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***Basic Study***

**Tolvaptan regulates aquaporin-2 and fecal water in cirrhotic rats with ascites**

Chen C *et al.* Tolvpatan effect in distal colon

Chao Chen, Ren-Pin Chen, Hai-Hua Lin, Wen-You Zhang, Xie-Lin Huang, Zhi-Ming Huang

**Chao Chen, Ren-Pin Chen, Hai-Hua Lin, Wen-You Zhang, Xie-Lin Huang, Zhi-Ming Huang,** Department of Gastroenterology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

**Xie-Lin Huang**, Ren-Ji College of Wenzhou Medical University, Wenzhou 325035, Zhejiang Province, China

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**Correspondence to:** **Zhi-Ming Huang,** **Professor,** Department of Gastroenterology, the First Affiliated Hospital of Wenzhou Medical University, Shangcai Village, Ouhai District, Wenzhou 325000, Zhejiang Province, China. wyyyhzm@126.com

**Telephone:** +86-577-55578033

**Fax**: +86-577-55578033

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**Abstract**

**AIM:** To investigate the role of tolvaptan in regulating aquaporin (AQP)-2 expression and fecal water content in cirrhotic rats with ascites.

**METHODS:** Cirrhotic rats with ascites were induced by repetitive dorsal injection of CCl4 for 14 wk. In total, 84 cirrhotic rats with ascites treated with tolvaptan were divided into three groups (vehicle, and 3 mg/kg and 5 mg/kg tolvaptan), and then further grouped into five subgroups (days 1, 2, 3, 4 and 5). Rats were killed after administration of tolvaptan, and blood samples were obtained to measure vasopressin and sodium concentrations. Colonic mucosa was scraped for protein expression and transcriptional level of aquaporin-2. The whole layer was fixed for hematoxylin–eosin (HE) staining and feces were collected for calculation of fecal water content.

**RESULTS:** Vasopressin decreased compared to the vehicle group from day 2, but eventually reached a similar level in each treatment group, despite the different doses of tolvaptan. AQP-2 showed significant upregulation in cirrhotic rats with ascites compared to the control group (100 ± 17.23% *vs* 22.2 ± 10.23%, *P* < 0.01). After administration of tolvaptan, expression of AQP-2 began to decrease from day 2 in each treatment group, but no significant difference was found finally. Fecal water content in the distal colon was increased by 5 mg/kg tolvaptan on day 1 (67.3 ± 4.32% *vs* 42.2 ± 5.26% in the vehicle group, *P* < 0.05). Fecal water content returned to the baseline at day 4 at the latest, and it was not correspondent with the change of AQP-2 expression. HE staining of colonic mucosa was also utilized, but no mucosal damage was detected.

**CONCLUSION:** Upregulation of AQP-2 in distal colon is found in cirrhotic rats with ascites. Tolvaptan inhibits its expression and might decrease water reabsorption and induce diarrhea.

**Key words:** Tolvaptan; Aquaporin-2; Cirrhosis; Ascites

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**Core tip:** Aquaporin (AQP)-2 is mainly expressed in the kidneys, although it has been detected in the distal colon. We confirmed its expression in the distal colon and upregulation in cirrhotic rats with ascites. Tolvaptan is a highly potent and selective AQP-2 antagonist and used to treat cirrhotic ascites and hyponatremia. It blocked AQP-2 expression in the distal colon after oral administration, and increased fecal water content temporarily. But fecal water content returned to baseline quickly. The results suggest that tolvaptan inhibited AQP-2 expression in the distal colon and may induce diarrhea to clear abundant water.

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**INTRODUCTION**

Ascites is a serious complication often seen in patients secondary to advanced liver cirrhosis with portal hypertension. The onset and development of cirrhotic ascites are multifactorial. Liver function deterioration, portal hypertension and systemic vasodilation lead to activation of the renin–angiotensin–aldosterone system, sympathetic nervous system, and vasopressin[1]. Ascites and water retention are associated with increased mortality rates[2], and redistribution of liquid forms a considerable part of physiological and pathological changes.

