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the synthesis and secretion of ANP are augmented mainly in the atrium, but those of BNP increase in the atrium as well as the ventricle. In addition, ANP and BNP are likely to be useful clinicopathological markers of the severity of

heart failure and the evaluation of therapeutic efficacies in canine clinical cases with heart diseases. Further clinical investigations of canine ANP and BNP are worthwhile and warranted.

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Generation of Reactive Oxygen Species by Neutrophils in Host Defense Mechanisms —Spectrophotometric Analyses—

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Generation of reactive oxygen species (ROS) by leukocyte is helpful for the first defense mechanism in case of bacterial infections and others, while it also induces tissue damages, and plays an important role in systemic inflammatory response syndrome, multiple organ dysfunction syndrome and multiple organ failure.

Phagocytic leukocytes include neutrophils, eosinophils, monocytes and macrophages. Among them, neutrophils and eosinophils, both of which contain specific peroxidases in exceptionally high concentration, exhibit the strongest microbicidal function. Generation of ROS by neutrophils consists of two step reactions. They are the superoxide anion (O_2^-) generation derived from reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-catalyzing reaction, and the following disinfection and decomposition mediated by myeloperoxidase (MPO). In this mechanism, the drop of bactericidal activity may be considered as a possible result of defect and abnormality. However, the most useful ROS in disinfection have not been determined. The fact that the defect of NADPH oxidase deficiency,

chronic granulomatous disease (CGD), leads to clinically severe infectious disease, whereas MPO deficiency, usually, but not always, tends to indicate no symptoms of infection shows the complexity of the ROS generation system in vivo.

To realize physiological production of singlet molecular oxygen (1O_2) in porcine MPO-hydrogen peroxide (H_2O_2)-chloride ion (Cl^-) system, the research group I am working with have developed a novel detection technique in the chapter 1 of this thesis.

Neutrophils kill ingested microorganisms by releasing microbicidal proteins from cytoplasmic granules and by generating O_2^- and other ROS into the intracellular phagosomal compartment. The formation of O_2^- is catalyzed by a membrane-associated NADPH oxidase, and in the subsequent reactions O_2^- is dismutated to H_2O_2 . All mammalian phagocytes have the oxidative metabolism. Following phagocytosis, the membranes of azurophilic granules fuse with the membrane of the phagocytic vacuole, which phagolysosomes release MPO into the vacuole containing the ingested microorganisms. The

heme enzyme MPO has a capacity to generate an array of oxidizing species with considerable cytotoxic potential such as hypochlorous acid (HOCl) as a major oxidant. Another toxic oxidant, electronic excited state oxygen, $^1\text{O}_2$, has been originally proposed as the source of chemiluminescence from neutrophils during the respiratory burst through the MPO catalyzing reaction. However, no evidence for the production of $^1\text{O}_2$ was found in the visible light emission produced by activated neutrophils with various kinds of stimulators and $^1\text{O}_2$ inhibitors. In stead of the visible region, the most convincing evidence for $^1\text{O}_2$ formation is to detect the near infrared light emission at 1,268 nm, which is a characteristic wavelength derived from the delta singlet oxygen ($^1\Delta g\text{O}_2$) to grand state oxygen ($^3\Sigma g-\text{O}_2$) transition. So far the detection result of MPO mediated $^1\text{O}_2$ generation in unphysiological condition with the use of Ge detector in the near infrared region implied that the physiological production of $^1\text{O}_2$ by an MPO mediated reaction is unlikely.

The putative role of $^1\text{O}_2$ in the respiratory burst of neutrophils has remained elusive due to lack of reliable means to study its quantitative production. To realize direct measurement of $^1\text{O}_2$ from biological or chemical reactions in the near infrared region, the research group I am working with have developed a highly sensitive detection system which employs two InGaAs/InP pin photodiodes incorporated with a dual charge integrating amplifier circuit. By using this detection system, I detected light emission derived from porcine MPO mediated reaction in physiological conditions; pH 7.4, 1–30 nM MPO, 10–100 mM H_2O_2 and 100–130 mM Cl^- in place of Br^- without the use of deuterium oxide. The porcine MPO- H_2O_2 - Cl^- system exhibited a single emission peak at 1.27 μm with a spectral distribution identical to that of $^1\Delta g\text{O}_2$. Three antibiotics which is administrated in case of inflammatory skin disease in human, showed

remarkable $^1\text{O}_2$ quenching activity in the near infrared region. The result suggests the possible physiological production of $^1\text{O}_2$ in the MPO- H_2O_2 - Cl^- system at an intravacuolar neutral pH, and it also shows the anti-inflammatory effect of antibiotics. In contrast to the previous aspects, the MPO mediated generation of $^1\text{O}_2$ may act an important role in host defense mechanism. However, the proof has yet to be obtained by using cellular $^1\text{O}_2$ detection system in the future.

