Effect of aquaporin-1 deletion on pleural fluid transport[1]

JIANG Jin-Jun, BAI Chun-Xue[2], HONG Qun-Ying, ZHANG Min, SONG Yuan-Lin[3]

Department of Pulmonary Medicine, Zhongshan Hospital, Fudan University, Shanghai 200032, China; [3]Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco CA 94143-0521, USA

KEY WORDS pleura; aquaporins; sodium channels; terbutaline; amiloride; pleural effusion

ABSTRACT

AIM: To investigate the role of aquaporin-1 (AQP1) and sodium channel on pleural fluid transport. **METHODS:** Wild-type and AQP1 null mice were used in this study. After the mice were briefly anesthetized, 0.25 mL of hyperosmolar or isosmolar solution (containing terbutaline, amiloride or saline only) was infused into the pleural space. Then mice were sacrificed at scheduled times for measurement of pleural fluid osmolality or volume. **RESULTS:** After instillation of hyperosmolar fluid into the pleural space, the osmolality of pleural fluid in wild-type mice was higher than that in AQP1 null mice killed at the same time (1, 2, 5 min). There was no difference in the isosmolar clearance between the wild-type and AQP1 null mice after injection of 0.25 mL isosmolar fluid into the pleural space. Terbutaline increased the osmotic and isosmolar fluid transport across pleura, but these effects were not influenced by AQP1 deletion. In contrast, amiloride reduced osmotic and isosmolar pleural fluid transport, and these effects were not influenced by AQP1 deletion. **CONCLUSION**: AQP1 water channels facilitated osmotic fluid transport across the pleural surface. However, AQP1 did not play an important role in pleural isosmolar fluid clearance. Sodium channel may play a role in osmotic and isosmolar pleural fluid transport. The effects of sodium channel on fluid transport across pleural space were not influenced by aquaporin-1 deletion.

INTRODUCTION

The aquaporins (AQP) are a family of small membrane-spanning proteins that are expressed at plasma membranes in many cell types involved in fluid transport[1]. Water movement across aquaporins can be driven by osmotic, oncotic, or hydrostatic forces. Recent studies in mice lacking specific aquaporins have indicated an important role for aquaporins in several organs[2]. For example, mice lacking AQP1 or AQP3 have nephrogenic diabetes insipidus with marked polyuria[3,4], mice lacking AQP4 manifest reduced cerebral edema in response to brain injury[5], and mice lacking AQP5 have defective saliva secretion[6]. Humans with mutation of AQP2, the vasopressin-regulated water channel, have a rare autosomal form of hereditary nephrogenic diabetes insipidus[7]. But the role of AQP1 on pleural fluid transport remains unknown.

AQP1 is strongly expressed in microvessels near diaphragmatic, visceral, and parietal pleura of wild-type mice. AQP1 is also expressed in surface mesothelial cells of visceral pleura and to a lesser extent in parietal and diaphragmatic pleura[8]. Previous study showed that AQP1 deletion could reduce alveolar-capillary osmotic water permeability 10-fold[9]. AQP1 deletion produced

[1] Project supported by the grants from Ministry of Education (No 2000026548) and grants from Science and Technology Commission of Shanghai Municipality (No 00JC14041).
[2] Correspondence to Prof BAI Chun-Xue.
Phn 86-21-6404-1990, ext 2445. Fax 86-21-6418-7165.
E-mail cxbai@zshospital.com
Received 2002-07-02 Accepted 2003-01-15

a small but significant 1.4-fold decrease in lung fluid accumulation in response to a hydrostatic stress[10]. The results from previous studies suggest that aquaporin-1 play a major role in osmotic water movement in the lung. Therefore we postulated that AQP1 might play a role in pleural fluid dynamics.

Amiloride is served as a sodium channel antagonist and could reduce the clearance of isosmolar fluid in rabbit pleural space[11]. In contrast, terbutaline could facilitate the clearance, which was inhibited by amiloride[12,13]. These findings demonstrated that sodium channel appeared to play a role in pleural isomolar liquid transport. So far as we know, there is no research on the relation between sodium channel and osmotic fluid transport. Since both AQP1 and sodium channel might be involved in pleural fluid transport, we hypothesized that AQP1 and sodium channel might influence each other in pleural fluid dynamics.