The colon is a vital organ in regulating water and salt balance. A total of 6–8 L of digestive fluids is secreted into intestinal tract every day, which mainly comes from plasma and tissue fluid, but few are excreted. Water and salt homeostasis are mainly regulated by colonic epithelium. The aquaporin (AQP) family plays an important role in modulating absorption and secretion of water and electrolytes. The major AQPs in the colon include AQP-1, 2, 3, 4 and 8[3-5]. AQP-2 is mainly expressed in the kidneys, and was first discovered by Preston *et al*[6,7] in 1992. Since then, it has been found in human and rat colons. In the kidneys, activation of vasopressin V2 receptor increases water reabsorption, both by short- and long-term regulation[1]. Whether AQP-2 regulates the water balance in the intestinal tract remains obscure.

Tolvaptan is a novel vaptan and characterized as a highly effective and selective non-peptide vasopressin V2 receptor antagonist. It binds to vasopressin V2 receptor and induces aquaresis in humans and rats after oral administration[8]. Tolvaptan increases free water clearance and shows its unique curative effect in kidney and cardiovascular diseases[9-11]. Tolvaptan has been shown to alleviate syndrome of inappropriate antidiuretic hormone or ascites[12-14].

In our clinical cases, some patients with hyponatremia appeared to have diarrhea during aquaretic therapy. We suspected that they might respond to tolvaptan administration, and we designed the present study to investigate the correlation between tolvaptan and fecal water content.

**MATERIALS AND METHODS**

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University in 2013 (No. wydw2013-0071). Tolvaptan was a gift from Otsuka Pharmaceutical Company, Japan.

***Modeling***

The study was performed in 84 conscious adult male Sprague–Dawley rats with cirrhosis and ascites. Ascites was induced by repeated injection of CCl4 (1 mg/kg), dissolved in paraffin (1:1; v/v), by dorsal subcutaneous injection twice weekly (Monday and Friday) for 14 wk. Rats weighing 200–250 g were fed *ad libitum* with standard chow and filtrated water containing phenobarbital (0.3 g/L) as drinking fluid. After 1 wk phenobarbital induction, CCl4 treatment began. One hundred and thirty rats were submitted to the model protocol, but 46 of these could not be included in the study for following reasons: 20 died during the protocol and 26 failed to develop ascites. After developing ascites, all rats were observed for one more week to judge the stability of ascites. All the animals with ascites were submitted to metabolic cages for 3 d before tolvaptan (dissolved in 1% hydroxypropyl methylcellulose) treatment.

***Experimental protocols***

In total, 84 cirrhotic rats with ascites were included in this protocol and randomly assigned to three groups (vehicle, and 3 mg/kg and 5 mg/kg tolvaptan). Each group was further divided into five subgroups (1, 2, 3, 4 and 5 d). Tolvaptan was applied by oral gavage. Body weight was measured daily at 09:00 AM prior to treatment. Animals were sacrificed at 9 AM to obtain blood samples, distal colon and feces.

***Tissue preparation***

At the end of the experiment, rats were anesthetized intraperitoneally with 10% chloral hydrate; blood samples were obtained by postcaval puncture, and the descending colon was removed rapidly, feces was collected, and the colon was washed with PBS at 4 °C three times. Animals were killed by cervical dislocation. Colonic mucosa was isolated from underlying serosa by scraping with a glass slide and placing it into liquid nitrogen and then storing at −80 °C for Western blotting and real-time polymerase chain reaction (PCR); the whole layer was fixed in 4% paraformaldehyde for 24 h and prepared for hematoxylin–eosin (HE) staining. Blood was collected in centrifuge tubes containing EDTA (1.5 mg/mL) and proteinase inhibitor (Roche, Switzerland), and then kept on ice until centrifugation at 4 °C to obtain plasma. The plasma was stored at −20 °C for subsequent assay of plasma vasopressin and ion concentrations.

***Hormone determination***

Hormone levels were determined by avidin–biotin ELISA using commercially available kits, vasopressin ELISA kit (Westang Bio-Tech, Shanghai, China) for plasma vasopressin concentration. Na+ concentration was detected by potentiometric method (AU5800; Beckman Coulter, CA, United States).

