Tropical Journal of Pharmaceutical Research April 2018; 17 (4): 669-673
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i4.15

Original Research Article

Galantamine protects against hydrochloric acid aspiration-induced acute respiratory distress syndrome in rabbits

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Sent for review: 5 February 2018 *Revised accepted: 28 March 2018*

Abstract

Purpose: *To study the effect of galantamine on anti-inflammatory responses in acid aspiration-induced acute respiratory syndrome (ARDS), and the underlying mechanism.*
Methods: *Six groups of male rabbits (156), i.e., control group, ARDS group, galantamine + ARDS (GAL) group, galantamine + ARDS + methyllycaconitine (MLA) group, galantamine + ARDS + vagotomy (Vag) group, and galantamine + ARDS + atropine sulfate (ATS) group. ARDS model was produced by acid aspiration. After 4 h, TNF-α, IL-6 and IL-1β were assayed in lung tissue, while corticosterone levels were determined in blood. Histopathological lesions and wet-to-dry (W/D) weight ratio of lung tissue (n = 10) were assessed. After 12 h, HMGB1 protein and corticosterone levels were determined in lung tissue and blood, respectively. Mortality rate was determined after 72 h.*
Results: *Acid aspiration-induced ARDS induced disorganization of lung structure, and elevated TNF-α, IL-6, IL-1β, and HMGB1activities, and lung W/D weight ratio. The acid-induced ARDS, as well as increases in W/D weight ratio, pro-inflammatory cytokines and lung lesions were significantly decreased by galantamine pretreatment. Methyllycaconitin, vagotomy and atropine sulfate abolished the galantamine-induced suppression of acute inflammatory response, pathological changes in lungs, and W/D weight ratio. However, serum corticosterone levels were not significantly altered in each group.*
Conclusion: *Galantamine reduces inflammation in acid aspiration-induced ARDS by the cholinergic anti-inflammatory pathway.*

Keywords: *Galantamine, Hydrochloric acid, Aspiration-induced, Respiratory distress syndrome, Methyllycaconitin, Vagotomy, Atropine sulfate*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The etiology of acute respiratory distress syndrome (ARDS) is associated with damage to the membrane of capillaries of the alveoli which compromises their permeability and results in edema [1]. Aspiration pneumonitis is one of the important causes of ARDS [2]. Studies so far are yet to unravel the signal pathways implicated in the pathogenesis of ARDS. Nonetheless, research findings indicate that ARDS may be triggered on and amplified by cytokine mediation through enhanced expression of high mobility group box 1 (HMGB1), interleukin (IL)-1, IL-6,

and tumor necrosis factor (TNF)-α in the lungs [3].

In situations such as autoimmune diseases and sepsis, the anti-inflammatory functions of the cholinergic nervous system results in blockage of uncontrolled expression of pro-inflammatory mediators, through the vagus nerve [4,5]. Vagal efferent stimulation can protect against *Mesobuthus tamulus* venom-induced ARDS through anti-inflammatory mechanism [6]. The regulatory influence of the efferent vagus nerve on expression of pro-inflammatory cytokines is mediated through the cholinergic anti-inflammatory route which involves α7nAChR-dependent signal cascade [7]. This anti-inflammatory route is under the control of neuronal muscarinic mechanisms [8]. It has been demonstrated that galantamine (GAL), a negative effector of cholinesterase, mitigated lipopolysaccharide-induced inflammatory responses in animal models through the cholinergic route [9].

The present study investigated the protective effects of GAL against acid aspiration-induced ARDS, and the mechanism involved.

EXPERIMENTAL

Animals

Adult New Zealand white rabbits (male, weight range: 1.9 - 2.5 kg) were subjected to pentobarbital anesthesia (3 %, 25 mg/kg body weight), followed by uninterrupted administration at the rates range of 5 - 10 mg/kg body weight/h. The rabbit muscles were relaxed by administration of 0.2 mg/kg body weight pipecuronium bromide. The rabbits were ventilated during tracheotomy in SIMV mode with the aid of a Drager Evita 2 Dura ventilator (Drager Medical AG & Co., Germany), under the following settings: 6 ml/kg body weight VT; maintenance of Pa_{CO_2} within 35 - 45 mmHg via RR adjustments; F_{IO_2} = 0.3; inspiratory-to-expiratory ratio of 1:1, and 2 cmH_2O positive end-pressure.