In this study, our goal is to investigate the role of AQP1 and its interaction with sodium channel in fluid transport across pleural surface.

MATERIALS AND METHODS

Transgenic mice Transgenic knockout mice deficient in AQP1 were generated by targeted gene disruption as described previously[4,14]. Measurements were done in litter-matched mice (8-10 weeks of age) produced by intercrossing of heterozygous mice in a CD1 genetic background. The investigators were blinded to genotype information for all physiological measurements. All the mice were generously provided by Dr Verkman (UCSF). Protocols were approved by Committee on Animal Research, Fudan University.

Osmotic water permeability measurements Mice were briefly anesthetized by halothane inhalation. The 0.25 mL of fluid (Ringer's solution containing sucrose 200 mmol/L and 5 % bovine serum albumin, 500 Osmmol/kg) was infused into the right pleural cavity using a 1-mL syringe and 27-gauge needle. The chest wall was punctured laterally between the 5th and 6th ribs, with the tip of needle parallel to the lung surface to avoid lung puncture. After discontinuation of anesthesia, mice resumed normal activity without signs of distress. Mice were given free access to water. At scheduled times (1, 2, and 5min), mice were reanesthetized and the abdominal cavity was rapidly exposed and the descending aorta was transected. The chest was opened by a midline incision, and pleural fluid was withdrawn using a micropipette. After low-speed centrifugation (112×*g*, 5 min), supernatant osmolality was measured in duplicate using a freezing-point depression osmometer (Precision System, Natick, MA). The wild-type and AQP1 null mice were divided into the following 3 groups, respectively: terbutaline group, amiloride group, and control group. In terbutaline group, the fluid instilled into the pleural space contained terbutaline 100 μmol/L. And in amiloride group, the fluid contained amiloride 200 μmol/L.

Isosmolar pleural fluid clearance Mice were briefly anesthetized by halothane inhalation. Isosmolar fluid 0.25 mL (Ringer's solution containing 5 % bovine serum albumin, 300 Osmmol/kg) was infused into the right pleural cavity. After discontinuation of anesthesia, mice resumed normal activity without signs of distress. Mice were given free access to water. After 30, 60, or 90 min, mice were reanesthetized, and pleural fluid was withdrawn as described above. Care was taken to withdraw as much fluid as possible. After centrifugation, the volume of the supernatant fluid was recorded. The groups and drug concentrations were the same as described above.

Statistics Statistical analysis was performed with SPSS for Windows. Statistical comparison was determined by analysis of variance. $P<0.05$ was considered as being significantly different.

RESULT

Role of AQP1 in pleural fluid transport Osmotically driven water transport across the pleural surface was measured in anesthetized mice in which 0.25 mL of hyperosmolar (500 Osmmol/kg) solution was instilled into the pleural space. Then mice were killed at scheduled times (1, 2, and 5min), and pleural fluid was collected from the chest cavity for measurement of osmolality. After instillation of 500 Osmmol/kg fluid into the pleural space, the pleural fluid osmolality in wild-type mice was significantly lower than that in AQP1 null mice ($P<0.01$, Fig 1), indicating more osmotic water entry and hence higher osmotic water permeability. These findings suggested that AQP1 was contributed to pleural osmotic fluid transport.

To determine whether the decreased pleural osmotic water permeability resulted in impaired clearance of pleural fluid, the pleural space was instilled with isosmolar fluid, and the pleural fluid volumes remaining at scheduled time points were measured. Fig 2 shows an approximately linear decrease in collected pleural fluid volume, and there was no difference in pleural absorp-

