# Seawater-drowning-induced acute lung injury: From molecular mechanisms to potential treatments (Review)

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**Abstract.** Drowning is a crucial public safety problem and is the third leading cause of accidental fatality, claiming ~372,000 lives annually, worldwide. In near-drowning patients, acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) is one of the most common complications. Approximately 1/3 of near-drowning patients fulfill the criteria for ALI or ARDS. In the present article, the current literature of near-drowning, pathophysiologic changes and the molecular mechanisms of seawater-drowning-induced ALI and ARDS was reviewed. Seawater is three times more hyperosmolar than plasma, and following inhalation of seawater the hyperosmotic seawater may cause serious injury in the lung and alveoli. The perturbing effects of seawater may be primarily categorized into insufficiency of pulmonary surfactant, blood-air barrier disruption, formation of pulmonary edema, inflammation, oxidative stress, autophagy, apoptosis and various other hypertonic stimulation. Potential treatments for seawater-induced ALI/ARDS were also presented, in addition to suggestions for further studies. A total of nine therapeutic strategies had been tested and all had focused on modulating the over-activated immunoreactions. In conclusion, seawater drowning is a complex injury process and the exact mechanisms and potential treatments require further exploration.

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*Key words:* acute respiratory distress syndrome, acute lung injury, near-drowning, seawater

## 1. Introduction

Drowning is the third leading cause of accidental fatality (1) and claims ~372,000 lives annually, worldwide (2,3). Over 50% of the drowning victims that result in fatality are <25 years-old (1,2); however, this serious health threat is often neglected. In near-drowning patients, lung injury is one of the most common complications (4,5). Furthermore, ~1/3 of near-drowning patients fulfill the criteria for acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) (4). ALI/ARDS has been acknowledged as a common and lethal disease since it was first described in 1967 (6). It is a life-threatening disorder with a mortality rate ranging from 25-40% and so cannot be ignored (6). Pneumonia, aspiration, shock and severe sepsis are the primary triggers (7). Furthermore, a result of this complication is the infiltration of neutrophils into the alveolar space, the release pro-inflammatory cytokines, which causes leakage of edema fluid and mismatch of ventilation and perfusion (6,7). In recent years, taking vacations close to the sea or exploiting marine resources has becoming more popular and with this the frequency of seawater drowning accidents have correspondingly increased. Therefore, it is essential that the mechanisms of seawater-drowning-induced ALI be fully elucidated.

## 2. Pathophysiology of seawater-drowning-induced acute lung injury

*Physiologic changes of water drowning.* As stated by Layon and Modell (1), a detrimental consequence of drowning is primary respiratory impairment from submersion/immersion in a liquid medium. When suddenly immersed in water, victims will hold their breath, which results in oxygen depletion and carbon dioxide accumulation, a condition that is not sustainable. Subsequently, victims eventually become hypercarbic and hypoxemic; thus, in response, victims may begin to breathe, subsequently inhaling water. This further impairs the victims' ability to breathe and exchange air normally (1,3,8). Drowning or near-drowning prevents victims from breathing, which subsequently induces hypoxia. It is widely acknowledged

that hypoxia provokes multiple complications, such as cerebral hypoxia and cardiovascular disorders (5); however, hypoxia has also been associated with initiating ALI/ARDS (9,10).

*Pathophysiological changes in the lung after seawater inhalation*. Seawater is a hyperosmotic liquid that is low in temperature and contains a high content of sodium, calcium and substantial quantities of bacteria (11). Additionally, seawater is three times more hyperosmolar than plasma (942 vs. 300 mOsm/kg, respectively) (11,12). Following inhalation of seawater, the hyperosmotic and cold seawater may produce a strong injury stimulus (such as inflammation, DNA damage and apoptosis) in the lung and alveoli (13-15). As the osmotic pressure of seawater is higher than that of plasma, the fluid in the surrounding tissue space and pulmonary capillaries enters the alveolar space, resulting in pulmonary edema and hypoxia (16-18), which may result in ALI/ARDS.

