

國立屏東科技大學生物科技系
Department of Biological Science and Technology
National Pingtung University of Science and Technology

碩士學位論文
Master Thesis

膽紅素對在葡聚糖硫酸鈉（DSS）小鼠結腸炎模型的影響

The Effects of Bilirubin in the Dextran Sulfate Sodium (DSS)
Mouse Colitis Model

指導教授：黃卓治 博士
Adviser：Tzou-Chi Huang, Ph.D.

研究生：農氏芳絨
Graduate student：Nong Thi Phuong Nhung

中華民國 104 年 6 月 29 日
June 29, 2015

摘要

學號：M10218032

研究計劃：膽紅素對在葡聚醣硫酸鈉 (DSS) 小鼠結腸炎模型的影響

總頁數：64 頁

學院名稱：國立屏東科技大學

系別：生物科技系

畢業時間及摘要別：一百零三學年度第二學期碩士學位論文摘要

研究生：農氏芳絨

指導教授：黃卓治 教授

摘要內容:

BALB/c 小鼠分為正常組、模型對照組(500 kDa 的和 40 kDa 的 DSS)和三個不同濃度的膽紅素處理組。口服給藥膽紅素(10, 50, 100 毫克/公斤體重)一周後，動物給予 3%DSS(40 kDa)的飲用水，除了正常組外，進一步 8 個連續天給藥膽紅素有或沒有膽紅素治療。40 kDa 的 DSS 造成小鼠嚴重的瀰漫性結腸炎，而 500 kDa 的 DSS 處理的小鼠沒有病變。膽紅素可防止體重減輕和 DSS 誘導的結腸炎增加的疾病活動指數(DAI)。三種不同濃度的膽紅素中，10mg / kg 的膽紅素組可取得最好治療的結果。膽紅素抑制 DSS-引導的粘膜水腫、粘膜下糜爛和結腸及多種組織的損害。本研究發現，膽紅素給藥可改善臨床症狀，並降低小鼠模型中潰瘍性結腸炎(UC)的損害。

關鍵詞：膽紅素 (BR) ;葡聚醣硫酸鈉 (DSS) ;結腸炎;炎性腸病。

Abstract

Student ID : M10218032
Title of thesis : **The Effects of Bilirubin in the Dextran Sulphate Sodium (DSS) Mouse Colitis Model**
Total Page : 64 pages
Name of Institute : National Pingtung University of Science and Technology
Department of Biological Science and Technology
Graduate Date : June 29th, 2015 **Degree Conferred:** Master
Name of Student : Nong Thi Phuong Nhung **Adviser:**
Tzou – Chi Huang, Ph.D

The content of abstract in this thesis:

BALB/c mice were divided into normal group, colitis control group (500 kDa and 40 kDa DSS), and three different concentrations of bilirubin-treated groups. Bilirubin (10 or 50 or 100 mg/kg body weight) was administered orally. After one week, animals were given 3% DSS (40 kDa) in drinking water, except those of the normal group, and for a further 8 consecutive days with or without bilirubin treatment. Mice treated with 40 kDa DSS developed most severe diffuse colitis, while mice treated with DSS of 500 kDa had no lesions. Bilirubin prevents body weight loss and an increase in disease activity index (DAI) scores in mice with DSS-induced colitis. Among three different concentrations of bilirubin, 10 mg/kg bilirubin group was achieved the best result. Bilirubin treatment inhibited DSS-induced mucosal edema, submucosal erosions and colon damage in various tissues. Bilirubin administration improves clinical signs and reduces the damage of colonic inflammation in a murine model of ulcerative colitis (UC).

Keywords: Bilirubin (BR); dextran sodium sulfate (DSS); Colitis;
Inflammatory bowel disease.

Acknowledgements

First of all, I have to thank to my principle advisor, Professor Huang Tzou - Chi, for supporting my research with ideas, providing me the opportunity to further my scientific knowledge in such an excellent lab and giving me a push in the right direction in life when I needed the most. Also, I wish to extend my gratitude to Mr. Ellis Huang for his helps during the experiment in Veterinary Department.

Secondly, many thanks to all Professors from College of Biological Science and Technology who give me a lot of interesting lectures and good supports when I attended classes in Master program as well as in research. My master would have remained a dream if I did not receive a wonderful chance to study at National Pingtung University of Science and Technology. I appreciate all your advices and it will prepare me for whatever obstacles I will face in the future.