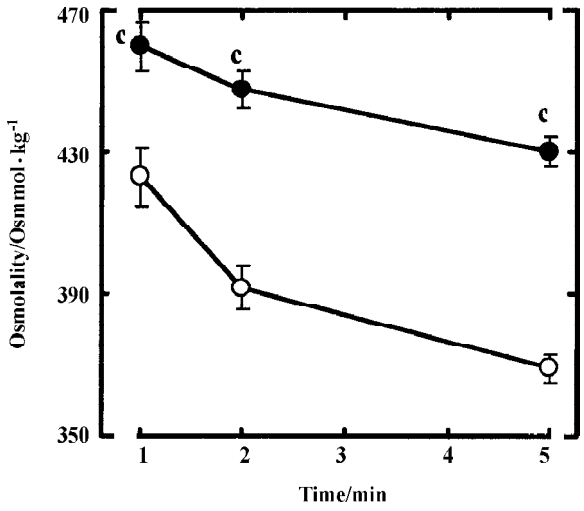

Fig 1. Osmotically induced water transport across the pleural barrier in wild-type (○) and AQP1 null (●) mice. Time course of pleural fluid osmolality. *n*=4 mice. Mean±SD. $^{c}P<0.01$ *vs* wild-type group.

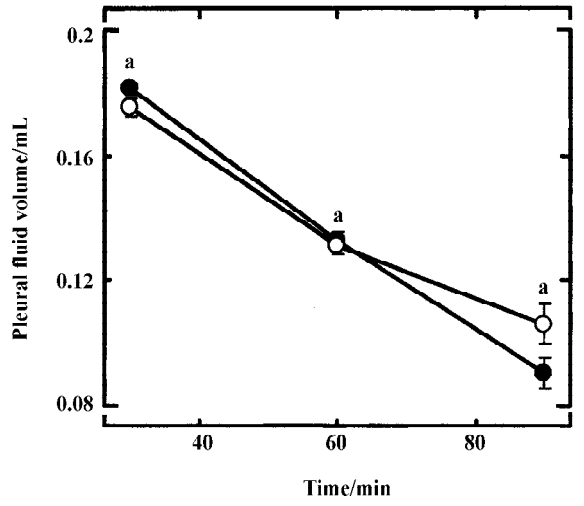

Fig 2. Isosmolar fluid absorption from the pleural space in wild-type (○) and AQP1 null (●) mice. Time course of pleural fluid volume in wild-type and AQP1 null mice. *n*=4 mice. Mean±SD. $^{a}P>0.05$ *vs* wild-type group.

tion between the wild-type and aquaporin-1 null mice ($P>0.05$). It was concluded that AQP1 deletion did not affect isosmolar pleural fluid clearance. Since measurements were comparative, the validity of this conclusion was unlikely to be affected by the imperfect recovery of pleural fluid by the micropipette collection method (0.23 mL recovered at time 0 of 0.25 mL instilled).

Interaction of AQP1 and sodium channel in the pleural fluid transport

Terbutaline After instillation of 500 Osmmol/kg fluid into the pleural space of the wild-type mice, the pleural fluid osmolality of terbutaline group was lower than that of control group in both wild-type and AQP1 null mice ($P<0.05$, Fig 3). The result demonstrated that terbutaline increased the osmotically driven pleural fluid transport and AQP1 deletion did not affect terbutaline role on osmotic pleural liquid transport. Terbutaline facilitated isosmolar fluid clearance in pleural cavity of these two gene-types mice ($P<0.05$, Fig 5 and 6). And fluid absorption in terbutaline group of wild-type mice was not different from that of AQP1 null mice ($P>0.05$). AQP1 deletion did not influence terbutaline increasing isosmolar pleural fluid clearance.

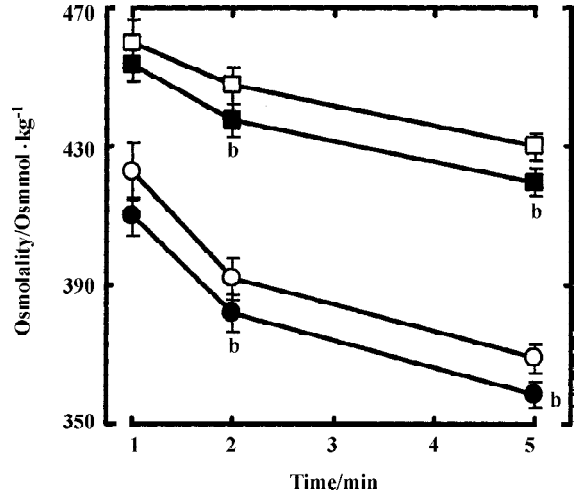

Fig 3. Osmotically induced water transport across the pleural barrier in wild-type (control: ○, terbutaline: ●) and AQP1 null (control: □, terbutaline: ■) mice. Time course of pleural fluid osmolality. *n*=4 mice. Mean±SD. $^{b}P<0.05$ *vs* control group.