The presence of water in the alveoli and airways obstructs ventilation and blood-gas exchange, triggering immediate hypoxia (19). As reported in multiple studies (12,18,19), the severity of seawater-induced pulmonary edema is 3-fold higher when compared with edema caused by freshwater. Pathological alterations occur when lungs are perfused with seawater, including alveolar septum widening, alveolar collapse and alveolar-capillary membrane damage (15,20). Folkesson *et al* (12) indicated that instilling a hyperosmotic liquid, such as seawater, into the trachea of rabbits may increase alveolar-capillary membrane permeability, resulting in the exudation of water, ions and proteins, as well as neutrophils and macrophages (20). As a consequence, massive inflammatory mediators are released and elicit further damage to lung tissue and cells.

## 3. Perturbing effects of seawater

Seawater drowning may cause ALI/ARDS; however, the underlying molecular mechanisms have not yet been clearly elucidated. The present review has a predominant focus on the molecular networks affected by seawater and the resulting cellular alterations. Seawater-induced changes are summarized in Table I (13-17,19-32) and are elucidated in detail.

*Insufficiency of pulmonary surfactant*. Pulmonary surfactant is a lipid-protein complex synthetized and secreted by type II alveolar epithelial cells (33). It is comprised of four types of protein: Surfactant protein (SP)-A, SP-B, SP-C and SP-D (34). Pulmonary surfactant secretion aids in the reduction of alveolar surface tension, maintains alveolar opening and prevents lung interstitial proteins and fluids leaking into the alveolar cavity (35). Multiple studies have proposed that the content of pulmonary surfactant may be an important severity indicator in critically ill patients with lung diseases (36,37). Seawater aspiration and interstitial fluid exudation dilute and wash out pulmonary surfactant (1,3,30). Inhalation of seawater may cause lung inflammation and type II alveolar epithelial cell damage. This damage may result from perturbing the physiological function of epithelial cells and inhibiting surfactant synthesis and secretion (30). Alternatively, a deficiency of pulmonary surfactants subsequently triggers a decrease in lung compliance, which increases the risk of alveolar collapse, in addition to increasing cell permeability to fluid. Furthermore, this event provokes alterations in lung ventilation/perfusion ratios (38,39), which ultimately may result in ALI/ARDS.

*Blood-air barrier disruption*. Gas exchange between alveoli and the blood in surrounding capillaries is dependent on the lung blood-air barrier (40). Various causes of lung diseases may inflict damage on lung blood-air barrier function, thereby affecting the respiratory function of the lungs (41,42). However, barrier disruption differs with injury type. Pulmonary edema causes pulmonary interstitium thickening and alters the gas exchange process by increasing the distance for gas exchange to occur (43), whereas lipopolysaccharide-induced acute lung injury predominantly damages pulmonary microvascular endothelial cells and destroys their barrier function, thereby causing inflammatory cell aggregation, adhesion, exudation and secretion of various inflammatory cytokines and chemokines (44). As a result, the blood barrier is further damaged.

In seawater-induced lung injury, the components of seawater, including bacteria and viruses, affect the regulation of pulmonary surfactant and the alveolar epithelium directly. Alteration of the pulmonary surfactant layer may generate pulmonary surfactant deficiency (30) and may also affect the composition of alveoli by promoting alveolar epithelial cell damage and apoptosis and subsequently resulting in barrier function alteration (13). The hypertonic nature of seawater elicits direct epithelial cell stimulation and causes the cells to contract (31), increasing the gap between cells and therefore increasing the permeability of these cells (12). The RhoA/Rho kinase pathway participates in the cytoskeletal contractile response (45). Rho-associated coiled-coil forming protein kinase (ROCK) promotes phosphorylation of the light-chain of myosin (MLC) though MLC phosphatase (46). Multiple studies (45,47) have demonstrated that the RhoA/ROCK pathway regulates cell contraction and modulates the actin cytoskeleton. Seawater has been indicated to induce the RhoA/Rho kinase pathway and promotes the phosphorylation of myosin phosphatase target subunit 1, both *in vivo* and *in vitro* (31).