***Western blotting***

Colonic mucosal epithelium was obtained by scraping with a glass slide, and homogenized in lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China) with a glass homogenizer. The successive process followed the instructions of theMem-PER Eukaryotic Membrane Protein Extraction Kit (Thermo Scientific).The supernatant was collected and its protein concentration was analyzed by BCA kit (Tiangen Biotech, Beijing, China), and then diluted with loading buffer (FUDE Biological Technology, Hangzhou, China) to 4 μg/μL. Each mixture was boiled at 100 °C for 5 min and chilled on ice. The mixture was stored at −20 °C and equal volumes were loaded in SDS-PAGE, together with molecular markers. The protein was transferred from the gel to a PVDF membrane, and the membrane was blocked for 1 h at room temperature using 5% bovine serum albumin. Membranes were incubated with AQP-2 antibody (Cell Signaling Technology, Danvers, MA, United States) overnight at 4 °C. After washing the membrane with TBST, horseradish peroxidase was added for signal development. Images were obtained by darkroom development techniques for chemiluminescence by ECL Plus (Advansta, Menlo Park, CA, United States).

***mRNA detection for target genes***

Total RNA was isolated from the colonic mucosal epithelium samples using TRIzol reagent (Invitrogen, Carlsbad, CA, United States). Reverse transcription was carried out using a Fist-Strand cDNA Synthesis kit (Thermo Scientific) in a total volume of 20 μL. The resultant cDNA was amplified using a SYBR Green qPCR kit (Applied Biosystems, Carlsbad, CA, United States), and quantified using an ABI PRISM 7500 sequence detection system (Applied Biosystems). For PCR, the following sense and antisense primers were designed from rat AQP-2 cDNA sequence: sense, 5’-TGGGTTGCCATGTCTCCTTC-3’; and antisense, 5’-GCGTTGTTGTGGAGAGCATT-3’. The PCR system consisted of 1 μL cDNA, 0.5 μL each of specific primers (10 μmol/L), 5 μL 2 × SYBR Green Mix and 3 μL water in a total volume of 10 μL. The PCR cycling conditions for AQP-2 and GAPDH were as follows: an initial denaturation and activation at 95°C for 10 min, followed by 40 amplification cycles of 95 °C for 15 s and 60 °C for 60 s. mRNA expression of the target genes was standardized by reference gene GAPDH. The relative expression of each gene was calculated by the comparative Ct method. The 2−ΔΔCt method was adopted to quantify the mRNA expression level between each group, and ΔΔCt was defined by the following equation: ΔΔCt= [(Ct of AQP-2 – Ct of GAPDH)tolvaptan − (Ct of AQP-2 − Ct of GAPDH)vehicle].

***HE staining***

Colonic mucosal epithelium samples from cirrhotic rats with ascites were fixed overnight in 4% paraformaldehyde at 4 °C and routinely processed for paraffin embedding. Histological sections (5 μm) were then prepared and stained with HE. Images (at × 400 magnification) of the distal colon were acquired with a biological imaging microscope (Olympus, Tokyo, Japan).

***Fecal water content***

Fecal samples were collected at the time of sacrifice, and dried in an oven at 60 °C for 24 h. The fecal water content was calculated based on the following equation: wet weights (g) − dry weights (g)/ wet weights (g) ± 100%.

***Statistical analysis***

The results were expressed as the mean ± SEM, and the analyses were performed using SPSS version 17.0 software. Student’s *t* test was used for two-group comparisons. Differences were considered statistically significant at *P* < 0.05. All the statistical review of the study was performed by a biomedical statistician.

**RESULTS**

By pathological examination, all liver specimens from cirrhotic rats with ascites showed cirrhosis, but we failed to distinguish the significant differences between rats receiving tolvaptan or vehicle. HE staining of the colon tissue sections showed no mucosal damage to the colon attributable to the administration of tolvaptan (Figure 1).

Table 1 shows the effect of different doses of tolvaptan at different times on cirrhotic rats. Animals were divided into three groups according to drug levels, and we chose the first 5 d to observe plasma vasopressin, sodium concentration, and body weight. No significant differences were observed in body weight and no change was identified for sodium and vasopressin concentrations based on different doses of tolvaptan and vehicle, respectively.

