Studies on Novel Pathways for Activation of Nitrile(s)

A Thesis Submitted in Partial Fulfillment of the Requirements for the Masters Degree in Pharmaceutical Sciences (Pharmacology and Toxicology)

By

Sherry Nasralla Tawfik Nasralla

B.Sc. in Pharmaceutical Sciences., Ain Shams University (2002)
Demonstrator of Pharmacology and Toxicology
Faculty of Pharmacy, Ain Shams University

Under the Supervision of

Prof. Amani E. Khalifa

Professor and Head of the Department of Pharmacology & Toxicology,
Faculty of Pharmacy, Ain Shams University

Prof. Mohammed Z. Gad

Professor of Biochemistry, Faculties of Pharmacy, Ain Shams University and The German University in Cairo

Dr. Asser I. Ghoneim

Lecturer, Department of Pharmacology and Toxicology,
Faculty of Pharmacy, Ain Shams University

Department of Pharmacology and Toxicology
Faculty of Pharmacy, Ain Shams University, Cairo, Egypt
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Examination Board Approval Sheet

Name of Candidate

Sherry Nasralla Tawfik Nasralla

Title of Thesis

Studies on Novel Pathways for Activation of Nitrile(s)

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Ain Shams University

Department of Pharmacology and Toxicology

Approved by the committee in charge:

1. **Prof. Amani E. Khalifa**
2. **Prof. Mohammed Z. Gad**
3. **Prof. Osama A. Badary**
4. **Prof. Evan. I. Saad**

Date: 26 / 7 / 2009

Pre-requisite Post-Graduate Courses

Besides the work presented in this thesis, the candidate has attended the following courses:

General courses:

1. Statistics
2. Instrumental Analysis
3. Computer Sciences
4. Physical Chemistry

Special Courses:

1. Neuropharmacology
2. Clinical Toxicology
3. Experimental Pharmacology
4. Molecular Pharmacology
5. Selected Topics in Pharmacology and Toxicology

She has successfully passed examinations in these courses with the general grade of *Excellent*.

Head of Pharmacology and Toxicology Department

Prof. Amani Emam Khalifa

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Table of Contents

Contents	*Page*
List of Tables	*vii*
List of Figures	*ix*
Abstract	*xii*
List of abbreviations	*xv*
Introduction	*1*
Acrylonitrile	*2*
Lactoperoxidase	*24*
Aim of the Work	*35*
Materials and Methods	*40*
Materials	*41*
Design of the Work	*48*
Methods	*51*
Results	*67*
Discussion	*111*
Summary and Conclusions	*148*
References	*152*
Arabic Summary and Conclusions	*183*

List of Tables

Tables	*Page*
Table 3.1 *Effect of Fe^{+2} concentration on CN^- production from ACN by the Fenton Reaction*	***64***
Table 4.1 *Effect of temperature on CN^- production by the LPO/H_2O_2/ACN System*	***70***
Table 4.2 *Effect of pH on CN^- production by the LPO/H_2O_2/ACN System*	***73***
Table 4.3 *Effect of Incubation Time on CN^- production by the LPO/H_2O_2/ACN System*	***76***
Table 4.4 *Effect of LPO concentration on CN^- production by the LPO/H_2O_2/ACN System*	***79***
Table 4.5 *Effect of H_2O_2 concentration on CN^- production by the LPO/H_2O_2/ACN System*	***82***
Table 4.6 *Effect of ACN concentration on CN^- production by the LPO/H_2O_2/ACN System*	***85***
Table 4.7 *Optimum parameters for CN^- production by the LPO/H_2O_2/ACN System*	***87***

Table 4.8 *Effect of NO_2^- concentration on CN^- production by the LPO/H_2O_2/ACN System* ***91***

Table 4.9 *Effect of Inhibitors on CN^- production by the LPO/H_2O_2/ACN System* ***96***

Table 4.10 *Effect of Halides on CN^- production by the LPO/H_2O_2/ACN System* ***101***

Table 4.11 *Effect of Sulfhydryl Compounds on CN^- production by the LPO/H_2O_2/ACN System* ***105***

Table 4.12 *Effect of SOD and Mannitol CN^- production by the LPO/H_2O_2/ACN System in the presence of GSH* ***109***

List of Figures

Figures	*Page*
Figure 1.1 *SOHIO Process for the production of ACN*	***3***
Figure 1.2 *Biotransformation of ACN*	***18***
Figure 1.3 *The prosthetic heme group in LPO is bound to the protein through ester bonds between two of its methyl groups and Glu375 and Asp225 of the protein*	***25***
Figure 1.4 *The general reaction scheme of mammalian peroxidases*	***28***
Figure 1.5 *The general mechanism of peroxidases*	***29***
Figure 1.6 *Reactions of LPO with nitrite*	***32***
Figure 1.7 *Reactions of LPO with thiocyanate*	***33***
Figure 3.1 *Conway Microdiffusion Cell*	***46***
Figure 3.2 *Wheaton Scintillation Vials*	***46***
Figure 3.3 *Orion Double Junction Silver/Silver chloride Reference Electrode*	***47***