Previous reports have revealed that seawater inhalation damages tight junctions and gap junctions between cells, thus affecting alveolar cell permeability, function and communication, ultimately promoting lung edema formation (16,26). Connexin 43 is located in gap junction channels and connects the cytoplasm between adjacent cells (48). Furthermore, connexin 43 is able to rapidly exchange ions and intracellular signaling molecules. Notably, in the presence of seawater, connexin 43 has been indicated to upregulate the phosphorylation of Ser368, while p-connexin 43 downregulation protects the barrier function and palliates lung edema (16,26).

*Formation of pulmonary edema*. Pulmonary edema formation is an additional critical mechanism associated with ALI and ARDS (29). Following inhalation of seawater, water flows directly into alveoli and the osmotic pressure gradient promotes water retention within alveoli (12). If the fluid is not rapidly cleared, alveolar edema occurs; thus, alveolar fluid clearance (AFC) is critical in preventing ALI/ARDS. Specific ion and water channels are known to participate in AFC. Epithelial $Na^+$ channels (ENaC) are ion channels composed of

Table I. Seawater-induced changes.

| Name | Category | Cell/tissue | Change | (Refs.) |
|---|---|---|---|---|
| B cell lymphoma-2 | Apoptosis-related protein | *In vivo* | ↑P | (14) |
| Cleaved caspase-3 | Apoptosis-related protein | *In vivo*, A549, PAT2 | ↑P | (13,14) |
| Cleaved caspase-8 | Apoptosis-related protein | *In vivo*, A549, PAT2 | ↑P | (13) |
| Fas | Apoptosis-related protein | *In vivo*, A549, PAT2 | ↑P | (13) |
| FasL | Apoptosis-related protein | *In vivo*, A549, PAT2 | ↑P | (13) |
| 4E-BP1 | Binding protein | AEC | ↑Ph | (27) |
| AQP1 | Channel | *In vivo*, A549, PAT2 | ↑R,P | (21,23) |
| AQP5 | Channel | *In vivo*, A549, PAT2 | ↑R,P | (21,23) |
| α-ENaC | Channel | *In vivo* | ↓R | (30) |
| IL-10 | Cytokine | *In vivo* | ↑P | (15-17,28) |
| IL-1β | Cytokine | *In vivo*, NR8383 | ↑P | (15-17,27) |
| IL-6 | Cytokine | *In vivo*, NR8383 | ↑P | (20,27) |
| IL-8 | Cytokine | *In vivo* | ↑P | (24,25) |
| MIF | Cytokine | *In vivo* | ↑P | (20) |
| TNF-α | Cytokine | *In vivo*, NR8383 | ↑P | (16,17,20,27,28,30) |
| MPO | Enzyme | *In vivo* | ↑A | (20,28,30) |
| MYPT-1 | Enzyme | *In vivo*, A549, RPMVECs | ↑Ph | (31) |
| T-SOD | Enzyme | *In vivo* | ↓A | (17) |
| VEGF | Growth regulator | *In vivo* | ↑R,P | (19,22) |
| Akt | Kinase | *In vivo*, AEC | ↑Ph | (14,19) |
| ATM | Kinase | AEC | ↑Ph | (19) |
| eIF4E | Kinase | AEC | ↑Ph | (27) |
| JAK1 | Kinase | NR8383 | ↑Ph | (15) |
| JAK2 | Kinase | NR8383 | ↑Ph | (15) |
| p70S6K1 | Kinase | AEC | ↑Ph | (27) |
| PI3K | Kinase | AEC | ↑Ph | (19) |
| PKC | Kinase | *In vivo*, A549 | ↑Ph | (26) |
| ERK1/2 | MAP kinase | *In vivo* | ↑Ph | (14) |
| p38 | MAP kinase | AEC | ↑Ph | (19) |
| LC3 | Microtubule associated protein | *In vivo* | ↑R | (25) |
| LC3-II | Microtubule associated protein | *In vivo* | ↑P | (25) |
| SEMA7A | Neuronal guidance protein | *In vivo*, RPMVECs | ↑P | (32) |
| MDA | Peroxidation product | *In vivo* | ↑A | (17) |
| NE | Protease | *In vivo* | ↑P | (28) |
| ERβ | Receptor | *In vivo* | ↓P | (21) |
| $sVEGFR_1$ | Receptor | *In vivo* | ↑P | (22) |
| VDR | Receptor | *In vivo*, A549, RPMVECs | ↑R, P | (31) |
| S6 ribosomal | Ribosomal protein | AEC | ↑Ph | (27) |
| SP-A | Secretory protein | *In vivo* | ↓R | (30) |
| GTP-RhoA | Signaling G protein | *In vivo*, A549, RPMVECs | ↑P | (31) |
| HIF-1α | Transcription factor | *In vivo*, A549, AEC | ↑P | (17,19,27) |
| NF-κB | Transcription factor | *In vivo*, A549 | ↑Ph, TL | (17,20,28,31) |
| STAT1 | Transcription factor | *In vivo*, NR8383 | ↑P, Ph | (15) |
| Cx43 | Transmembrane channel | *In vivo*, A549 | ↑R, Ph | (16,26) |
| Cx43 | Transmembrane channel | *In vivo* | ↓P | (16) |
| $Na^+/K^+$-ATPase | Transporter | *In vivo* | ↓A, P | (29,30) |

