学校编码：10384　　分类号＿＿＿密级＿＿
学号：24520141153576　　UDC ＿＿

厦门大学

硕 士 学 位 论 文

湿热应激对ETEC诱导腹泻小鼠HSPs介导TLR4信号通路的影响及三仁汤干预作用

Influence of Hot-humid Stress on HSPs mediated TLR4 signal channel in ETEC induced diarrhea mice and intervention effect of Sanren Decoction

单晓梅

指导教师姓名：　赖 鹏 华　副 教 授
专 业 名 称：　中 医 内 科 学
论文提交日期：　２０１７ 年　　月
论文答辩日期：　２０１７ 年　　月
学位授予日期：　２０１７ 年　　月

答辩委员会主席：＿＿＿＿＿＿
评　　阅　　人：＿＿＿＿＿＿

2017 年 5 月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外,该学位论文为(　　)课题(组)的研究成果，获得（　　）课题（组）经费或实验室的资助，在（　　）实验室完成。（请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。）

声明人（签名）：

年　　月　　日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

（　　）1.经厦门大学保密委员会审查核定的保密学位论文，于　　年　　月　　日解密，解密后适用上述授权。

（　　）2.不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年　　月　　日

摘 要

旅行者腹泻（Travelers'Diarrhea,TD)作为感染性腹泻病中的一种特殊类型，具有与地区、时间密切相关的流行病学特征。首先，TD 发生与旅游目的地密切相关，Dupont[1]根据旅行者腹泻发生率的高低，将全球分为三类地区，南亚、非洲、 拉丁美洲大多数的国家属高风险地区。其次，旅行者来源地亦影响 TD 发病率，即若旅行者来源地与目的地气候社会生活条件相仿，则 TD 发生率较低。且TD经常发生于逗留在外埠的早期阶段，停留时间越长，发生率和复发率越低。同样，若近期到过热带地区则再次到访时 TD 的发生率亦降低。专家推测这种现象与机体的应激和习服相关。TD 的病因有多种多样，但以生物性致病因子最为重要，其中主要病种以产肠毒素性大肠杆菌（ETEC)为主，约占 40%[2]。脂多糖(LPS)是大肠杆菌细胞壁最外层的主要物质，Toll 样受体（TLRs）是近年来发现的在宿主抗病原微生物的免疫应答中起重要作用的细胞表面受体，也是巨噬细胞参与炎症反应的重要受体，在免疫防御中起关键作用。Toll 样受体 4（TLR4）可接受 LPS 的刺激，从而导致单核-巨噬细胞活化，使其吞噬和杀菌能力增强，同时释放一系列细胞因子而引起炎症反应。髓样分化因子 88（MyD88）在 TLR 信号通路中属于节点位置，起着承上启下的作用，其传导的信号可以启动活化核转录因子-κB（NF-κB）信号通路，并在固有免疫防御、信号传递和疾病发生方面起着关键的作用。NF-kB 是一种能调节炎症和免疫基因表达的转录调节因子，其可由细菌脂多糖、氧化应激、细胞因子等多种刺激激活，从而产生一系列的病理现象。热休克蛋白 60（HSP60）、热休克蛋白 70（HSP70）属于热应激蛋白，是生物体在抵制亚致死温度（一般高于其正常生长温度 8～12℃）时体内合成的蛋白质。而对病原体的侵入，HSP60、HSP70 会充当免疫抗原来激活免疫系统。因此，本实验通过观察湿热应激对 ETEC 感染性腹泻 TLR4 相关信号通路的影响探讨旅行者腹泻的发病机制及应激在其间的作用。

“湿盛则濡泻”，湿热为腹泻的主因，亦有流行病学调查显示旅行者腹泻在夏季、雨季发病率较高[1, 2] 。三仁汤出自吴鞠通的《温病条辨》，是湿热证通用方，作用于上、中、下三焦的湿热之证，临床可化裁治疗多种与湿热有关的病症。

本实验还观察三仁汤对湿热应激下腹泻小鼠模型回肠组织 HSPs 及 TLR4 相关信号通路的影响以探讨发病机制和三仁汤的防治机制。

目的：本实验采用复合因素（外部湿热应激和大肠杆菌 ETEC 感染）建立湿热应激下的腹泻小鼠模型，并观察在湿热应激下三仁汤对 ETEC 诱导的腹泻小鼠的行为学改变， TLR4、MyD88、HSP60、HSP70、NF-κB 表达水平的变化，探讨湿热应激对 TLR4 胞内信号转导以及下游 NF-kB 信号转导的影响，及三仁汤的作用机理。

