CHAPTER I

INTRODUCTION

1.1 Background

Inflammation induced by biological, chemical, and physiological factors has long been associated with increased risk of human cancer in various organs (Coussens and Werb 2002). Epidemiological studies indicate a strong relationship between inflammation and carcinogenesis. For example, individuals with long-standing extensive ulcerative colitis and Crohn's disease have a significant risk of colorectal cancer (Siegel and Sands 2006), chronic hepatitis B and C infections in the liver predispose to hepatocellular carcinoma (Barazani, Hiatt et al. 2007), and *Helicobacter pylori* infection has been established to have a causal relationship to gastric cancer (Peter and Beglinger 2007). Within the lung, chronic inflammatory diseases, such as idiopathic pulmonary fibrosis, systemic sclerosis, certain pneumoconiosis and chronic obstructive pulmonary disease (COPD), have been implicated to lung carcinogenesis (Brody and Spira 2006).

Inflammatory processes in the lung are characterized by the influx of neutrophils into the airways. At this moment it is suggested that increased neutrophil accumulation, although necessary as part of the lung's secondary defense systems, is crucially involved in the development of lung cancer (Quint and Wedzicha 2007). Weitzman and Stossel first demonstrated that activated polymorphonuclear neutrophils (PMNs) are able to cause both mutations (Weitzman and Stossel 1981) and malignant transformations in vitro (Weitzman, Weitberg et al. 1985). Further studies have supported the important role of PMNs in carcinogenesis by the ability of PMNs to induce DNA single strand breaks (Shacter, Beecham et al. 1988; Knaapen, Schins et al. 2002) and DNA base modification (Dizdaroglu, Olinski et al. 1993; Knaapen, Seiler et al. 1999). Although the mechanisms whereby

inflammation may initiate or promote carcinogenesis has not been fully elucidated, production of DNA damaging reactive oxygen species (ROS) by activated inflammatory cells has been proposed to contribute significantly to inflammation-associated carcinogenesis (Ohshima, Tatemichi et al. 2003). Reactive oxygen species are nowadays considered to participate in cancer initiation, promotion and progression. Reactive oxygen species are highly reactive molecules or molecular fragments that are continuously produced in all aerobic organisms, mostly as a consequence of mitochondrial respiratory chain reaction. Besides oxidative phosphorylation, ROS are continuously formed in peroxisomes, the cytochrome P450 system and by inflammatory cells, including neutrophils, eosinophils and macrophages (Karihtala and Soini 2007).

Currently, it is generally accepted that the mutagenic capacity of ROS-derived neutrophils is mediated by H_2O_2 through the formation of highly reactive hydroxyl radical ($^{\bullet}OH$) (Spencer, Jenner et al. 1995). In contrast, until recently HOCl was believed to have no contribution in DNA damage and mutagenesis mediated by activated neutrophils (Shacter, Beecham et al. 1988). However, recently we found that hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation frequency was increased in HOCl-exposed A549 human alveolar epithelial cells (Güngör *et al.*, Unpublished). In line with this observation, Güngör *et al.* showed that neutrophils were potent inhibitors of nucleotide excision repair (NER) in human pulmonary epithelial cells and MPO-catalysed formation of HOCl was thought to be the most likely ROS responsible for these inhibitory effects (Gungor, Godschalk et al. 2007). In fact, HOCl is the major oxidant produced by neutrophils, since MPO consumes up to 70% of neutrophil-derived H_2O_2 to generate HOCl (Hampton, Kettle et al. 1998). Hypochlorous acid plays an important role in bacterial cell killing, but excessive or misplaced generation of HOCl is known to cause damage to tissues. HOCl is capable to react with a number of biological molecules including DNA, proteins, lipids and

cholesterol (Hawkins and Davies 2002). In addition, in the presence of superoxide ($O_2^{\bullet-}$) or reduced metal ions (Masuda, Suzuki et al. 2001), HOCl may generate hydroxyl radicals. Shen *et al.* reported that incubation of DNA with either isolated myeloperoxidase (MPO) or eosinophil peroxidase (EPO), together with plasma levels of halides (Cl- and Br-), and a cell-free $O_2^{\bullet-}$ -generating system resulted in oxidative DNA damage (Shen, Wu et al. 2000). However, despite the multitude of cellular and extracellular targets with which HOCl can react, its ability to cause DNA damage in intact cells is still poorly investigated (Spencer, Whiteman et al. 2000). Therefore, our recent findings regarding HOCl-induced mutagenicity, warrant further studies to investigate the oxidative DNA damage effects of HOCl in order to find a possible explanation for the mutagenic effects of HOCl.

The p53 tumor suppressor is a universal sensor of genotoxic stress that regulates the transcription of genes required for cell-cycle arrest and apoptosis. In response to DNA damage, p53 protein is phosphorylated at its amino-terminus and becomes stabilized upon disruption of an interaction with its negative regulator, MDM2. Upon activation, p53 promotes different interactions with other proteins and with target gene regulatory elements to facilitate cell-cycle arrest, apoptosis, or adaptation in response to DNA damage. Among the transcriptional targets of p53, the cyclin-dependent kinase (CDK) inhibitor $p21^{Cip1}$ and growth arrest and DNA damage (Gadd) genes play a key role in mediating G1 arrest. p21 inhibits growth of proliferating cells by inhibiting G1 cyclin-dependent kinases and proliferating cell nuclear antigen (PCNA)-dependent DNA polymerase activity at DNA replication forks (Colman, Afshari et al. 2000). Like p53, the Gadd genes are induced in cells exposed to genotoxic stress. In the lung, Gadd45 is selectively expressed in alveolar cells during hyperoxia. Gadd45 binds DNA replication and repair proteins cdc2, p21, and PCNA, suggesting a potential role in cell cycle control and DNA repair. Altogether, it suggested that

p21 and Gadd45 may serve to coordinate DNA repair and replication in damaged cells (Roper, Gehen et al. 2005), and are considered as a sensitive indicator of genotoxic stress.

1.2 Research Plans

In the present study, we will examine oxidative DNA damage induced by HOCl in pulmonary cells. For this purpose, the effects of HOCl on cellular DNA were investigated in A549 human alveolar epithelial cells by measuring both DNA single strand breaks and 8-OHdG DNA lesions. DNA strand breakage was measured by the comet assay which is regarded as a rapid and sensitive method for measuring DNA damage. 8-OHdG was measured as biomarker of oxidative DNA damage using high-performance liquid chromatography with electrochemical detection (HPLC-ECD) (Floyd 1990). As a further potential consequence of the cellular DNA damage, we will also investigate whether HOCl alters the expression p21 and Gadd45 using quantitative real time PCR.

1.3 Hypothesis

HOCl can induce mutagenesis in A549 human alveolar epithelial cells through the development of oxidative DNA damage and DNA strand breakage.

1.4 Societal Relevance

This study will gain more insight into the mechanism whereby neutrophils involve in the development of cancer. This might provide a better understanding in the pathogenesis of inflammation-induced carcinogenesis. Finally, it may also aid in the improvement of existing and/or the development of cancer therapies.