4E-BP, eukaryotic translation initiation factor 4E binding protein 1; AQP, aquaporin; α-ENaC, epithelial sodium channel subunit alpha; IL, interleukin; MIF, macrophage migration inhibitory factor; TNF-α, tumor necrosis factor alpha; MPO, myeloperoxidase; MYPT-1, myosin light-chain phosphatase-1; T-SOD, total superoxide dismutase; VEGF, vascular endothelial growth factor; Akt, protein kinase B; eIF4E, eukaryotic translation initiation factor 4E; JAK, janus kinase; P70S6K1, ribosomal protein S6 kinase beta-1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PKC, protein kinase C; ERK, extracellular signal-regulated kinase; LC3, microtubule-associated protein 1 light chain 3 alpha; SEMA7A, semaphorin 7A; MDA, malondialdehyde; NE, neutrophil elastase; ERβ, estrogen receptor beta; $sVEGFR_1$, soluble vascular endothelial growth factor receptor 1; VDR, vitamin D receptor; SP-A, secretory protein A; GTP-RhoA, guanosine-5'-triphosphate-ras homolog gene family member A; HIF-1α, hypoxia-inducible factor 1-alpha; NF-κB, nuclear factor-kappa B; STAT1, signal transducer and activator of transcription 1; Cx43, connexin 43; A, activity; P, protein abundance; Ph, phosphorylation; A, activity; R, mRNA abundance; TL, translocation; PAT2, primary alveolar type II cells; AEC, alveolar epithelial cells; RPMVECs, rat pulmonary microvascular endothelial cells.

Table II. Treatment of seawater-induced acute lung injury.