The chapter 2 of this thesis reports a safe and accurate spectrophotometric technique to determine neutrophil cytochrome b₅₅₈ (cyt b₅₅₈) in minimal amount of sample from human CGD.

CGD is an inherent disease characterized clinically by severe recurrent bacterial infections from infancy. This disease is a disorder of formation of O_2^- by neutrophil NADPH oxidase system, mostly due to defects in cyt b₅₅₈, that is one of the oxidase components. Diagnosis of CGD has been performed by the assay of the O_2^- -forming activity, immunological determination of defects in the oxidase components, and/or spectrophotometry of cyt b₅₅₈. However, spectrophotometric analysis of the b-type heme is difficult with small amounts of blood from human infant CGD patients, as the limited amounts of neutrophils are contaminated with a relatively high ratio of hemoglobin (Hb) which interferes with the heme spectrum of cyt b₅₅₈.

In this thesis, I report an accurate method for the spectrophotometric analysis of cyt b₅₅₈ in a small amount of CGD neutrophils that were treated with CO gas in a safe procedure instead of the previously reported CO-bubbling method. The difference of the reduced minus oxidized cyt b₅₅₈ spectrum was measured under no interference from oxy Hb at the α and β bands and differentiated as $d[\Delta A]/d\lambda$ (λ = wavelength) to obtain further evidence for the defects of the cyt b₅₅₈ heme spectrum. The interference from CO-insensitive met Hb was eliminated by subtracting the absorption peak at the Soret (γ)

band of the contaminating met Hb, which was estimated from the CO-treated and untreated spectra of the same, hemolyzed sample.

This spectrophotometric method is feasible for the determination of heme content of cyt *b*₅₅₈ with a small amount of CGD neutrophils in 10–20 ml of blood even in the presence of contaminating Hb.

Cyt *b*₅₅₈ of human, bovine and porcine have the same absorption peaks in a region. Comparative analysis of cDNA between human and bovine, porcine and murine shows that the highest degree of homology in cyt *b*₅₅₈. Cyt *b*₅₅₈ of mammalian species may have the same properties of absorption spectrum. Therefore, this procedure is applicable to another mamma-

lian.

In conclusion, the results of this thesis suggest the MPO-mediated ¹O₂ generation in neutrophil phagosome and the anti-inflammatory effect of antibiotics. The spectrophotometric determination of cyt *b*₅₅₈ is applicable to minimal amount of neutrophil from CGD.

The advancement of the research in generation of ROS by neutrophils will be further accelerated by the development of cellular ¹O₂ detection system. With the realization of such a detection system, the study of generation of ¹O₂ *in vitro* will become feasible, and generation of ROS by neutrophils in host defense mechanisms will be cleared.

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Pathological studies on distal axonopathy caused by 2,5-hexanedione :
comparative study using normal and neurofilament-deficient quail

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2,5-hexanedione (2,5-HD) is the common γ -diketone metabolite of neurotoxic chemicals such as n-hexane or methyl n-butyl ketone, and has been widely utilized as the most convenient compound for experimental studies of γ -diketone neuropathy. Traditionally, γ -diketone neuropathy is classified as a distal axonopathy, which is characterized by distal axonal swellings with neurofilament (NF) accumulation and degeneration in long tracts of the central nervous system (CNS) and long nerves of the peripheral nervous system (PNS). The relationship between NF accumulation and axonal degeneration, however, has not been adequately

elucidated in this toxic neuropathy. In the present study, this relationship was examined using normal and neurofilament-deficient (Quv) quail.

Both normal and Quv quail were inoculated intraperitoneally with 350 mg/kg per day 2,5-HD for 6 consecutive weeks. 2,5-HD induced distal axonopathy in about 4–6 weeks in normal quail and acute neurotoxicity in Quv quail. Although all treated Quv quail showed neurological signs, there were no recognizable 2,5-HD-induced lesions in the nervous system. Two explanations for the absence of the distal axonopathy in Quv quail treated with 2,5-HD are possible. The development of axonopathy may require an accu-