After administration of both doses of tolvaptan, there was a significant decrease in body weight at day 1, as well as the successive days (Table 2). After drug treatment for 4–5 d, ascites was eliminated (confirmed by abdominal laparotomy when sacrificed). Vasopressin decreased from day 2, and there was a significant difference between tolvaptan doses (3 mg/kg *vs* 5mg/kg) on day 2 (11.41 ± 0.62 pg/ mL *vs* 10.12 ± 0.42 pg/mL, *P* < 0.05) and day 3 (10.21 ± 0.41 pg/ mL *vs* 8.12 ± 0.22 pg/ mL, *P* < 0.05). No significant difference was found on days 4 and 5. The results suggest that the higher dose of tolvaptan exerted its desired effects faster, but the water diuretic effect of tolvaptan is not increased with dose.

AQP-2 expression in the distal colon was detected in each group. Two bands of AQP-2 protein were detected by Western blotting. One band appeared at 23 kDa and the other at 39 kDa, representing the nonphosphorylated and phosphorylated forms, respectively. The total expression of the two bands was analyzed as the protein level in the distal colon (Figure 2). The protein expression level of AQP-2 after 1 d tolvaptan administration showed no significant difference compared to that with vehicle. Significant downregulation was found on day 2 (3 mg/kg: 100% ± 22.9% *vs* 54.7%±11.7, *P* < 0.05; 5mg/kg: 100% ± 22.9% *vs* 53.0% ± 9.4%, *P* < 0.05). And successive days, with both 3 mg/kg and 5 mg/kg However, no difference was found in AQP-2 expression between the 3 mg/kg and 5 mg/kg groups. Transcriptional AQP-2 in the distal colon was also measured (Figure 3). There was a significant difference on day 1 between the tolvaptan and vehicle groups (3 mg/kg: 100% ± 16.3 *vs* 68.50% ± 19.20, *P* < 0.05; 5 mg/kg: 100% ± 16.3 *vs* 34.16% ± 15.14, *P* < 0.01), as well as on successive days. Expression of AQP-2 mRNA before and after administration of tolvaptan showed a consistent trend to reflect protein expression, but the alteration in mRNA level appeared more quickly.

Fecal water content after tolvaptan administration increased on days 2 and 3 with both doses of tolvaptan, but only the higher dose caused an increase on day 1 (Figure 4). After 3 d of tolvaptan administration, the fecal water content returned to baseline.

**DISCUSSION**

The effect of tolvaptan on AQP-2 in the distal colon has been reported in rats in order to explore the roles of vasopressin and aldosterone in physiological adaption[15-16]. In our study, to investigate the colonic expression of AQP-2 in cirrhotic rats with ascites, and to explore the role of tolvaptan in regulation of fecal water content, different doses of tolvaptan were administered for different times, and the findings presented here indicated that level of AQP-2 expression increased in cirrhotic rats with ascites, and it was downregulated by oral administration of tolvaptan. Furthermore, the fecal water content rose temporarily during tolvaptan therapy.

Every AQP protein consists of six transmembrane domains, and the AQP family selectively transports water, glycerin, and other compounds through the membrane. It is generally accepted in the kidneys that transmembrane free water is regulated by water channel, namely AQP-2. Both water deprivation[17-18] and deamino-arg8-vasopressin (dAVP)[19-20] infusion increases AQP-2 expression in the kidney. AQP-2 was first observed in the distal colon in dehydrated rats by Gallardo *et al*[3] in 2001. They suggested that the colonic expression of AQP-2 in apical membranes of rats helps regulate transepithelial water transport from the intestinal side to the vascular side. This mechanism was strongly involved in water absorption and fecal dehydration.

In our study, colonic expression of AQP-2 was confirmed and we found that it was upregulated in cirrhotic rats with ascites. Ascites is a state of water retention, which is a combined effect of endocrine and hemodynamic systems. We attributed the increase of AQP-2 expression to the development of ascites. High hemodynamic state accelerates hypothalamus secretion of excessive vasopressin[21], and deterioration of liver function reduces metabolism of vasopressin and aldosterone. Both of these contribute to the high level of vasopressin and aldosterone in blood stream. Excessive vasopressin stimulates the V2 receptors and activates adenylyl cyclase. Adenylyl cyclase increases the concentration of cAMP in the cytoplasm, which facilitates phosphorylation of AQP-2. The accumulation of AQP-2 is induced by vesicle trafficking from storage vesicles to apical surface membranes within several tens of minutes (short-term regulation)[22-25], and in the long-term, upregulation of AQP-2 in response to vasopressin plays a major role[26,27].

