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Expression of Aquaporin 2, Aquaporin 3 and Aquaporin 4 in Renal Medulla of Bactrian Camel (*Camelus bactrianus*)

Expresión de Acuaporina 2, Acuaporina 3 y Acuaporina 4 en la Médula Renal del Camello Bactriano (*Camelus bactrianus*)

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WANG, J.; YANG, M.; WANG, Z.; WU, J.; CHEN, J.; YANG, W. & WANG, J. Expression of aquaporin 2, aquaporin 3 and aquaporin 4 in renal medulla of Bactrian camel (*Camelus bactrianus*). *Int. J. Morphol., 36(1)*:303-309, 2018.

SUMMARY: Aquaporins (AQPs) are members of the aquaporin water channel family that play an important role in reabsorption of water from the renal tubular fluid to concentrate urine. Using immunohistochemical staining on paraffin sections, We studied expression of AQP2, AQP3 and AQP4 in renal medulla of Bactrian camel (*Camelus bactrianus*). The renal medulla of cattle (Bos taurus) acted as the control. Compared with the control, strong expression of AQP2 was observed at the apical plasma membrane and intracellular vesicles, in both the outer medullary collecting duct (OMCD) and the inner medullary collecting duct (IMCD) of camel. Strong expression of AQP3 was observed at the basolateral plasma membrane of the IMCD of camel. Strong AQP4 expression, however, was observed at the basolateral plasma membrane in the OMCD of camel. Moreover, moderate AQP4 expression was detected in endothelium of capillary in medullary region of camels, whereas very weak/absent expression was detected in endothelium of capillary of cattle. We concluded that expression of AQP2, AQP3 and AQP4 in the camel kidney showed some differences from cattle in renal trans-epithelial water transport. It may enhance our better understanding of special water metabolism mechanisms that enable camels to survive in extreme environments.

KEY WORDS: Bactrian camel (*Camelus bactrianus*); AQP2; AQP3; AQP4; Renal medulla.

INTRODUCTION

Bactrian camel (*Camelus bactrianus*), as a large mammal belonging to Camelus, Camelidae, Tylopoda and Pacentalia (Corbet & Hill, 1991), is located in the desert, semi-desert regions in the north and northwest China. Bactrian camels serve as a very important part in the production of agriculture and the development of animal husbandry in those regions. To adapt to the harsh conditions-hot and arid of deserts or semi-deserts, camels acquire a number of special characteristics. They can store energy in their humps and abdomen in the form of fat, enabling them to survive long periods without any food (Emmanuel & Nahapetian, 1980). Camel's body temperature may vary from 34 to 41 °C throughout the day (Schmidt-Nielsen, 1965). Blood glucose levels in camels are twice those of other ruminants (Al-Ali *et al*., 1988). Furthermore, it is vitally important that camels possess another feature of resistance to thirst and can produce highly concentrated urine to the maintenance of body water homeostasis (Chen & Liu, 2002). Camels tolerate a high dietary intake of salt, consuming eight times more than cattle and sheep (Food and Agriculture Organization of the United Nations, 1994). It has been shown that even when loss of water accounts for 30 % to 40 % of body weight in camels, there is no danger to their lives (Zhao, 1995).

Aquaporins (AQPs) are a family of small, approximately 30 kDa integral membrane proteins that act as water channels. AQPs are responsible for facilitated trans-membrane water transport in a variety of epithelial and nonepithelial cells (Huang *et al*., 2001). AQPs have been cloned in microorganisms (Calamita, 2000), animals (Agre & Kozono, 2003; King *et al*., 2004) and plants (Johansson *et al*., 2000). In mammals, 13 AQP subtypes, named AQP0–12, have been identified and these are expressed in various tissues such as the kidney, brain, liver, lungs and salivary glands (Ishibashi *et al*., 2009). Eight isoforms of AQPs (1–4, 6–8, 11) are expressed in the kidney of which five have been shown to play a role in body water balance. AQP1, AQP2, AQP3, AQP4

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and AQP7 are distributed differentially and strategically along the nephron to facilitate water reabsorption and urine concentration (Kortenoeven & Fenton, 2014).

To our knowledge, ours is the first report that shows AQPs expression in the renal medulla of Bactrian camels. We believe that AQPs expression and water transport in the kidney of camels in deserts or semi-deserts zone differ from other species for reduction of renal fluid loss and maintenance of body fluid balance. It has been reported that AQP2 is regulated both in trafficking (short-term regulation) and in abundance (long-term regulation) via arginine vasopressin (AVP) (Nielsen *et al*., 1993; Terris *et al*., 1996). AVP is a peptide hormone synthesized in the supraoptic and paraventricular nuclei of hypothalamus and regulated body water homeostasis (Bisset & Chowdrey, 1988). Interestingly, both AQP3 and AQP4 are regulated in abundance by AVP in long-term (Poulsen *et al*., 2013). Therefore, we will aim to determine AQP2, AQP3 and AQP4 localization and related functions in camel kidneys.