Physiological saline infusion was maintained at a constant rate of 8 ml/kg body weight/h, to ensure that arterial pressure values were > 80 mmHg. No vasoactive drugs were applied during the study which was approved by the Animal Ethical Committee of The People's Hospital of Chongqing City (approval no. 2017040502), and implemented according to "Principles of Laboratory Animal Care" (NIH publication no. 85 - 23, revised 1985) [10].

Reagents

The reagents used and their sources were: GAL (EMD Biosciences Inc.,USA); *α7* nicotinic receptor blocking agent i.e. methyllycaconitine (MLA) as well as corticosterone and cytokine assay kits (R & D Systems, USA); atropine sulfate (ATS, Hefeng Pharmaceutical Co., China); rabbit anti-HMGB1 polyclonal antibody (Boster Biotechnology Company, China), and HMGBI kits (Boster Biotech. Company Ltd, USA). Western blotting antibodies were product of Pierce Biotechnology, USA).

Vagotomy

In each rabbit, the right cervical vagus nerve was exposed, ligated using a 4 - 0 silk suture, and parted.

Preparation of ARDS model

Intra-tracheal instillation of 10^{-1} M HCL was done in the two lateral positions (1.5 ml/kg per side) prior to a pause for about 5 sec at a peak pressure of 25 cmH_2O. The ARDS model was considered stable 30 min later if Pa_{O_2}/F_{IO_2} was less than 200, otherwise, the procedure was repeated until Pa_{O_2}/F_{IO_2} reached the predefined standard level.

Experimental protocols

Rabbits were observed for 30 min for stabilization after cannulation, and were then randomized into six groups (26 rabbits/group). Group 1: ARDS group; group 2 (ARDS + GAL group), in which GAL (4 mg/kg) was given *i.p.* 30 min prior to hydrochloric acid aspiration; group 3 (ARDS + GAL + MLA), in which MLA (4 mg/kg, *i.p.*) was administered 15 min before GAL; group 4 (ARDS + GAL + Vag) in which vagotomy was done 15 min before intraperitoneal GAL; group 5 (ARDS + GAL + ATS), in which ATS (4 mg/kg, *i.p.*) was administered 15 min before intraperitoneal GAL; group 6 (control), given in place of treatment, equivalent volume of saline intra-tracheally.

Survival study

The population of rabbits that survived in the six groups were noted 72 h after the hydrochloric acid aspiration.

Histological analysis

Four (4) h after hydrochloric acid aspiration, the rabbits were euthanized (n = 10). Lung tissue samples were processed for light microscopy in

accordance with standard procedures [11]. Histological changes were scored by a pathologist naïve to the animal treatments, on a 5-point scale based on presence of, and extent of lesions such as edema, hemorrhage, and neutrophils: *absent = 0, mild = 1, moderate =2, severe = 3, and 4 = overwhelming,* with a maximum score of 12 points [12].

Wet-to-dry weight ratio

Following a 4-h euthanization, the right lungs were ligated, excised and washed with phosphate buffered saline (PBS). They were then blotted and weighed (wet weights). Thereafter, the samples were oven-dried to constant weights for 48 h at a temperature of 80 °C (dry weights). These measurements allowed for determination of the ratio of wet-to-dry (W/D).

Corticosterone assay

Blood was taken from the euthanized rabbits through cardiac puncture at 4 and 12 h, and used for assay of corticosterone levels in serum in line with instructions contained in corticosterone assay kits.

Cytokine assay

Lung tissue homogenate was prepared at pH 7.2 in PBS treated with protease inhibitor, using lung samples obtained 4 h after hydrochloric acid aspiration, rinsed of blood, and homogenized using Polytron (Brinkman) in homogenization buffer (PBS, containing a protease inhibitor cocktail, pH 7.2) (n = 10). The homogenates were clarified by centrifugation, and the resultant supernatants were subjected to ELISA assays for TNF-α, IL-1β and IL-6. The protein contents of the supernatants were estimated using the Bradford procedure.

Western blot analysis for HMGB1

Lung tissue protein extracts prepared from samples taken after 12 h of HCL exposure were subjected to Western blot analysis in line with standard procedures. Following sequential incubation of the membrane with the respective primary and secondary antibodies, the membranes were washed thrice in TTBS buffer and visualized through chemiluminescence.

Statistical analysis

All data are presented mean ± standard deviation (SD. Comparisons between different groups were done with analysis of variance (ANOVA) and Student-Newman-Keuls q-test. Values of $p <$ 0.05 were taken as indicative of statistically significant differences.