Tolvaptan is a highly effective and selective non-peptide vasopressin V2 receptor antagonist. It was reported that oral tolvaptan (1 and 3 mg/kg) promoted a marked diuretic effect in dimethyl nitrosamine (DMNA)-induced cirrhotic rats with ascites[28]. Cristia *et al*[16] reported that 3 mg/kg tolvaptan significantly inhibited AQP-2 expression in the distal colon. In our preliminary experiment, we selected different doses of tolvaptan and found that 1 mg/kg showed aquaresis but had no significant effect on colonic AQP-2 expression. So, we tried higher doses of 3 mg/kg and 5 mg/kg. Ascites was eliminated in most of the rats receiving tolvaptan therapy at the end of the protocol. Serum sodium increased and no hypernatremia was present. Some rats died of dehydration. We confirmed elevation of vasopressin in cirrhotic rats[[21](http://www.ncbi.nlm.nih.gov/pubmed/?term=Kim%20JK%5BAuthor%5D&cauthor=true&cauthor_uid=8423035)] and different doses of tolvaptan decreased it to a similar level. It seems that in the cirrhotic ascites model, elevation of vasopressin is mainly induced by a nonosmotic mechanism, because hyponatremia should inhibit vasopressin excretion in healthy individuals. After the circulation was improved, vasopressin decreased.

As in the kidneys, the effect of vasopressin on AQP-2 regulation in the colon is a response to V2 receptor activation. Our results confirmed that the reduction of colonic expression of AQP-2 was a direct response to oral tolvaptan administration. In the present study, both doses of tolvaptan affected AQP-2 regulation but different doses eventually resulted in similar expression levels in the distal colon.

It is reported that the colon could be a target of action for vasopressin, as it has been shown that vasopressin stimulates Na+ and water absorption[15]. Water is removed from luminal feces by the surface mucosa and the crypts[29]. Cristià *et al*[16] suggested that the stimulation of myofibroblast growth and the increase of AQP-2 expression are consistent with the antidiuretic role of tolvaptan in the distal colon. The studies above discussed the colonic microscopic function and electrochemical changes of tolvaptan, but the biological function has not been explored.

We showed that the fecal water content increased and we attributed it to tolvaptan administration. It is known that mucosal damage in the colon causes diarrhea[30,31]. We were not sure whether a higher dose of tolvaptan would cause mucosal damage. So, we investigated HE staining of the colon tissue sections, but no mucosal damage was found that was attributable to administration of tolvaptan. However, after a few days of therapy, the fecal water content returned to normal (4–5 d). This phenomenon did not correspond to the alteration of AQP-2 expression in the distal colon. As tolvaptan was the only variable in this experiment, we suggested there would be compensatory mechanisms of water balance.

The vasopressin-dependent water flow is through AQP-2 from the intestinal tract, and outflow to the intercellular space via AQP-3 and/or AQP-4. But it appears that neither AQP-3 nor AQP-4 is regulated by vasopressin in the distal colon[32-33]. It has been suggested that AQP-4 has little or no effect on colonic fluid secretion or fecal dehydration. However, AQP-3 is the most dominantly expressed AQP in the colon and plays an important role in the absorption of water[34-35], and its abundance appears to be regulated on a long-term basis in a manner similar to the long-term regulation of AQP-2. When exposed to an environment of possible water overload[36], AQP-3 could serve as a water channel to reabsorb water from the enteric cavity. When AQP-3 was specifically blocked by HgCl2 or CuSO4, diarrhea was induced[37]. We speculated that AQP-3 upregulation might be one of the mechanisms to balance water absorption in the distal colon after tolvaptan administration.

Our experiment explored fecal water content in response to oral tolvaptan administration in the therapy of cirrhotic ascites. Tolvaptan reduces water reabsorption and might promote clearance of redundant water. This mechanism could induce diarrhea in some conditions. However, the precise mechanism needs to be further explored. Besides the long-term regulation, short-term regulation should also be detected in the distal colon and its biological function should be confirmed.

**COMMENTS**

***Background***

Aquaporin (AQP)-2 is one of the AQP family and marked free-water clearance is induced when it is blocked. Its potent and selective antagonist, tolvaptan, can be used to treat cirrhotic ascites and hyponatremia. AQP-2 is found in the distal colon but its biological function has not been established. So, the authors designed this study to detect the colonic expression of AQP-2 and to investigate the correlation between tolvaptan administration and fecal water content.