MATERIAL AND METHOD

The kidneys belonging to healthy Bactrian camels (4 castrated 6-8 years old) were collected immediately after slaughter from a slaughter house of the Right Alasan Banner Food Company in Inner Mongolia Autonomous Region, China. For comparison, the kidneys of healthy cattle (Bos taurus; 4 castrated, 4-6 years old) were collected immediately after slaughter from a slaughter house of Linxia Hui autonomous prefecture, Gansu Province, China. The kidneys were fixed with 4 % Paraformaldehyde solution (PH 7.4), and shortly afterward, blocks were cut from various parts of the kidneys.

All research protocols used in the current experiment were approved by the Animal Ethics Committee of the Inner Mongolia Autonomous Region and Gansu Province, China.

For immunolight microscopy, specimens were dehydrated through a graded series of alcohol, cleared and embedded in paraffin wax, and sectioned at 5mm used for immunohistochemical staining. The sections were dewaxed and rehydrated, an antigen retrieval procedure was performed to unmask antigens, by treating the samples three times in a microwave oven at medium-high fire for 5 min each time in 10 mM citrate buffer, pH 6. After cooling to room temperature, the sections were treated with 3 % hydrogen peroxide (H_2O_2) in distilled water for 30 min, then washed three times with PBS for 10 min each. The sections were incubated in blocking reagent for 1 h and incubated overnight at 4 °C with anti-AQP2 diluted 1:150 (Boster, Wuhan, China), anti-AQP3 diluted 1:150 (Boster, Wuhan, China) and anti-AQP4 diluted 1:500 (Santa Cruz Biotechnology, Santa Cruz). The primary antibody was diluted in 10 mM PBS supplemented with 0.1 % BSA and 0.3 % Triton X-100 [10 mM PBS (7 mM Na_2HPO4,3 mM NaH2PO4, 150 mM NaCl, pH 7.4)]. Subsequently, the sections were rinsed with 10 mM PBS for 3x10 min and incubated with biotinylated anti-rabbit IgG (ZSGB-BIO, Beijing, China, dilution: 1:200) secondary antibody for 2 h at room temperature. Then, the sections were rinsed with 10 mM PBS for 3x10 min and incubated with horseradish peroxidase streptavidin (ZSGB-BIO, Beijing, China, dilution: 1:200) tertiary antibody for 2h at room temperature followed by coloration with diaminobenzidine (DAB; ZSGB-BIO, Beijing, China) for 30s to visualize positive reaction. Counterstaining was with hematoxylin staining. The sections were carried out using bright microscopy (BH-2, Olympus, Nagano, Japan).

RESULTS

We examined the localization of AQP2, AQP3 and AQP4 in renal medulla of cattle (the control) and camel. Counterstaining with hematoxylin staining was used to identify negative segments. In this study, renal medulla was divided into the outer and inner medulla, for collecting duct, thereby forming the outer medullary collecting duct (OMCD) and the inner medullary collecting duct (IMCD).

In the IMCD and OMCD Immunolight microscopy revealed weak expression of AQP2 at the apical plasma membrane and intracellular vesicles of principal cells of cattle, respectively (Figs. 1A and B), whereas strong expression of AQP2 was observed at the apical plasma membrane and intracellular vesicles of camel compared with the control, respectively (Figs. 1C and D).

We investigated whether the expression of AQP3 in abundance was parallel with AQP2 at the apical plasma membrane of the IMCD and the OMCD of camel compared with the control. However, in the OMCD, moderate AQP3 expression was observed at the basolateral plasma membrane of camel compared with the control (Figs. 2A and C), whereas strong expression of AQP3 was observed at the basolateral plasma membrane of the IMCD of camel (Figs. 2B and D).

To investigate whether AQP4 expression was similar to the AQP3 at the basolateral plasma membrane of the IMCD and the OMCD of camel, respectively. We examined AQP4 expression in the IMCD and the OMCD of cattle and camel, simultaneously. It was observed different AQP4 expression in medulla between the control cattle and camel. Strong AQP4 expression was observed at the basolateral plasma

membrane in the OMCD for camel compared with the control (Figs. 3A and C), whereas moderate expression of AQP4 was observed at the basolateral plasma membrane of the IMCD of camel (Figs. 3B and D). Moreover, moderate AQP4 expression was detected in endothelium of capillary in medullary region of camels (Fig. 3D), whereas weak/absent expression was detected in endothelium of capillary of cattle (Fig. 3C).

