ORIGINAL ARTICLE

Effects of intratracheal captopril on severely meconium-injured piglet lungs

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Abstract

Background: Severe meconium aspiration syndrome (MAS) may cause intractable respiratory failure in neonates. Targeting the renin-angiotensin system may be an effective way to treat such pulmonary dysfunction. Captopril has the potential to mitigate the severity of lung injury by inhibiting angiotensin-converting enzyme.

Methods: Twelve newborn piglets were intratracheally instilled with human meconium to induce severe MAS and were randomly treated with IT administration of captopril (0.5 mg/kg) (IT-Cap group, n = 6), or sham air instillation (Control group, n = 6). Cardiopulmonary profiles were monitored for a total of 5 hours. Pulmonary history was examined to compare lung injury severity between groups.

Results: There were no significant differences between the two study groups in gas exchange and lung compliance, peak inspiratory pressure, heart rate, and mean arterial blood pressure over the 5-h experimental period, but there were trends toward lower blood pressure and pH in the IT-Cap group. Histopathological examinations revealed significantly higher lung injury scores in the dependent site of the control group than in the nondependent site of the control group and both sites of the IT-Cap group.

Conclusion: Intratracheal captopril did not present significant beneficial effects on severe meconium-injured lungs within 5 hours after injury. Further studies with different disease severities and dosing strategies are required.

Keywords: Acute lung injury; Captopril; Intratracheal instillation; Meconium aspiration syndrome; Piglet

1. INTRODUCTION

Acute lung injury (ALI) can be induced by aspirated meconium in neonates. Meconium aspiration syndrome (MAS) is one of the major causes of severe respiratory failure in ill newborn babies and may cause high morbidity and even mortality. Severe pulmonary inflammation caused by aspirated meconium contributes to poor outcomes in neonates with severe MAS.[1] In addition to removal of the aspirated meconium,[2] decreasing pulmonary inflammation is a way of improving pulmonary outcome in neonates with severe MAS. To this end, a search for agents effective in this regard is worthwhile.

The pathogenesis of ALI, as in any other pulmonary dysfunction, is complex; therefore, a single target to effectively treat ALI is difficult to find. The renin-angiotensin system (RAS) is known to be implicated in the regulation of inflammation, proliferation, and fibrosis in pulmonary diseases, including ALI, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, pulmonary arterial hypertension, and asthma.[3] It is generally accepted that RAS mainly regulates blood pressure and fluid balance in the human body, but a locally expressed RAS exists in tissues other than the kidney, including the lungs.[4,5] This local RAS in lung tissues was considered to play a vital role in the fibrotic response to ALI.[4,5] There is a growing interest in the use of treatments targeting the RAS to treat pulmonary diseases.[3]

In the RAS, angiotensin I is produced from plasma globulin by the action of renin and then converted by the angiotensin-converting enzyme (ACE) to angiotensin II. Angiotensin II can be generated locally within the lungs and plays a role in the fibrotic response to ALI.[4] ACE inhibitors and angiotensin II receptor antagonists have been considered as potential therapies for fibrotic lung disease.[6,7] Increased expression of ACE in ALI was found with inflammation, pulmonary edema, and histological changes.[8] In meconium-contaminated lung tissue, investigators found that pulmonary apoptotic cell death was related to angiotensin II receptor action.[7] Therefore, an ACE inhibitor or angiotensin receptor antagonist may benefit meconium-injured lungs.

Captopril is a longstanding drug that prevents the conversion of angiotensin I to angiotensin II by inhibiting ACE. Its effectiveness in mitigating radiation-induced lung injury has been demonstrated for many years.[9–11] Previously, investigators demonstrated that captopril pretreatment could significantly inhibit meconium-induced lung cell death, cytokine production, and specific gene expression in newborn lungs.[6] Another published report demonstrated that captopril pretreatment could protect the lungs against acute pancreatitis-associated ALI.[12] Therefore, we believe the use of captopril in lung tissues may be able to

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Conflicts of Interest The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2019) 82: 505-509.

Received June 17, 2018; accepted July 10, 2018.

doi: 10.1097/JCMA.0000000000000044.

decrease the pulmonary reaction to aspirated meconium and reduce the severity of MAS.