***Research frontiers***

AQP-2 is a member of the AQP family, and it helps to absorb water in the kidneys. It was also found in the colon and is involved in water and electrolyte transportation. Tolvaptan is a specific antagonist of AQP-2 and has widespread clinical application.

***Innovations and breakthroughs***

This is the first study to explore colonic expression in cirrhotic rats with ascites, and tolvaptan inhibited its expression and increased fecal water content.

***Applications***

This study suggests that tolvaptan may be useful in upregulating fecal water content and indicates a way to clear abundant water.

***Peer-review***

It is an interesting study with appropriate methodology and the results are clear and of great importance.

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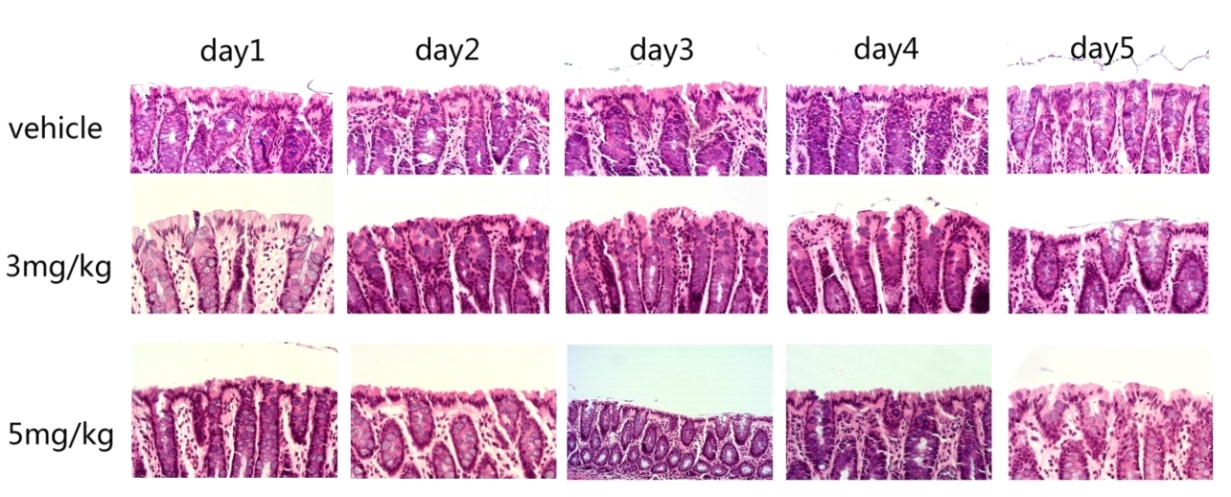
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**P-Reviewer:** **Lee SH, Tekin F,** Wang JS **S-Editor:** Qi Y

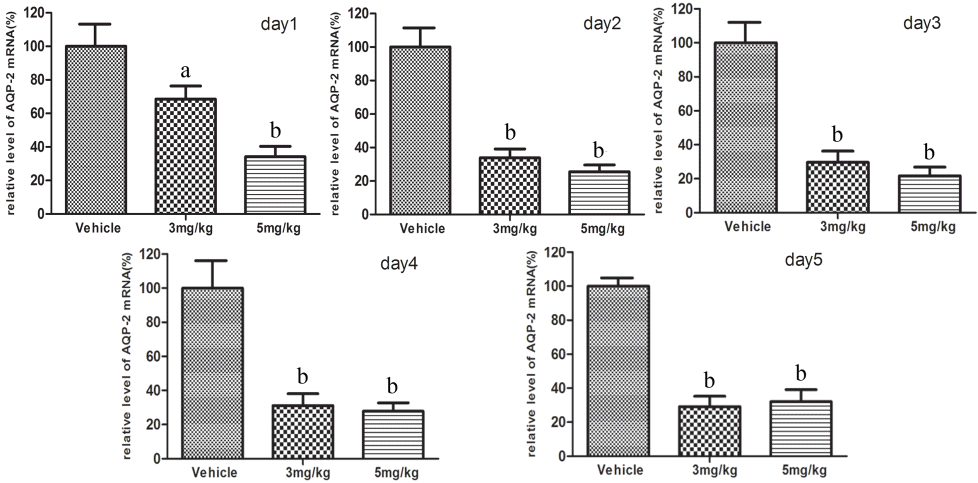
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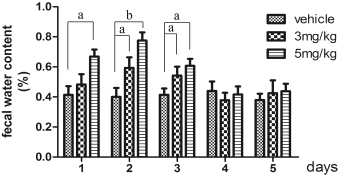
**Figure 1** **HE staining of distal colon of cirrhotic rats with ascites receiving different doses of tolvaptan for different times (× 400).** No mucosal damage was attributable to administration of tolvaptan.