RESULTS

Mortality

There was no mortality in the control group. However, mortalities in ARDS, ARDS + GAL, MLA, and ATS groups were 87.5, 12.5, 50 and 50 %, respectively.

Pathological changes in lung tissue

No lesions were evident in the photomicrograph of lung tissue from the control group. On the other hand, HCL aspiration for 4 h was associated with clear evidence of histopathological changes such as inflammation, hemorrhage, thickened alveolar walls, and pulmonary edema. However, GAL pretreatment led to significant reductions in the HCL-induced pulmonary lesions. Methyllycaconitine, vagotomy and atropine sulfate reduced GAL-induced reduction in lung lesions and inflammation. These results are shown in Figures 1A - 1F. The degree of pulmonary damage was significantly higher in ARDS rabbits than in the GAL group ($p < 0.05$). Rabbits in the MLA, Vag and ATS groups had significantly higher aggregate scores in lung lesions, when compared with corresponding scores for GAL group ($p < 0.05$, Figure 1G).

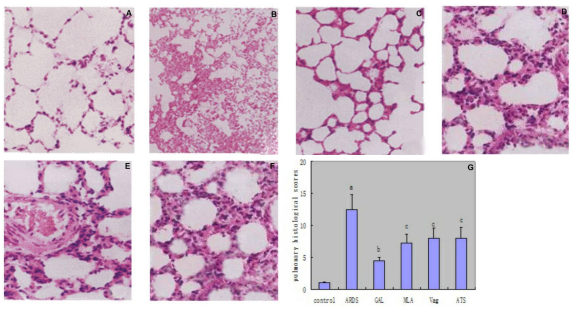

Figure 1: Photomicrographs of lung tissues from the 3 groups, and scores for pulmonary lesions. A: Control group; B: ARDS group; C: GAL group; D: MLA group; E: Vag group; F: ATS group (H & E, x 200). G: scores for pulmonary lesions in the six groups. [a]$p < 0.05$, ARDS group compared to control group; [b]$p < 0.05$, GAL group compared to ARDS group (ANOVA); [c]$p < 0.05$, MLA, Vag, ATS groups compared to GAL group

Influence of GAL on W/D index

The W/D ratio of the control group was significantly lower than that of the ARDS ($p <$ 0.05). However, the GAL treatment led to

significant decrease in W/D relative to the ARDS group ($p < 0.05$).The W/D ratios in the MLA, Vag and ATS groups were significantly higher than that of the GAL group, indicating that methyllycaconitine, vagotomy and atropine sulfate reduced GAL-induced attenuation of the degree of pulmonary edema in ARDS ($p < 0.05$, Figure 2).

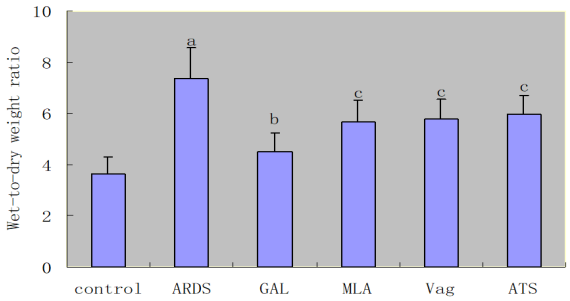

Figure 2: W/D ratios in the 6 groups. [a]***p* < 0.05,** ARDS group compared to control group; [b]***p* < 0.05,** GAL group compared to ARDS group; [c]***p* < 0.05,** MLA, Vag and ATS groups compared to GAL group. Values are mean ± SD

GAL suppressed HMGB1 expression in lungs

HMGBI was expressed at a significantly higher level in the ARDS group than in control, but HMGBI expression was significantly reduced in the GAL group relative to the ARDS group ($p < 0.05$). In addition, HMGB1 protein expression in MLA, Vag and ATS groups were higher, when compared with corresponding expressions in the control, indicating that methyllycaconitine, vagotomy and atropine sulfate reduced GAL-induced down-regulation of HMGB1 expression ($p < 0.05$, Figure 3).