We hypothesized that intratracheal (IT) instillation of captopril directly into the lungs may improve gas exchange and reduce lung injury. The purpose of this study was to test the pulmonary effects of IT-instilled captopril in an animal model of newborn piglets with severe MAS.

2. METHODS

The administration and supervision of animals in this study followed the guidelines of the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University (YMIACUC number: 1030313).

2.1. Animal preparation and physiological monitoring

Newborn male <2-weeks-old piglets were placed in a plastic box, and 4% isoflurane was administered by inhalation until they reached a state of unconsciousness. Then, they received an intramuscular injection of 0.1 mg atropine sulfate (Sintong, Taoyuan, Taiwan) and 25 mg/kg ketamine (Pfizer, New York, NY) for anesthesia. All animals were placed on a servo-controlled heating blanket in a supine position to maintain their body temperature at 38.5°C ± 0.5°C throughout the experiment. A subcutaneous injection of 1 to 2 mL of 2% lidocaine hydrochloride (AstraZeneca, Karlskoga, Sweden) was injected at the surgical site for local analgesia. After tracheostomy and intubation, the animals were paralyzed and sedated with an intramuscular injection of 1 mL/kg mixture of 0.2 mg/mL pancuronium bromide (Schering-Plough, Kenilworth, NJ), 0.5 mg/mL midazolam (Roche, Kaiseraugst, Switzerland), and 5 mg/mL ketamine. Then, conventional ventilation was established by connecting the animals to a volume-controlled animal ventilator (Model 683, Harvard Apparatus, Holliston, MA), set to a tidal volume of 8 mL/kg at an inspiratory vs expiratory ratio of 1:1. The positive end-expiratory pressure was 5 cm H_2O, and the fraction of inspired oxygen (FiO_2) was 1.0 throughout the experiment. The initial ventilation rate was set at 35 breaths/min with an increment or decrement of 5 breaths/min to maintain the arterial blood partial pressure of CO_2 ($PaCO_2$) within 40 and 55 mmHg. The right femoral artery and vein were cannulated with umbilical vessel catheters for monitoring arterial pressure, arterial blood sampling, and drug administration. A mixture of ketamine (5 mg/mL), midazolam (0.5 mg/mL), and pancuronium bromide (0.2 mg/mL) was continually infused intravenously at a rate of 1 mL/kg/h to maintain anesthesia until the experiment was completed.

Electrocardiography, arterial pressure, peripheral oxygen saturation, and rectal temperature were constantly monitored (M1205A Omni-Care 24/24C, Hewlett-Packard, Essex, MA). Respiratory flow and airway pressure signals from a heated capillary tube pneumotachograph-coupled pressure transducers (MP-45-16, Validyne) were recorded by using PowerLab 16/30 (AD Instruments, Australia) to integrate tidal volume, record peak inspiratory pressure (PIP), and calculate respiratory system compliance (Crs). Arterial blood gas data were collected using a gas analyzer (OPTI CCA-TS, OPTI Medical, Atlanta, GA).

2.2. Meconium slurry preparation and lung injury induction

Fresh meconium from unrelated healthy neonates was collected, pooled, and diluted with 0.9% $NaCl_{(aq)}$ to a 25% (w/v) slurry. The meconium slurry was stored in refrigerator at –20°C and then reheated to 37°C before use. After baseline cardiopulmonary function measurement, six aliquots of 1 mL/kg meconium slurry, including four aliquots in four quadrantal positions and other two aliquots in the supine position, were instilled intratracheally into the lungs of all piglets via endotracheal tube using an 8-Fr feeding tube. Additional meconium slurry was given to reach the target of arterial blood O_2 partial pressure (PaO_2) < 100 mmHg (at FiO_2 = 1.0) and Crs < 50% of the baseline value. An additional 15-min period was allowed to ensure postinjury stabilization before further treatments, and the end of this period was designated as T = 0.