Amiloride In both wild-type and AQP1 null mice, amiloride reduced osmotic pleural liquid transport ($P<0.05$, Fig 4). Therefore, the effect of amiloride on osmotically driven pleural liquid transport was not affected by AQP1 deletion. Amiloride decreased isosmolar pleural fluid absorption in these two gene-types mice ($P>0.05$, Fig 5 and 6). AQP1 deletion did not affect amiloride reducing isosmolar pleural fluid clearance.

DISCUSSION

Aquaporin knockout mice serve as sophisticated tools to analysis of aquaporin function. The AQP1 null mouse thus provided a useful model with which to examine the role of AQP1 in the various processes asso-

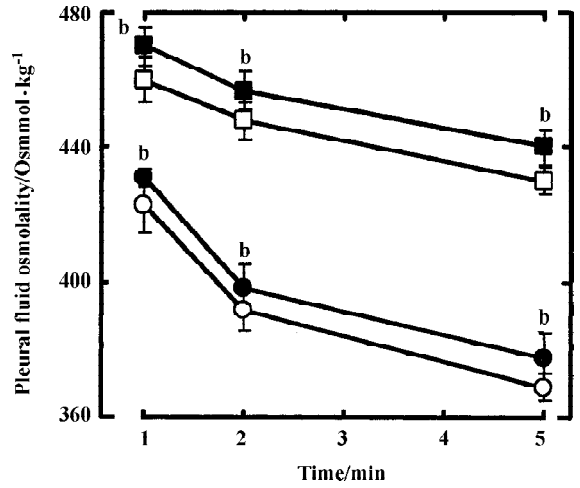

Fig 4. Osmotically induced water transport across the pleural barrier in wild-type (control: ○, amiloride: ●) and AQP1 null (control: □, amiloride: ■) mice. Time course of pleural fluid osmolality. *n*=4 mice. Mean±SD. $^bP<0.05$ *vs* control group.

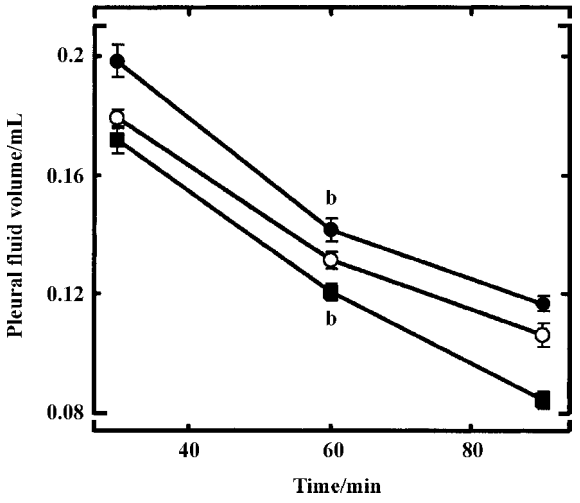

Fig 5. Isosmolar fluid absorption from the pleural space in wild-type mice (control: ○, amiloride: ●, terbutaline: ■). Time course of pleural fluid volume in wild-type and AQP1 null mice. *n*=4 mice. Mean±SD. $^bP<0.05$ *vs* control group.

ciated with pleural fluid balance. One finding of this study was that though AQP1 provided a major pathway for osmotically driven water transport across the pleural barrier, AQP1 did not appear to play a role in isosmolar pleural fluid clearance. Since AQP1 is expressed primarily in microvascular endothelial cells near the pleural surface, the reduced osmotic water permeability in AQP1 null mice indicates that the microvascular endothelium is a principal barrier for osmosis. The results here indicate that pleural osmotic water permeability is high and significantly reduced by AQP1 deletion.