方法：使用清洁级 BABL/c 小鼠 140 只，随机分组。通过高温高湿刺激、产毒性大肠杆菌腹腔注射，综合构建湿热证腹泻动物模型，给予三仁汤干预治疗，给予利福昔明作为阳性对照。用实时荧光定量 PCR 的生物学方法检测 TLR4、MyD88、HSP60、HSP70、NF-κB 的表达，HE 染色法观察回肠病理改变。

结果：1. 湿热模型组出现发热、嗜卧、行动呆滞、肛周红肿、便塘或粘液便等表现；湿热模型鼠回肠组织 HE 染色见粘膜充血、水肿、渗出、淋巴组织增生、大量炎症细胞浸润，提示造模成功。三仁汤治疗组症状较轻，光镜显示肠道炎症反应明显减轻。三仁汤组和阳性对照组之间无明显差异。

2. 第 6h 湿热模型组回肠组织中 TRL4、MyD88、NF-kB、HSP60、HSP70 的信使 RNA （mRNA）水平比正常对照组显著升高（$p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p<0.01$）。三仁汤组第 6h 回肠组织中 TRL4、NF-kB 的 mRNA 水平比湿热模型组显著降低($p<0.001$, $p<0.01$)；TRL4 的 mRNA 水平比阳性对照组显著降低($p<0.01$)，三仁汤组和阳性对照组在其他指标上无明显差异。

3. 第 18h 湿热模型组回肠组织中 TRL4、MyD88 的 mRNA 水平比正常对照组升高（$p<0.05$,$p<0.01$,），NF-kB 的 mRNA 水平比正常对照组降低（$p<0.05$）。三仁汤组第 18h 回肠组织中 NF-kB、HSP60 的 mRNA 水平比湿热模型组升高（$p<0.001$, $p<0.001$）；TRL4 的 mRNA 水平比阳性对照组降低($p<0.05$)，三仁汤组和阳性对照组在其他指标上无明显差异。

结论：1. 在大肠杆菌腹腔注射的基础上，使用人工智能气候箱创建高温高湿的环境，建立湿热证腹泻模型，体现了中医湿热证理论，脾胃内伤、再感客邪的“内外合邪”的致病特点。2. 湿热应激和大肠杆菌 ETEC 感染共同作用于小鼠，可引起前期（6h）小鼠回肠组织 HSP60、HSP70 的过度表达和 TLR4 水平的

增高，从而加重炎症反应。表明 HSP60、HSP70 对 TLR4 和 NF-kB 的活化可能有调节作用。3. 三仁汤可能通过维持 HSP60、HSP70 的高表达，保护宿主细胞，抑制 LPS-TLR4 信号通路的传导以及延缓下游 NF-kB 表达趋势，从而控制机体炎症，达到干预的效果。并且效果要优于利福昔明、拆方 1 和拆方 2。

关键词：湿热应激； 腹泻； 三仁汤； HSPs ； TLR4 信号通路

Abstract

Travelers' diarrhea (TD), as a special type of infectious diarrhea, has epidemiological characteristics that are closely related to the region and time. First, TD occurs closely with the tourist destination. Dupont divides the earth into three categories according to the incidence of dike diarrhea. Most countries in South Asia, Africa and Latin America are high risk areas. Second, the source of the traveler also affects the incidence of TD. If the source of the traveler is similar to the social climate of the destination climate, the incidence of TD is lower. And TD often occurs in the early stages of the stay outside the place, the longer the stay, the lower the incidence and recurrence rate. Similarly, the incidence of TD is also reduced if the recent to the tropical areas are re-visit. Experts speculate that this phenomenon is related to the body's stress and acclimatization. TD has a variety of factors, but the most important biological factors, of which the main disease to enterotoxigenic Escherichia coli (ETEC), accounting for about 40%. Lipopolysaccharide (LPS) is the main material of the outermost layer of E. coli cell wall.Toll-like receptors (TLRs) are located on the cell surface and play an important role in the immune response. It is also an important receptor for macrophages involved in the inflammatory response and plays a key role in immune defense. When TLR4 (Toll like receptor 4) receives LPS (Lipopolysaccharide) stimulation, it can lead to mononuclear - macrophage activation and release of a series of cytokines, so that it can enhance phagocytic and bactericidal ability. MyD88 (myeloid differentiation primary response protein 88) is a node position in the TLR signaling pathway, and it can activate the NF-kB signal pathway and plays a critical role in inherent immune defense, signal pathway and disease process. NF-kB is a transcriptional regulator that regulates the expression of inflammation and immune genes, which is activated by a variety of stimuli, such as bacterial lipopolysaccharide, oxidative stress, cytokines and so on. HSP60 and HSP70 belong to the heat shock protein, which is the protein synthesized in vivo when the

organism resists the sub-lethal temperature (generally higher than its normal growth temperature 8 ~ 12 ℃). when pathogens infected, HSP60 (heat shock protein 60) and HSP70 (heat shock protein 70) can act as immune antigens to activate the immune system. Therefore, in this study, we investigated the effects of damp-heat stress on the TLR4-related signal pathway in ETEC-infected diarrhea. The pathogenesis and the role of stress in diarrhea were discussed.