2.3. Experimental protocols

Experimental newborn piglets were randomly assigned to two groups (n = 6 per group), including a control group, which received sham treatment by air instillation (2 mL/kg), and an IT-Cap group, which received one dose of 0.5 mg/kg captopril (Sigma-Aldrich, USA) by IT administration. An 8-Fr feeding tube was used for IT administration of medications. Each dose of captopril was dissolved into normal saline to give a concentration of 0.25 mg/mL, and the 2 mL/kg dosing volume was divided into two aliquots for separate instillation with the piglet body in either the right or the left decubitus position. Six breaths of artificial respiration were administered via Ambu bag to assist with instillation. The interval between aliquots was 1 minute to allow stabilization. The posttreated piglets were kept in the supine position throughout the experiment. Physiological data were recorded hourly for 5 hours or until death. The time points were designated as T1-T5. During the experimental period, 1 mEq/kg/dose of sodium bicarbonate was given to any animal that showed acidosis. A bolus of 10 mL/kg normal saline was given to any animal with a MAP < 40 mmHg. If pneumothorax was found, a chest tube was inserted to drain the leaked air. If asystole occurred, intravenous injection of one dose of 0.01 mg/kg epinephrine for every 3 minutes was administered along with cardiac massage being performed until the heart rate exceeded 60 beats/min to a maximum of 30 minutes. When the animals reached the end point of the experiment, an IV bolus of 1 mL/kg ketamine/midazolam/pancuronium mixture was given to enhance anesthesia, followed by an IV bolus of 2 mL/kg 15% potassium chloride for euthanasia.

2.4. Histological examination

After euthanasia, the chest of each piglet was opened, and the gross morphology of the chest cavity was inspected. Lung tissue samples of approximately 1.0 cm^3 were collected from the most nondependent segment of the right middle lobe and the most dependent segment of the right inferior lobe, and were immersed immediately in buffered 10% formaldehyde, incubated overnight, and embedded in paraffin. The paraffin-sectioned specimens were stained with hematoxylin and eosin. The lung injury variables of alveolar and interstitial inflammation, hemorrhage, atelectasis, and necrosis were examined by a physician who was blinded to the study protocol. The lung injury score was graded as 0, 1, 2, 3, or 4 for abnormalities noted in 0%, 25%, 50%, 75%, or diffusely covering the field, respectively.

2.5. Statistical analysis

The data were presented as mean ± SEM or median with interquartile range, as appropriate. A Student's *t* test was used to compare physiological data between the two groups at the same time point, and a paired *t* test was used to compare pre and postinjury data of the same group. The serial cardiopulmonary function data over 5-h experiments were analyzed by a two-way repeated-measures ANOVA and post hoc Student–Newman–Keuls test. Kruskal–Wallis test followed by post hoc Student–Newman–Keuls test was used to compare lung injury scores among groups and different sites. A two-tailed $p < 0.05$ was considered to indicate significance.

3. RESULTS

A total of 12 piglets were enrolled in this study. Mean body weight was 1.6 ± 0.4 kg in the control group and 1.5 ± 0.2 kg

Table 1

Pre and postinjury cardiopulmonary profiles of the experimental groups

	$PaCO_2$, mmHg	PaO_2, mmHg	PIP, cmH_2O	Crs, mL/cmH_2O/kg	Heart Rate, beats/min	MBP, mmHg
Preinjury						
Control	7.43 ± 0.06	37 ± 4	441 ± 51	14 ± 2	0.87 ± 0.13	183 ± 45
IT-Cap	7.40 ± 0.11	39 ± 6	428 ± 94	12 ± 1	1.13 ± 0.16	187 ± 21
Postinjury						
Control	7.35 ± 0.03	44 ± 4	83 ± 31[a]	20 ± 1[a]	0.50 ± 0.05[a]	184 ± 40
IT-Cap	7.34 ± 0.09	46 ± 11	88 ± 13[a]	21 ± 4[a]	0.51 ± 0.10[a]	190 ± 17

Data are presented as mean ± SD.

Crs = compliance of respiratory system; IT-Cap = intratracheal captopril; MBP = mean arterial blood pressure; $PaCO_2$ = partial pressure of CO_2; PIP = peak inspiratory pressure.

[a]$p < 0.05$ vs preinjury data of the same group.