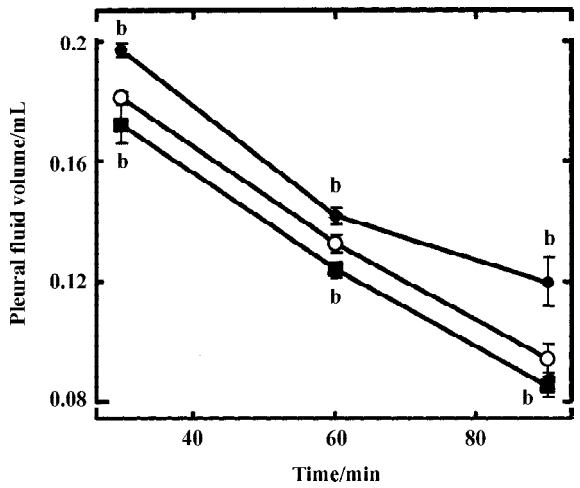

Fig 6. Isosmolar fluid absorption from the pleural space in AQP1 null mice (control: ○, amiloride: ●, terbutaline: ■). Time course of pleural fluid volume in wild-type and AQP1 null mice. *n*=4 mice. Mean±SD. $^bP<0.05$ *vs* control group.

Amiloride blocked epithelial sodium channel (ENaC), Na^+/H^+ exchanger, Na^+-K^+-ATPase and Na^+-cotransport in different organs of different animals[14-16]. In rabbit expriments, amiloride decreased net rate of Na^+ and liquid absorption in pleural space[11]. Terbutaline increased the activity of Na^+-K^+-ATPase and ENaC by cAMP[11,12]. Furthermore, terbutaline could increase the net rate of Na^+ and fluid absorption in rabbit pleural space[18,19], and this effect could be inhibited by amiloride. It showed that amiloride and terbutaline could modulate the sodium absorption in rabbit pleural fluid transport by sodium channel. Our study suggested that terbutaline may not only facilitate osmotic fluid entry into the pleural space, but also increase isosmolar pleural fluid clearance. In contrast, amiloride decreased both osmotic and isosmolar pleural liquid transport. Furthermore, aqauporin-1 deletion did not affect the function of terbutaline and amiloride on pleural fluid transport. The results suggested that the role of sodium channel on pleural fluid transport could not be influenced by aquaporin-1 lacking.

Our study showed that AQP1 deletion did not affect clearance of isosmolar fluid in the pleural space. As Starling forces favor fluid accumulation, it is likely that the majority of fluid existing in the pleural space passes through lymphatics located throughout the parietal pleura[19-21]. Thus the expression of AQP1 in pleural microvasculature does not facilitate pleural fluid clearance. Another reason might be due to the slow fluid clearance rate. As found in the study of alveolar

fluid clearance, maximum stimulation of alveolar fluid clearance was not affected by AQP1 deletion, given much lower fluid transport rate in the lung than that in the kidney proximal tubes[10]. Though terbutaline could increase the isosmolar fluid pleural clearance, the fluid transport rate was till lower compare to osmotic fluid transport. Our results showed that terbutaline and amiloride could affect pleural fluid transport in mice. It indicates that sodium channel may play a role in pleural fluid dynamics. It is assumed that sodium channel function might be up-regulated after deletion of AQP1 as an compensatory mechanism, but from this study, aqauporin-1 deletion did not affect pleural fluid transport in control or treated groups. As we know, it was the first time to show the relation between aquaporin-1 and sodium channel in pleural fluid transport.

In summary, aquaporin-1 may play a major role in osmotic fluid pleural transport, but it does not play an important role in isosmolar fluid pleural clearance. Since AQP1 is expressed in a number of microvascular beds in pleural space and expressed higher in metastasis tumor tissue[22], maybe it plays a role in the process of malignant pleural effusion in which pleural capillary is invaded by tumor cell or other acute pleural filtration disease in which large amount of accumulation occurs. Sodium channel activities are associated with osmotic and isosmolar fluid transport in pleural space. By increasing or decreasing activities of pleural sodium channel, the fluid transport rate could be modulated. The role of sodium channel in pleural fluid transport may be unaffected by aquaporin-1 deletion.