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**Figure 2** **Phosphorylated (39kDa) and nonphosphorylated (23kDa) protein expression of AQP-2 in distal colon in cirrhotic rats with ascites treated with oral gavage of tolvaptan (3 or 5 mg/kg), or vehicle.** After 1 d of both doses of tolvaptan, no significant difference was found between each group, and protein expression level of AQP-2 showed significant differences since day 2 (3 mg/kg: 100% ± 22.9% *vs* 54.7% ± 11.7, *P* < 0.05; 5 mg/kg: 100% ± 22.9% *vs* 53.0% ± 9.4%, *P* < 0.05) and successive days. AQP-2 expression in the control group (no treatment) was also measured, which was significantly lower than that on day 2 (22.2 ± 10.23% *vs* 100 ± 17.23%, *P* < 0.01). No significant difference was found between the groups treated with different doses of tolvaptan. Data are mean ± SEM; a*P* < 0.05, tolvaptan *vs* vehicle; b*P* < 0.01, tolvaptan *vs* vehicle, respectively.



**Figure 3 Relative mRNA expression of aquaporin -2 in the distal colon in the tolvaptan and vehicle groups.** Transcription of AQP-2 in the distal colon in cirrhotic rats with ascites treated with different doses of tolvaptan (3 and 5 mg/kg) was measured. A significant difference was detected since day 1 (3 mg/kg: 100% ± 16.3 *vs* 68.50 ± 19.20, *P* < 0.05; 5mg/kg: 100% ± 16.3 *vs* 34.16 ± 15.14, *P* < 0.01) and successive days. There was no significant difference between the respective tolvaptan groups. Data are mean ± SEM; a*P* < 0.05, tolvaptan *vs* vehicle, respectively; b*P* < 0.01, tolvaptan *vs* vehicle, respectively.



**Figure 4** **Fecal water content in the distal colon of cirrhotic rats with ascites.** The fecal water content increased from day 1 in the 5 mg/kg tolvaptan group (41.4% ± 12.8% *vs* 66.8% ± 10.3%, *P* < 0.05), and from day 2 in the 3 mg/kg group (40.0% ± 13.3% *vs* 59.2% ± 15.8%, *P* < 0.05). However, it returned to baseline after 3 d administration of both doses of tolvaptan. No significant difference was found between different doses of tolvaptan. Data are mean ± SEM; a*P* < 0.05, tolvaptan *vs* vehicle, respectively; b*P* < 0.01, tolvaptan *vs* vehicle, respectively.