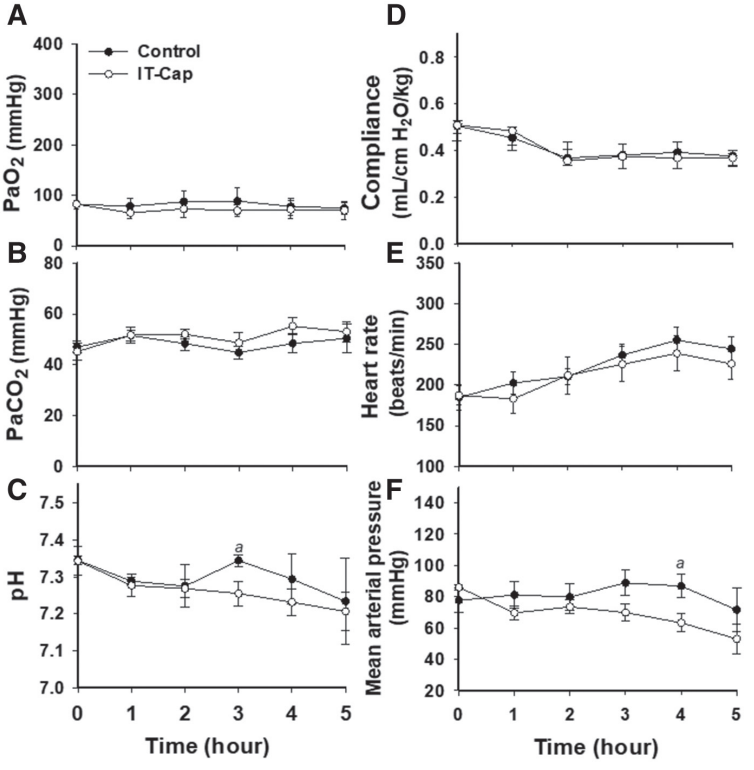

Fig. 1 Changes in cardiopulmonary parameters during the 5-h experiments in the two study groups. A, Arterial partial pressure of oxygen (PaO_2); B, Arterial partial pressure of carbon dioxide ($PaCO_2$); C, Arterial blood pH; D, Compliance of the respiratory system; E, Heart rates; F, Mean arterial blood pressures. Black circle: sham air instillation (control group); white circle: intratracheal instillation of captopril (IT-Cap group). There was no significant difference between the two study groups over the 5-h experiments for all cardiopulmonary parameters, in spite of relatively lower blood pH and mean arterial blood pressures in the IT-Cap vs the control group from the third hour of the experiment to the end. [a]$p < 0.05$ vs control group at the same time point.

in the IT-Cap group. There was no significant difference in cardiopulmonary baseline data between the two groups at baseline or after injury (Table 1) ($p > 0.05$). After lung injury, there were significant decreases in PaO_2 and Crs ($p < 0.05$), and there was a significant increase in PIP ($p < 0.05$) in the same group (Table 1).

The gas exchange measurements over our 5-h experiments revealed no significant difference between the control and IT-Cap groups ($p > 0.05$) (Fig. 1A–C). There was also no significant difference between the two groups in Crs, heart rate, and mean arterial blood pressure over 5 hours (Fig. 1D–F) ($p > 0.05$). However, we noted trends toward relatively lower pH and blood pressure in the IT-Cap group from the third hour to the end of the experiment (Fig. 1C, F).

Representative histological results are shown in Figure 2. As shown, instilled meconium slurry induced severe inflammatory cell infiltration and hemorrhage in lung tissue, especially in dependent sites (Fig. 2A, C). Less inflammatory cell occupation in the alveolar space could be found in nondependent sites (Fig. 2B, D). The total lung injury scores were the highest at the dependent site of the control group (Table 2) and significantly higher than at the nondependent site of the control group and both dependent and nondependent sites of the IT-Cap group ($p < 0.05$) (Fig. 2E, F).

4. DISCUSSION

The current study demonstrated that the use of IT captopril had little benefit in terms of gas exchange in the severe MAS model of newborn piglets. Conversely, we observed trends of relatively lower blood pressure and pH in animals that received IT captopril.