REFERENCES

1 Nielsen S, Smith BL, Christensen EI, Agre P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 1993; 90: 7275-9.

2 Verkman AS, Yang B, Song Y, Manley G, Ma T. Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. Exp Physiol 2000; 85 Suppl: 233S-41S.

3 Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J Biol Chem 1998; 273: 4296-9.

4 Schnermann J, Chou J, Ma T, Knepper MA, Verkman AS. Defective proximal tubule reabsorption in transgenic aquaporin-1 null mice. Proc Natl Acad Sci USA 1998; 95: 9660-4.

5 Manley GT, Fujimura M, Ma T, Filiz F, Bollen A, Chan P, Verkman AS. Aquaporin-4 deletion in mice reduces brain edema following acute water intoxication and ischemic stroke. Nat Med 2000; 6: 159-63.

6 Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 1999; 274: 20071-4.

7 Deen PM, Verkijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH, *et al*. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. Science 1994; 264: 92-5.

8 Song Y, Yang B, Matthay MA, Ma T, Verkman AS. Role of aquaporin water channels in pleural fluid dynamics. Am J Physiol Cell Physiol 2000; 279: C1744-50.

9 Bai C, Fukuda N, Song Y, Ma T, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. J Clin Invest 1999; 103: 555-61.

10 Song Y, Ma T, Matthay MA, Verkman AS. Role of aquaporin-4 in air space-to-capillary water permeability in intact mouse lung measured by a novel gravimetric method. J Gen Physiol 2000; 115: 17-27.

11 Zocchi L, Agostoni E & Cremaschi D. β–Agonist activation of an amiloride-insensitive transport mechanism in rabbit pleura. Respir Physiol 1995; 100: 7-13.

12 Zocchi L, Agostoni E. Effect of β-adrenergic blockade or stimulation on net rate of hydrothorax absorption. Respir Physiol 1994; 97: 347-56.

13 Ma T, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, *et al*. Nephrogenic diabetes insipidus in mice deficient in aquaporin-3 water channels. Proc Natl Acad Sci USA 2000; 97: 4386-91.

14 Cremaschi D, Meyer G, Botta G, Rossetti C. Uncoupling of Na/H from Cl/HCO_3 exchange under some steady state conditions in rabbit gallbladder. Pflugers Arch 1988; 413:102-4.

15 Soltoff SP, Mandel IJ. Amiloride directly inhibits the Na-K-ATPase activity of rabbit kidney proximal tubules. Science 1983; 220: 957-9.

16 Renner EL, Lake JR, Cragoe EJ Jr, Scharschmidt BF. Amiloride and amiloride analogs inhibit Na/K-transporting ATPase and Na^+-coupled alanine transport in rat hepatocytes. Biochim Biophys Acta 1988; 938: 386-94.

17 Matalon S, O'Brodovich H. Sodium channels in alveolar epithelial cells: molecular characterization, biophysical properties, and physiological significance. Ann Rev Physiol 1999; 61: 627-61.

18 Suzuki S, Zuege D, Berthiaume Y. Sodium-independent modulation of Na-K-ATPase activity by beta-adrenergic agonist in alveolar type II cells. Am J Physiol 1995; 268: L983-90.

19 Broaddus VC, Wiener-Kronish JP, Berthiaume Y, Staub NC. Removal of pleural liquid and protein by lymphatics in awake sheep. J Appl Physiol 1988; 64: 384-90.

20 Agostoni E, Zocchi L. Active Na^+ transport and coupled liquid outflow from hydrothorances of various size. Respir Physiol 1993; 92: 101-13.

21 Kinasewitz GT, Groome LJ, Marshall RP, Diana JN. Role of pulmonary lymphatics and interstitium in visceral pleural fluid exchange. J Appl Physiol 1984; 56: 355-63.

22 Xiang Y, Ma B, Li T, Yu HM, Li XJ. Acetazolamide suppresses tumor metastasis and related protein expression in mice bearing Lewis lung carcinoma. Acta Pharmacol Sin. 2002; 23: 745-51.