at a lower level when compared with other enterobacterial lipid A preparations (Ogawa et al., 1994). IL-1β and TNF-α are well recognized for their role in the bone. Both cytokines stimulate bone resorption and inhibit bone formation (Stashenko et al., 1987; Nguyen et al., 1991; Tatakis, 1993). In addition IL-1 synergizes the bone resorptive actions of TNF-α (Bertolini et al., 1986; Van der Pluijm et al., 1991). Indeed, local production of the two cytokines was evident in inflamed gingiva.

Our study showed that *P. gingivalis* could stimulate PBMC to secrete IL-1β and TNF-α. This is consistent with the study by Reddi et al. (1996). They demonstrated the ability of surface-associated material of *P. gingivalis* to induce IL-1β and TNF-α production from PBMC. In addition, Gemmell and Seymour (1998) reported increased mean percentages of IL-1β+CD19 cells and IL-1β+CD14 cells in PBMC cultures after stimulation with *P. gingivalis*-outer membrane antigens. Interestingly, our data further showed that when WF10 combined with *P. gingivalis*, the combination appeared to have synergistic effect for TNF-α production. The statistically significant enhancement of TNF-α production was found at 0.001 μg/ml and 10 μg/ml *P. gingivalis* as compared to the effect of *P. gingivalis* alone. On the other hand, WF10 markedly down-regulated *P. gingivalis*-induced IL-1β production. The significant suppressive effect on IL-1β production was found at 1 μg/ml *P. gingivalis* but not at other concentrations as compared to the effect of *P. gingivalis* alone. It is however very difficult at this stage to interpret our *in vitro* results of up-regulation of TNF-α and down-regulation of IL-1β in WF10 and *P. gingivalis*-stimulated PBMC cultures. Perhaps, this may be partly due to small numbers of subjects in our study (n=2), therefore further experiments using more subjects are needed.

Unfortunately, there were quite a few limitations in our experiments due to very low budget and a short period of time. The first part of our study showed that WF10 and *P. gingivalis* effect could stimulate moderate amount of TNF-α but low IFN-γ and IL-12. Then in the latter part, we

decided to further investigate how WF10 and *P. gingivalis* affected TNF-α production but not IFN-γ and IL-12. The factors affected our decision was the unavailable commercial ELISA kit for IFN-γ and IL-12, and the prices of the kits were too expensive to obtain. However, the ELISA kit for IL-1β was available in our laboratory at that time and this cytokine is known to play an important role in the pathogenesis of periodontitis. We, therefore, decided to measure both TNF-α and IL-1β production in the latter part of our experiments

In conclusion our findings confirm the immunostimulatory effect of WF10 on human immune cells as shown by up-regulation of CD69 expression on lymphocyte subsets as well as cytokine production. However, it is too early to draw any conclusions from the opposite outcome on *P. gingivalis* induced TNF-α and IL-1 production by WF10. Clinical application of WF10 is likely to have some immunomodulatory effects to local tissue if being administered into deep periodontal pocket which consists of abundant of bacterial plaque pathogens. We do not know the effects of WF10 when combined with other periodontopathic bacteria e.g. *A. actinomycetemcomitans, B. forsythus.* Also we do not know whether WF10 has the ability to induce other cytokines (e.g. growth factors) apart from the pro-inflammatory cytokines. Obviously, these questions need to be answered. Future research of WF10 is required to better understand the immunomodulatory effects of WF10 with periodontopathic bacteria before the drug could find its place as a therapeutic agent in periodontal therapy.