| Name | Category | Cell/tissue | Target | Dose | Time | Refs. |
|---|---|---|---|---|---|---|
| Dexamethasone | Anti-inflammation | *In vivo* | $Na^+/K^+$-ATPase, SP-A, α-ENaC | 1 mg/kg | Post-treated | (30) |
| Tanshinone II A | Anti-edema | *In vivo*, A549, PAT2 | AQP1, AQP5 | 50 mg/kg, 25 μg/ml | Post-treated | (23) |
| Tanshinone II A | Anti-inflammation | *In vivo*, NR8383 | MIF, NF-κB | 10 mg/kg, 20 μg/ml | Post-treated | (20) |
| Tanshinone II A | Anti-apoptosis | *In vivo* | Bcl-2, Caspase-3, Akt, ERK1/2 | 50 mg/kg | Post-treated | (14) |
| 17β-Estradiol | Anti-edema | *In vivo* | ERβ, AQP1, AQP5 | 5 mg/kg | Post-treated | (21) |
| Urinary trypsin inhibitor | Anti-inflammation | *In vivo* | NF-κB, NE | 50,000 U/kg | Post-treated | (28) |
| 3,5,4'-Tri-O-acetylresveratrol | Anti-inflammation, snti-oxidative | *In vivo*, A549 | NF-κB, HIF-1α | 150 mg/kg, 450 mg/kg | Pretreated | (17) |
| 3,5,4'-Tri-O-acetylresveratrol | Cell-interaction | *In vivo*, A549 | Connexin 43 | 50 mg/kg. 150 mg/kg, 450 mg/kg | Pretreated | (16) |
| Epigallocatechin-3-gallate | Anti-inflammation | *In vivo*, NR8383 | STAT1, JAK1, JAK2 | 10 mg/kg | Pretreated | (15) |
| 4-hydroxyphenylacetic acid | Anti-inflammation, | *In vivo*, AEC, NR8383 | HIF-1α, p70S6K1, S6 ribosomal, 4E-BP1, eIF4E | 50 mg/kg, 100 mg/kg, 150 mg/kg | Pretreated anti-edema | (27) |
| 1α,25-Dihydroxyvitamin $D_3$ | Anti-inflammation | *In vivo*, A549, RPMVEC | NF-κB, RhoA, MYPT1 | 1 μg/kg, 5 μg/kg, 25 μg/kg | Pretreated | (31) |
| BMSCs | Anti-autophagy | *In vivo* | LC3 | $2x10^6$ cells | Post-treated | (25) |

Bcl-2, B cell lymphoma-2; AQP, aquaporin; α-ENaC, epithelial sodium channel subunit alpha; MIF, macrophage migration inhibitory factor; MYPT-1, myosin light-chain phosphatase-1; Akt, protein kinase B; eIF4E, eukaryotic translation initiation factor 4E; JAK, janus kinase; P70S6K1, ribosomal protein S6 kinase beta-1; LC3, microtubule-associated protein 1 light chain 3 alpha; NE, neutrophil elastase; ERβ, estrogen receptor beta; SP-A, secretory protein A; RhoA, ras homolog gene family member A; HIF-1α, hypoxia-inducible factor 1-alpha; NF-κB, nuclear factor-kappa B; STAT1, signal transducer and activator of transcription 1; Cx43, connexin 434 E-BP1, eukaryotic translation initiation factor 4E binding protein 1. RPMVEC, rat pulmonary microvascular endothelial cells.

three subunits (α, β and γ) (49,50). Their key function is $Na^+$ uptake and to aid the removal of excess pulmonary edema fluid from the alveolar space (51). A previous study indicated the importance of AFC by demonstrating that knockout α-EnaC mice succumbed within 48 h following birth, due to their inability to clear alveolar edema fluid (52). $Na^+/K^+$ adenosine triphosphatase ($Na^+/K^+$-ATPase) is a basolateral membrane protein that exchanges sodium and potassium (53). In concert with ENaC, $Na^+/K^+$-ATPase produces an osmotic gradient that aids in the reabsorption of alveolar fluid (51). As described in previous reports (54,55), impaired $Na^+/K^+$-ATPase may trigger severe lung edema. Aquaporins (AQPs) are a family of integral membrane proteins that contribute to transcellular and trans-epithelial water movement (56). The predominant types exhibited in the lung are AQP1 and AQP5 (57). AQP1 is primarily expressed in the microvascular endothelium, while AQP5 is located in the apical membrane of type II epithelium cells (57). Previous studies (58,59) have indicated that AQP levels are altered in multiple models of lung injury, likely contributing to lung edema formation. For seawater-drowning-induced ALI, the expression levels and activity of $Na^+/K^+$-ATPase in the lung tissue are reduced (29,30). In addition, ENaC transcription is also decreased (30). In contrast, seawater exposure increases AQP1 and AQP5 expression levels, indicating elevated water permeability of the blood-air barrier (21,23). All these changes promote lung edema.