In MAS, patients suffer from different degrees of airway obstruction, surfactant inhibition and inactivation, chemical

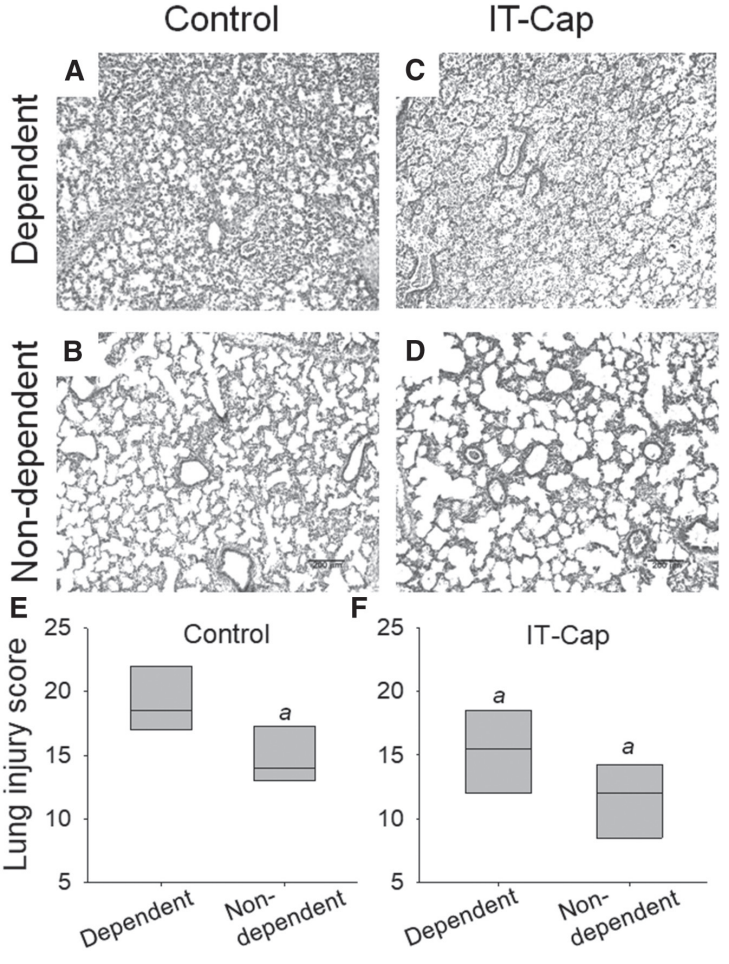

Fig. 2 Representative pulmonary histological graphics (hematoxylin and eosin stain) of animals in the two study groups. Top: Representative histological graphs in the dependent and nondependent sites of the control (A, B) and IT-Cap (C, D) groups. Bottom: Graphic lung injury scores in dependent and nondependent sites of the control (E) and IT-Cap (F) groups. Data are presented as median (interquartile range). Control, sham air instillation. IT-Cap, intratracheal instillation of captopril. [a]$p < 0.05$ vs the dependent site of the control group.

Table 2.

Comparisons of lung injury scores in different experimental groups

	Alveolar Inflammation	Interstitial Inflammation	Alveolar Hemorrhage	Interstitial Hemorrhage	Atelectasis	Necrosis	Total Injury Score
Dependent site							
Control	3.5 (3.0-4.0)	3.0 (3.0-4.0)	3.5 (3.0-4.0)	3.0 (2.8-3.0)	3.0 (3.0-4.0)	3.0 (2.0-3.0)	18.5 (17.0-22.0)
IT-Cap	3.0 (2.0-3.0)[a]	3.0 (2.0-3.0)	2.0 (2.0-3.3)[a]	2.5 (2.0-3.3)	2.5 (2.0-3.0)[a]	2.5 (2.0-3.0)	15.5 (12.0-18.5)[a]
Nondependent site							
Control	2.0 (2.0-2.3)[a]	3.0 (2.0-3.0)	3.0 (2.8-3.0)[a]	2.5 (2.0-3.0)	2.0 (1.8-3.0)[a]	2.0 (1.8-3.0)	14.0 (13.0-17.3)[a]
IT-Cap	2.0 (2.0-2.0)[a]	2.0 (1.8-2.3)[a]	2.0 (1.0-2.5)[a]	2.0 (1.8-3.0)	2.0 (1.0-2.3)[a]	1.5 (1.0-2.0)	12.0 (8.5-14.3)[a]

Data are presented as median (interquartile range).
IT-Cap = intratracheal captopril.
[a]$p < 0.05$ vs dependent site of the control group.

pneumonitis, and persistent pulmonary hypertension of the newborn may occur in the most severe cases.[13,14] Effective and reliable antiinflammatory strategies are one of the keys to successfully treat severe MAS.[1,14] However, MAS remains a significant problem all over the world, even after development of so many advanced respiratory therapies, especially in some underdeveloped areas, which lack modern medicines. Finding effective medications among traditional drugs may provide new therapeutic benefits in ill neonates in such places.

