CHAPTER I

INTRODUCTION

1.1 *Background of the present study*

WF10 and its properties

The compound WF10 (Immunokine®) is an intravenous formulation of a unique chlorite peroxide reaction product in a 1:10 dilution of Tetrachlorodecaoxygen anion complex (TCDO). It has been approved in Thailand for the treatment of inflammatory diseases such as cystitis, proctitis, mucositis etc. after cancer radiotherapy and claimed by the manufacturer that WF10 qualitatively and quantitatively modifies the immune response by influencing cell proliferation and function (OXO Chemie,1999). The major target cells for WF10 are macrophages, which are key cells of the immune system. The key features of WF10 are a). to activate macrophage which would lead to enhance phagocytosis and wound healing b). antimicrobial properties (OXO Chemie,1999).

There are a few TCDO formulations with varying formulations of TCDO as listed in Table 1.

Table 1. Summary of TCDO Formulations

Formulation (Other Names)	TCDO Concentration (%,w/v)	ClO_2^- Concentration (mM)
TCDO	100	693
WF10 (Immunokine®, Ryoxon®)	10	63
Oxoferin® (W100)	2	12.6

Immunokine® is provided in a sterile, clear, colorless, odorless, aqueous solution for intravenous infusion. Ryoxon® is also used for intravenous infusion. Interestingly,TCDO is used as a mouthwash for treatment of mucositis (Malik et al., 1997).

The major function of macrophage is to kill microorganisms and phagocytose cellular debris so that healing can proceed. In addition they release a number of cytokines including growth factors, interleukin-8 (IL-8), and tumor necrosis factor-α(TNF-α) needed for new tissue formation and shown to stimulate phagocytic cells such as macrophage itself and polymorphonuclear leukocyte (Schröder et al.,1990; Schopf et al.,1995). Hinz et al. (1986) demonstrated therapeutic effects of WF10 with regards to wound healing properties. It is thought to be due to its oxidative energy of the TCDO complex which is released after activation by biocatalysts such as hemoglobin, myoglobin, and peroxidases which are abundantly present in the body (Youngman et al., 1986). It is known that oxygenation of tissue is essential for the elimination of pathogens, the stimulation of phagocytosis as well as for the degradation of dead tissues and the synthesis of new tissue structures(Kühne et al.,1985). Therefore, WF10 may be suitable for the topical treatment of infected hypoxic wounds with chronic disturbance of wound healing like the situation in periodontitis lesion.

Antimicrobial effects are other important properties of WF10. Stoll et al. (1993) demonstrated that at therapeutic concentrations of the drug, the

growth of all 276 strains of bacteria derived from oropharyngeal flora was inhibited. Of interest, these bacteria included Gram negative periodontopathic bacteria such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* and *Wolinella recta* etc.

Taken together, this drug WF10 may have a modulatory effect on immune response in periodontal disease. However, more scientific data is still required prior to clinical use. Therefore, in the present study the immunomodulatory effect of WF10 will be investigated.

1.2 Objectives

To investigate the immunomodulatory effect of WF10 on immune cell population in PBMC culture. The specific aims are

1. To determine immune activation on lymphocyte subsets (T cells , B cells and NK cells) in WF10 stimulated PBMC cultures as monitored by CD69 expression.
2. To investigate cytokine production (TNF-α ,IFN-γ,and IL-12) in PBMC culture treated with WF10.
3. To evaluate the effect of periodontopathic bacteria, *P. gingivalis* and WF10 on pro-inflammatory cytokine production (TNF-α and IL-1β) in PBMC culture.

1.3 *Hypothesis*

WF10 may have immunomodulatory properties with regards to immune cell activation and cytokine production. The nature of periodontitis lesion consists of a large number of cellular infiltrates such as B cells, T cells and macrophage. High levels of pro-inflammatory cytokines including IL-1β and TNF-α have been consistently reported to be present in gingival crevicular fluid and periodontitis tissues. The lesion itself is in close proximity to subgingival plaque microorganisms and the majority of them are Gram negative bacteria including *P. gingivalis*, *A. actinomycetemcomitans* and *Bacteroides forsythus*. It is known that the interaction between host defense mechanisms and plaque bacteria plays a critical role in the pathogenesis of periodontitis (Page et al., 1997). Immunomodulation by WF10 together with the presence of periodontopathic bacteria, *P. gingivalis* could possibly alter the pathogenesis process of periodontal disease.

1.4 *Field of research*

To investigate immunomodulatory effect of WF 10 and its effect with *P.gingivalis* using an *in vitro* model of PBMC culture.

1.5 *Criteria inclusions*

1. Peripheral blood samples were collected from healthy adult volunteers.
2. Subjects who have healthy periodontium or gingivitis with probing pocket depth less than 4 mm will be included.
3. Subjects have not taken any antibiotics or anti-inflammatory drugs within 3 months prior to the treatment.

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4. Mononuclear cells were obtained from peripheral blood samples using gradient centrifugation.
5. *Porphyromonas gingivalis* FDC 381 were used.
6. Cell surface marker analysis was determined by flow cytometry (FACScan).
7. Cytokines production was analyzed by ELISA.

1.6 *Limitation of thesis*

This study cannot have many samples due to factors of time and expenses.

1.7 *Application and expectation of reseach*

WF10 is a very interesting immunomodulatory drug with good properties of enhancing phagocytosis, wound healing as well as antimicrobial effects (OXO Chemie, 1999). Periodontitis is a chronic inflammatory disease associated with Gram negative bacteria in subgingival plaque. It affects the gingiva, connective tissue and alveolar bone, thus leading to destruction of tooth supporting structures. According to the manufacturer's claim, this drug , WF10 seems to be very attractive for the use as an adjunct to periodontal therapy. Therefore, the present study will investigate the immunomodulatory effects of WF10 using an *in vitro* model of peripheral blood mononuclear cell (PBMC) culture. It is hoped that the results of the study would be beneficial for the decision making with regards to future clinical application of WF10 in periodontal therapy.