Figure 3.4 *Orion Silver/Sulfide Ion Selective Electrode* ***47***

Figure 3.5 *Design of the work* ***50***

Figure 3.6 *Standard Curve of CN^-* ***54***

Figure 3.7 *Fenton Reaction* ***61***

Figure 3.8 *Effect of Fe^{+2} concentration on CN^- Production from ACN by the Fenton Reaction* ***65***

Figure 4.1 *Effect of Temperature on CN^- Production by the LPO/H_2O_2/ACN System* ***71***

Figure 4.2 *Effect of pH on CN^- Production by the LPO/H_2O_2/ACN System* ***74***

Figure 4.3 *Effect of Incubation Time on CN^- Production by the LPO/H_2O_2/ACN System* ***77***

Figure 4.4 *Effect of LPO concentration on CN^- Production by the LPO/H_2O_2/ACN System* ***80***

Figure 4.5 *Effect of H_2O_2 concentration on CN^- Production by the LPO/H_2O_2/ACN System* ***83***

Figure 4.6 *Effect of ACN Concentration on CN^- Production by the LPO/H_2O_2/ACN System* ***86***

Figure 4.7 *Kinetics of ACN Metabolism to CN^- by the LPO/H_2O_2/ACN System* ***89***

Figure 4.8 *Effect of NO_2^- concentration on CN^- production by the LP* ***92***

Figure 4.9 *Effect of Inhibitors on CN^- production by the LPO/H_2O_2/ACN System* ***97***

Figure 4.10 *Effect of Halides (0.1 mM – 10 mM) on CN^- production by the LPO/H_2O_2/ACN System* ***102***

Figure 4.11 *Effect of Halides (>10 mM) on CN^- production by the LPO/H_2O_2/ACN System* ***103***

Figure 4.12 *Effect of Sulfhydryl Compounds on CN^- production by the LPO/H_2O_2/ACN System* ***106***

Figure 4.13 *Effect of SOD and Mannitol on CN^- production by the LPO/H_2O_2/ACNSystem in the presence of GSH* ***110***

Figure 5.1 *Proposed pathway for activation of ACN by the LPO/H_2O_2 System to generate CN^-* ***119***

Figure 5.2 *LPO redox states during interaction with ACN to generate CN^-* ***120***

Abstract

Acrylonitrile (ACN) is a well reported animal carcinogen and a reasonably anticipated human carcinogen. ACN poses a predicament for researchers who proved the necessity of its activation by the cytochrome P450 $2E_1$ enzyme, to induce carcinogenicity, yet, was reported to cause tumors in organs with diminished levels of the enzyme. The lactoperoxidase (LPO) enzyme is well reported for its multiple health benefits, however, the ability of the enzyme to activate pro-carcinogens is not fully explored. The aim of the present study was to investigate the ability of the LPO enzyme system to activate ACN *in vitro*, thereby resolving the challenge posed by the toxicant. Reaction mixtures containing the LPO enzyme system and ACN were incubated, in the presence and absence of reaction modulators, and assessed for the presence of cyanide ions (CN^-), used as a marker for the activation of ACN by the LPO enzyme system. Determination of CN^- was done by the electrochemical method described by Abreu and Ahmed (1980). Results revealed that when the reactants were incubated at a pH of 5 and a temperature of 37ºC in stoichiometric ratios (5 U/ml LPO, 0.5 mM H_2O_2 and 160 mM ACN) for 15 minutes, the LPO system was able to activate ACN proved by the generation of detectable levels of CN^-. Furthermore, the presence of nitrite (NO_2^-) was found to enhance the reaction while free radical scavengers and

competitors for LPO binding were found to inhibit it. Fluoride (F^-) and iodide (I^-) were potent inhibitors of LPO induced ACN activation, while the presence of bromide (Br^-) and chloride (Cl^-) was indifferent. The rate of CN^- generation was found to be enhanced in the presence of sulfhydryl compounds, but further investigation revealed a non-enzymatic CN^- extraction mechanism from ACN in the absence of free radical generation. A pathway depicting the activation of ACN by the LPO enzyme system was proposed, with the possible interventions and roles of various ions tested on the system. Collectively, results demonstrated the ability of the LPO enzyme system to activate ACN which may provide insight for elucidation of the mechanism of its carcinogenicity and presents a novel pathway for its activation.

Keywords: Acrylonitrile, Lactoperoxidase, Cyanide, Activation, in-vitro.

List of Abbreviations

ACN : Acrylonitrile

$ACN^{\bullet}$: Acrylonitrile radical

Cat. : Catalase

CEO : Cyanoethylene oxide

CN^{-} : Cyanide anion

CYP 450: Cytochrome P450

GSH : Reduced Glutathione

H_2O_2 : Hydrogen peroxide

HRP : Horseradish peroxidase

IMN. : Indomethacin

KCN : Potassium cyanide

LPO : Lactoperoxidase

MPO : Myeloperoxidase

NAC : N-acetyl L-cysteine

NaN_3 : Sodium azide

NO_2^{-}: Nitrite anion

$NO_2^{\bullet}$: Nitrite Radical

NOS : Nitric oxide synthases

$O_2^{\bullet -}$: Superoxide anion

OH^{-} : Hydroxyl anion

$OH^{\bullet}$: Hydroxyl radical

ROS : Reactive oxygen species

SA : Salicylic acid

SOD: Superoxide dismutase