I would like to thank all members in BT 204 laboratory who helped me so much when I first come Taiwan in both life and research.

To my beloved-family in Viet Nam, Mum, and Dad for their support, encouragement and understanding. For the countless times that I fell and stumbled, their unconditional love got my chin off the floor, helped me to overcome the obstacles and carry on with my long educational journey to get to where I am now.

Nong Thi Phuong Nhung

2015.06.29

Table of Content

摘要	I
Abstract	II
Acknowledgements.....	IV
Table of Content.....	V
List of Table.....	VIII
List of Figure	IX
I. INTRODUCTION	1
1.1. Background.....	1
1.2. Aim of the Study.....	3
1.3. Research Motivation	3
II. LITERATURE REVIEW	5
2.1. Inflammatory bowel diseases (IBD).....	5
2.1.1. Classification of IBD	5
2.1.2. Pathophysiology	6
2.2. DSS - Animal models of inflammatory bowel diseases	7
2.2.1. Dextran sulfate sodium (DSS).....	7
2.2.2. Advantage of DSS colitis mouse model	8
2.2.3. Colitis procedure.....	9

2.2.4. Dextran sulfate sodium colitis mouse model	9
2.2.5. The molecular weight of DSS	10
2.2.6. Clinical features	12
2.2.7. Pathological features of DSS colitis	12
2.2.8. Pathogenesis of DSS colitis	13
2.3. Bilirubin.....	14
2.3.1. Bilirubin: chemical structure and formation.....	14
2.3.2. Bilirubin metabolism	15
2.3.3. Toxicity of bilirubin.....	17
2.3.4. Bilirubin as an antioxidant	19
III. MATERIALS AND METHODS.....	22
3.1. Materials	22
3.1.1. Preparation of bilirubin	22
3.1.2. Preparation DSS solution	22
3.2. Experimental Design.....	22
3.3. Animals.....	24
3.4. Induction of colitis	24
3.5. Assessment of DSS colitis.....	25
3.6. Histopathological analysis.....	25

3.7. Statistical Analysis.....	27
IV. RESULTS AND DISCUSSIONS.....	27
4.1. The effects of DSS molecular weight to induce colitis in mice.....	27
4.2. Effects of bilirubin (BR) in the dextran sulphate sodium (DSS) mouse model – Induced experimental colitis.....	33
4.2.1. Oral administration of bilirubin prevents body weight loss in the DSS-induced colitis model	33
4.2.2. Assessment of disease activity index in mice.....	34
4.2.3. Bilirubin prevented the colonic shortening induced and prevented the reducing liver weight induced by DSS	36
4.2.4. Histologic findings in DSS-induced colitis	38
V. CONCLUSION	47
REFERENCE	48
APPENDIX.....	58
Information of Author	64

List of Table

Table 2.1: Variation of molecular weight and concentration of DSS used in the induction of colitis in some published studies.	11
Table 2.2: Effects of Bilirubin in animal model in some published studies...	20
Table 3.1: Disease activity index (DAI) scoring system	25



List of Figure

Figure 2.1: Conceptual framework for the pathogenesis of IBD.....	6
Figure 2.2: Molecular structure of Dextran sulfate sodium (DSS)	7
Figure 2.4: Chemical structure of the naturally occurring unconjugated BR..	15
Figure 2.5:Oxidation-reduction cycles for bilirubin and GSH	16
Figure 3.1: The flowchart of experimental design	24
Figure 4.1: Changes in the body weight of mice with DSS-induced colitis...	28
Figure 4.2: The disease activity index in mice	29
Figure 4.3: Histological analysis of mice organs	30
Figure 4.4: Oral administration of bilirubin prevents body weight loss in the DSS-induced colitis model.	33
Figure 4.5: The disease activity index in mice	34
Figure 4.6: Bilirubin prevented the colonic shortening induced and prevented the reducing liver weight induced by DSS.	36
Figure 4.7: Bilirubin reduces disease manifestation during DSS model in large intestine.	39
Figure 4.8: Histological analysis of large intestine in DSS mice group.....	39
Figure 4.9: Histological analysis of spleen..	42
Figure 4.10: Histological analysis of liver.....	43
Figure 4.11: Histological analysis of kidney.....	44