*Inflammation*. Development of ALI/ARDS is accompanied by increased inflammatory responses (60). Key ALI/ARDS characteristics induce inflammatory cell adhesion and exudation, which result in the release of large quantities of inflammatory cytokines and chemokines (15,17,20,32). These reaction cascades have an important role in the defense against pathogens (61). However, an abnormal hyperactive inflammatory response may promote and/or aggravate ALI/ARDS. Stimulation by seawater, pathogen invasion and pulmonary edema may all influence neutrophil activation (17), subsequently releasing large quantities of inflammatory cytokines, such as interleukin (IL)-1β (15-17), IL-6 (20,27), IL-8 (25) and tumor necrosis factor-α (20,25). In addition, neutrophilic activation also promotes the release of reactive oxygen species (ROS), in addition to some vasoactive substances (such as hypoxia-inducible factor-1α and vascular endothelial growth factor), which further aggravate lung injury (17).

Seawater-induced inflammatory cytokine release is associated with multiple pathways, including nuclear factor-κB (17), hypoxia-inducible factor -1α (16,19), macrophage migration inhibitory factor (20) and RhoA/Rho kinase signaling (31). Furthermore, seawater inhalation may promote lung injury through activating the Janus kinase/signal transducer and activator of transcription 1 (15) and p38 pathways (19). Inflammatory cytokine release in ALI may promote inflammatory cell activation, creating a vicious cycle, or even a 'cascade effect'.

*Oxidative stress*. Excessive generation of ROS and oxidative stress are processes that have been indicated in ALI/ARDS, promoting cell injury and apoptosis (62). Inflammatory reactions that occur within airways produce ROS and trigger a redox imbalance in the lungs (63). Indeed, scavenging of ROS significantly attenuates lipopolysaccharide (LPS)-induced lung injury (62). To date, no studies have demonstrated that seawater may promote ROS generation and induce oxidative stress; however, previous results have demonstrated that exposure to seawater induces myeloperoxidase and malondialdehyde activation and decreases total superoxide dismutase activity, indicating that seawater may cause oxidative stress in the lungs (17).

*Autophagy and apoptosis*. Autophagy is a vital process within the lysosomal degradation pathway. The predominant function of autophagy is the disposal of denatured proteins and damaged organelles (64). Furthermore, autophagy exhibits a homeostatic function at low basal levels and affects multiple critical cellular processes, including cell apoptosis, cell proliferation and immune function (65). Promotion of autophagy is triggered by various stressors, such as hypoxia, oxidative stress and hyperosmosis, which causes abnormal activation of inflammatory reactions or programmed cell death (66). Increasing evidence indicates that autophagy participates in multiple lung diseases (65). Previous studies have indicated that seawater aspiration may activate autophagy (24,25). Indeed, alveolar epithelial cells have been revealed to generate more autophagosomes following treatment with seawater (24) and notable upregulation of the autophagy protein, LC3-II was detected (25). Furthermore, autophagy inhibition by 3-methyladenine significantly attenuates seawater-inhalation-induced effects by reducing the partial pressure of oxygen, increasing the lung weight coefficient and destroying the alveolar structure (24).

Seawater aspiration is able to induce apoptosis in alveolar epithelial cells (13,14). Apoptosis may be initiated by two pathways: The extrinsic pathway, which is mediated through extracellular ligand binding, including Fas ligand (FasL), to specific receptors on the cell surface; and the intrinsic pathway, which is mediated by the mitochondria (67). Notably, seawater was revealed to activate the extrinsic pathway (13). A previous study indicated that, following seawater inhalation, Fas and FasL levels in the lung were increased to the extent where caspase-8 and caspase-3 cleavage was induced, which resulted in apoptosis (13). Alternative studies have identified that seawater induced apoptosis by significantly reducing the expression levels of the anti-apoptotic molecule, B-cell lymphoma 2 (13,14). Activation of Akt, which modulates cell survival and apoptosis, and extracellular signal-regulated kinases 1 and 2 that are involved in the protective action against cell death, was indicated to be triggered immediately following seawater exposure (14).