RAS was reported to be involved in the pathogenesis of lung injury.[15,16] Angiotensin II was demonstrated to stimulate DNA synthesis in lung fibroblasts and could promote fibroblast proliferation, resulting in fibrosis of lung tissue.[17] ACE inhibitors or angiotensin receptor antagonists were reported to attenuate lung injury severity in an animal model.[15] In MAS, severe chemical pneumonitis due to pulmonary inflammation is known as a major problem.[1] Aspirated meconium was reported to induce inflammatory cytokine expression and airway epithelial cell

apoptosis.[1,6,18,19] Activation of the angiotensin II receptor was also demonstrated to play a critical role in MAS.[20,21] Mitigation of inflammation to improve patient outcome and several antiinflammatory strategies have been reviewed in MAS.[1,22] Blocking the angiotensin receptor with a partial agonist (such as saralasin)[7] or antagonist (such as losartan)[23] before injury was reported to inhibit meconium-induced apoptosis. Also, inflammatory cytokine production and cell apoptosis were reported to be suppressed by pretreatment with an ACE inhibitor to reduce the angiotensin II level in meconium-injured cells.[6]

For decades, captopril, an ACE inhibitor, has been used to treat cardiovascular disease. Its protective effect against pulmonary diseases has been tested by some investigators.[3,4,9–12,17] Moreover, meconium-activated neutrophils and macrophages producing IL-13 were shown to be involved in signaling in lung fibrosis and airway hyper-responsiveness, and pretreatment with captopril to inhibit production of IL-13 was reported to ameliorate lung fibrosis and airway hyper-responsiveness.[24] Therefore, captopril may theoretically play a role in the pulmonary inflammation of MAS. However, none of the reported studies tested the effects of IT instillation of captopril in ALI.

To mimic the clinical conditions of MAS, we evaluated the effects of captopril on meconium-injured lungs in newborn piglets. To avoid the influence of captopril on systemic blood pressure, local administration by IT instillation is preferred over systemic routes. Unfortunately, in this study, IT captopril failed to improve gas exchange and lung mechanics. Moreover, decreased blood acidity and mild respiratory acidosis were observed with IT-instilled captopril. The high variability in the heart rate and significantly decreased mean arterial pressure also revealed the effects of captopril on systemic hemodynamics despite administration via the airway. Therefore, drug absorption by the IT route was proved, although it was not beneficial in gas exchange. Further studies in a less severe type of meconium-induced lung injury and employing different dosing strategies may provide different views in its efficacy.

To our knowledge, this is the first report to evaluate the efficacy of IT instillation of captopril on piglet lungs after meconium injury. Even though gas exchange and cardiopulmonary function did not improve but worsened with IT captopril treatment, histopathology supported the potency of captopril to slightly reduce lung injury severity in meconium-injured piglet lungs.

This study still has some limitations. First, we did not measure pulmonary pressures in the current study due to limitations of the laboratory facilities. The ACE is a potential therapeutic target for reducing pulmonary pressure,[25] and we suggest that future drug design should be based on this premise. Second, we only tested the effects of IT-instilled captopril without performing a comparison with another administration route because we believe captopril administered by the IT route may have the fewest adverse effects. Future studies with different dosing strategies and administration routes are suggested before clinical application of captopril to treat MAS.

In conclusion, although captopril has the potential to mitigate pulmonary inflammation, IT-instilled captopril did not present significant beneficial effects on severe meconium-injured lungs within 5 hours after injury. Further studies with different disease severities and dosing strategies are required before clinical application.

ACKNOWLEDGMENTS

We acknowledge the support of a grant from Taipei Veterans General hospital (Grant number: V105C-182 and V107C-062), Taiwan, Taiwan, ROC.

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