"Strong moisture will cause diarrhea"，Damp heat is the main cause of diarrhea. There is also an epidemiological survey that travelers have a higher incidence of diarrhea in the summer and rainy season. Sanren decoction from Wu Jutong's book "Wen disease differentiation", is the damp heat syndrome common side. Sanren decoction could treat the syndrome of dampness-heat from the triple energizer. In clinical practice, the modified Sanren decoction can treat a variety of damp-related diseases. This study also observed the effect of Sanren decoction on HSPs and TLR4-related signal transduction in ileal tissue of diarrhea mice under the effect of damp-heat stress. To explore the pathogenesis and Sanren decoction prevention and control mechanism.

Objective: In this study, the diarrhea mice model under damp-heat stress was induced by compound factors (external damp-heat stress and E. coli ETEC infection). To observe the behavioral changes of Sanren Decoction on ETEC - induced diarrhea in mice under damp - heat stress. And discussion Toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), heat shock protein 60 (HSP60), heat shock protein level changes (of HSP70) and nuclear factor -κB (NF-κB) expression. We analyze and discuss the mechanism of heat stress on TLR4 intracellular signal transduction and downstream NF-kB signal transduction, and the mechanism of Sanren decoction.

Methods: 140 clean BABL / c mice were used and randomized. Through the high temperature and high humidity stimulation, the toxicity of E. coli intraperitoneal injection, comprehensive construction of damp-heat diarrhea diarrhea animal model, given Sanren decoction treatment, given rifaximin as a positive control. The expression of TLR4, MyD88, HSP60, HSP70 and NF-κB were detected by real-time fluorescence quantitative PCR. The pathological changes of ileum were observed by

HE staining.

Results: 1. In the model group, the mice in the damp-heat model group were feverish, infatuated, dull, perianal swelling, pond or mucus. HE staining of ileum tissue in damp - heat model mice showed mucosal congestion, edema, exudation, lymphoid tissue hyperplasia and extensive inflammatory cell infiltration.

2. The mRNA levels of TRL4, MyD88, NF-kB, HSP60 and HSP70 in the ileum tissue of the 6th hour model were more increasingly than blank control group, and have significant statistical differences. The mRNA levels of TRL4 and NF-kB in ileum tissue of Sang Ren Tang were significantly decline than those in damp-heat model group. The mRNA level of TRL4 in ileum tissue of Sanren decoction group was significantly decline that of positive control group at the 6th hours. Sanren decoction group compared with the positive control group in the other indicators no significant difference.

Conclusion: 1. On the basis of the intraperitoneal injection of E. coli, artificial climate incubator, automatically adjust the temperature, humidity, heat diarrhea syndrome model system, reflecting the heat TCM syndrome stomach injuries, then induced the pathogenic characteristics.

2. Damp-heat stress and ETEC infection can cause excessive expression of HSP60, HSP70 and TLR4 in ileum tissue of mice at 6th hour, which is the reason for aggravating the inflammatory response. HSP60, HSP70 may modulate the activation of TLR4 and NF-kB.

3. Sanren decoction could protect the host cells, inhibit the transmission of LPS-TLR4 signaling pathway and delay the downstream NF-kB expression by maintaining high expression of HSP60 and HSP70. This will control the body inflammation, to achieve the effect of preventive treatment. And Sanren decoction is better than rifaximin, split 1 and split 2.

Keywords： Hot-humid stress ; diarrhea ; Sanren decoction ; HSPs ; TLR4 signal path

目 录

Table of Contents

英汉缩略语名词对照

英文缩写	英文全称	中文全称
TD	Travelers'Diarrhea	旅行者腹泻
ETEC	Enterotoxigenic Escherichia coli	产肠毒素性大肠杆菌
TLRs	Toll like receptor	Toll 样受体
TLR4	Toll like receptor 4	Toll 样受体 4
LPS	Lipopolysaccharide	脂多糖
LBP	LPS binding protein	LPS 结合蛋白
MyD88	Myeloid differentiation primary response protein 88	髓样分化因子 88
NF-kB	nuclear transcription factor kappa B	核转录因子 kappa B
HSP60	heat shock protein 60	热休克蛋白 60
HSP70	heat shock protein 70	热休克蛋白 70
mRNA	Messenger ribonucleic acid	信使 RNA
min	Minutes	分钟
ml	Milliliter	毫升
μl	Microlitre	微升
h	hour	小时
kg	Kilogram	千克