*Hypertonic stimulation*. High osmotic pressure is able to inflict cell damage and result in the loss of normal physiological function (68). Intestinal epithelial cells produce inflammatory mediators when exposed to hypertonic fluids, which promotes inflammatory bowel disease (69). Furthermore, stimulation with hypertonic fluids may cause a series of pathological perturbations in various cells in the lung tissue, including lung epithelial and vascular endothelial cell shrinkage, apoptosis, neutrophil chemotaxis, blood-gas barrier damage, as well as infiltration and secretion of inflammatory cytokines (12-15).

The osmosis-sensitive transcription factor, osmotic response element-binding protein (OREBP) is a member of the Rel transcriptional activators family, which has been previously described (69). OREBP transactivates several genes responsible for cell protection against injury derived from hyperosmosis, such as organic osmolytes (70), heat shock protein 70 (71) and vasopressin-activated urea transporters (72). Although no direct evidence has demonstrated that seawater activates OREBP in ALI/ARDS, it has been reported that multiple OREBP regulators are altered in seawater-drowning-induced ALI/ARDS (27,68).

Increased ROS levels are necessary for OREBP activation; however, these levels may be decreased following ROS reduction by antioxidants (73). Actin cytoskeleton reorganization has been indicated in the activation of OREBP though RhoA/Rho kinase signaling (68). Burg *et al* (68) reported that seawater-induced changes in p38, ataxia telagiectasia mutated kinase (ATM) and Phosphatidylinositol-4,5-bisphosphate 3-kinases (PI3Ks) regulated OREBP activity. p38 belongs to the mitogen-activated protein kinases family and its inhibition has been revealed to partially reduce hypertonicity-induced activation of OREBP (74). Furthermore, as does ATM, which participates in cell cycle regulation, DNA repair and cell survival. PI3Ks are intracellular lipid kinases may mediate OREBP activity though ATM (68). Therefore, we hypothesize that OREBP, an osmosis sensitive protein, is likely involved in seawater-drowning-induced ALI/ARDS.

*Other possible mechanisms*. Alternative possible mechanisms related to seawater-inhalation-induced ALI/ARDS include calcium oscillation and intracellular calcium overload. It has been indicated that high salt levels increase the production of pro-inflammatory molecules and potentiate LPS-induced macrophage activation (75); therefore, seawater may elicit similar effects.

## 4. Potential treatments and therapeutic targets of seawater-drowning-induced acute lung injury

*Hospital management*. Although the pathophysiological and molecular mechanisms of ALI/ARDS are well-acknowledged, no specific and effective treatments are currently available. The main treatment is supportive care, including pulmonary support, to avoid of complications (1).

*Potential treatments suggested by animal experiments*. Previous studies in rats and rabbits have indicated various treatments (as shown in Table II) that may be effective for seawater inhalation induced ALI/ARDS (Table II; 14-17,20,21,23,25,27,28,30,31). These therapeutic strategies predominantly focus on modulating the over-activated immunoreactions, such as dexamethasone, tanshinone II A and 1α, 25-dihydroxyvitamin D3. Additionally, some therapeutic agents are able to alleviate increased edema, such as 17β-Estradiol and tanshinone II A. However, many of these therapeutic strategies, including the therapeutic agents and their doses, have not been evaluated for safety in humans and the exact effects these may exert requires further investigation.

## 5. Conclusions

To conclude, seawater drowning is a complex injury process that involves pulmonary edema formation, inflammatory response enhancement, oxidative stress, hypertonic stimulation and pathogen invasion. In addition, other possible mechanisms require further exploration; these include the exact roles of OREBP, calcium oscillation and high salt concentrations. For potential treatments, further studies and confirmation are required.

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