**Table 1** **Body weight, vasopressin and serum sodium in baseline conditions in cirrhotic rats prior to receiving tolvaptan (3 mg/kg), tolvaptan (5 mg/kg) or vehicle**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **1 d** | **2 d** | **3 d** | **4 d** | **5 d** |
| Body weight (g) | Vehicle | 486.7 ± 12.57(5) | 492.1 ± 10.12(5) | 472.8 ± 18.21(5) | 475.2 ± 9.21(5) | 482.8 ± 13.24(5) |
| Tolvaptan  (3mg/kg) | 502.3 ± 11.91(6) | 487.3 ± 12.43(6) | 465.6 ± 16.59(6) | 465.6 ± 13.24(6) | 476.3 ± 16.00(5) |
| Tolvaptan  (5mg/kg) | 493.4 ± 16.34(6) | 485.2 ± 20.21(6) | 490.1 ± 13.12(6) | 472.1 ± 12.12(6) | 490.1 ± 11.12(6) |
| Vasopressin(pg/mL) | Vehicle | 13.25 ± 0.24(5) | 14.15 ± 0.41(5) | 15.44 ± 0.38(5) | 14.99 ± 0.24(5) | 13.05 ± 0.25(5) |
| Tolvaptan  (3mg/kg) | 15.29 ± 0.43(6) | 13.37 ± 0.22(6) | 16.87 ± 0.43(6) | 15.16 ± 0.17(6) | 14.33 ± 0.39(5) |
| Tolvaptan  (5mg/kg) | 14.85 ± 0.39(6) | 14.23 ± 0.31(6) | 15.12 ± 0.21(6) | 13.21 ± 0.21(6) | 15.23 ± 0.41(6) |
| Serum sodium (mol/L) | Vehicle | 141.2 ± 1.31(5) | 141.2 ± 1.11(5) | 139.2 ± 0.75(5) | 140.1 ± 0.92(5) | 140.2 ± 1.31(5) |
| Tolvaptan  (3mg/kg) | 139.2 ± 1.27 (6) | 141.3 ± 1.20(6) | 138.2 ± 1.85 (6) | 140.1 ± 1.23(6) | 138.2 ± 1.87(5) |
| Tolvaptan  (5mg/kg) | 141.2 ± 1.21(6) | 140.9 ± 1.12 (6) | 142.2 ± 1.22(6) | 138.1 ± 2.21(6) | 139.1 ± 1.55(6) |

Data are mean ± SEM (number of rats); no significant difference was found between different doses of tolvaptan and vehicle.

**Table 2** **Body weight, vasopressin and serum sodium in cirrhotic rats after receiving tolvaptan (3 mg/kg), tolvaptan (5 mg/kg) or vehicle**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **1 d** | **2 d** | **3 d** | **4 d** | **5 d** |
| Body weight (g) | Vehicle | 492.6 ± 11.22(5) | 498.5 ± 12.21(5) | 490.3 ± 16.21(5) | 500.1 ± 16.21(5) | 493.1 ± 11.12(5) |
| Tolvaptan  (3 mg/kg) | 482.2 ± 20.21(5) | 466.2 ± 10.19(6)a | 450.1 ± 18.21(5)a | 443.1 ± 20.12(6)b | 440.1 ± 30.12(5)a |
| Tolvaptan  (5 mg/kg) | 460.2 ± 12.31(5)a | 455.1 ± 16.12(5)b | 440.2 ± 23.12(6)a | 437.3 ± 32.21(5)b | 446.1 ± 25.21(4)b |
| Vasopressin(pg/mL) | Vehicle | 12.97 ± 0.02(5) | 13.81 ± 0.30(5) | 15.91 ± 0.29(5) | 16.97 ± 0.38(5) | 14.02 ± 0.48(5) |
| Tolvaptan  (3 mg/kg) | 12.25 ± 0.52(5) | 11.41 ± 0.62(6)a | 10.21 ± 0.41(5)b | 9.77 ± 0.21(6)b | 7.12 ± 0.32(5)b |
| Tolvaptan  (5 mg/kg) | 12.01 ± 0.21(5) | 10.12 ± 0.42(5)ac | 8.12 ± 0.22(6)bc | 7.01 ± 0.20(5)b | 7.33 ± 0.13(4)b |
| Serum sodium (mol/L) | Vehicle | 140.2 ± 1.25(5) | 140.2 ± 1.51(5) | 141.2 ± 0.65(5) | 141.1 ± 1.61(5) | 142.2 ± 0.81(5) |
| Tolvaptan  (3 mg/kg) | 143.2 ± 2.32(5) | 144.2 ± 1.17(6)a | 146.1 ± 1.13(5)b | 147.2 ± 2.11(6)b | 146.1 ± 2.47(5)b |
| Tolvaptan  (5 mg/kg) | 144.1 ± 1.22(5)a | 147.1 ± 2.51(5)bc | 147.9 ± 2.21(6)b | 146.2 ± 1.71(5)b | 147.2 ± 1.12(4)b |

Data are mean ± SEM (number of rats); a*P* < 0.05, tolvaptan *vs* vehicle, respectively; b*P* < 0.01, tolvaptan *vs* vehicle, respectively. c*P* < 0.05, tolvaptan (3 mg/kg) *vs* tolvaptan (5 mg/kg), respectively.