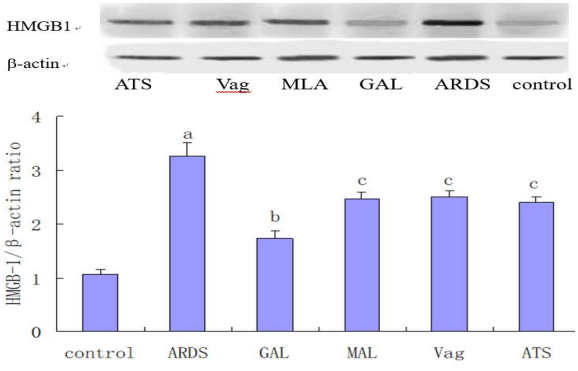

Figure 3: Expression levels of HMGB1 in pulmonary tissues of rats in the 6 groups at 12 h. [a]***p* < 0.05, ARDS group vs control group;** [b]**p < 0.05, GAL group vs ARDS group ;** [c]***p* < 0.05, MLA, Vag and ATS groups vs GAL group. Values are mean ± SD**

Pro-inflammatory cytokine expressions in lungs

In the ARDS group, TNF-α, IL-1β and IL-6 protein expressions at 4 h were significantly higher, relative to the control and GAL groups ($p < 0.05$)). This suggests that GAL suppressed the expressions of TNF-α, IL-1β and IL-6 in the lungs. In addition, TNF-α, IL-1β and IL-6 protein expressions were significantly higher in the MLA, Vag and ATS groups than in the GAL group, suggesting that methyllycaconitine, vagotomy and atropine sulfate reduced GAL-induced down-regulation of these cytokines ($p < 0.05$, Figure 4).

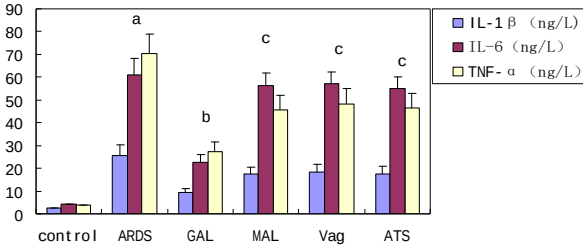

Figure 4: Effect of the various treatments on the levels of pro-inflammatory cytokines. [a]$p < 0.05$, ARDS group vs control group; [b]$p < 0.05$, GAL group vs ARDS group ; [c]$p < 0.05$, MLA, Vag and ATS groups vs GAL group. Values are mean ± SD

Corticosterone levels

There were no significant alterations in serum corticosterone levels amongst the various groups.

DISCUSSION

Acute acid aspiration-induced injury is a biphasic injury pattern at 1 h and 4 h. The first phase results from a direct physiochemical process, while the second phase is mediated by neutrophils, and is consistent with an acute inflammatory response [13]. Elevations in TNF-α, IL-1, and IL-6 are implicated in the etiology of ARDS. Neuronal cholinergic function is enhanced by GAL through inhibition of acetylcholinesterase (AChE), an action aided by its ability to traverse the blood brain barrier (BBB) [14]. In the present study, it was shown that GAL suppressed acute inflammatory response during the second phase of acute of acid aspiration-induced ARDS, and also suppressed the inflammation mediator HMGB1, but had no appreciable effect on serum concentrations of corticosterone. Notwithstanding the differences in animal models used, these results are in agreement with the report that GAL protects rats from LPS-provoked ALI [15].

The control of the neuronal cholinergic route of anti-inflammation in the efferent vagus nerve is poorly understood. However, it is known that this process is regulated in a central fashion. In the present study, it was demonstrated that GAL-associated mitigation of inflammation was diminished through vagus nerve transection. Signal transmission from the vagus nerve is regulated by the activities of muscarinic receptors, as well as AChE [8]. The findings of present study have shown that atropine sulfate, an antagonist of muscarinic receptors which can also pass through BBB, eliminated GAL-mediated mitigation of inflammation. It has been demonstrated that impulses from the vagus nerve regulate the inflammatory processes centrally and peripherally through the nicotinic ACh receptor [16]. In this study, it was shown that the α7nicotinic receptor blocking agent methyl lycaconitine reduced GAL-induced suppression of acute inflammatory response.

CONCLUSION

The results of this study demonstrate that GAL mitigates acid aspiration-induced ARDS via inhibition of pro-inflammatory cytokine availability whose mechanism involves an interplay between α-7nAChR, vagus nerve and muscarinic receptors. Thus, GAL has promising a potential for clinical application in suppressing inflammatory responses associated with ARDS.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work

Contributions of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Jin Yang conceived and designed the study, Yi Yang and Yan Peng collected and analyzed the data, Yi Yang wrote the manuscript.

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