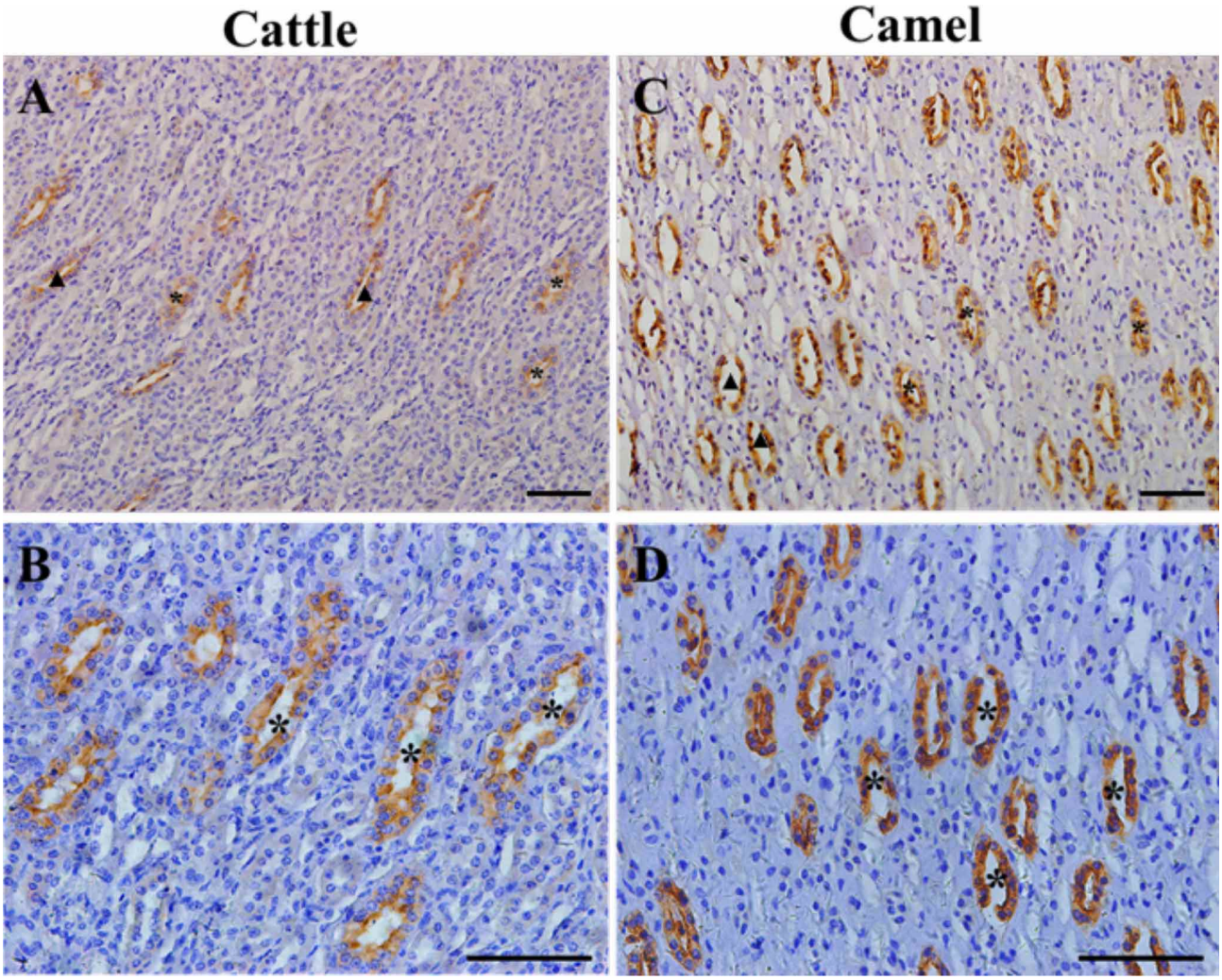

Fig. 1. AQP2 expression in the renal medulla of cattle (A-B) and camel (C-D). (Black triangle), Expression in the outer medullary collecting duct (IMCD); * , Expression in the inner medullary collecting duct (OMCD). Scale bar =100 mm.

DISCUSSION

Cattle and pig are the closest relatives of Bacterian camels, which belong to artiodactyla (even-toed ungulates) in taxonomy (Bactrian Camels Genome Sequencing and Analysis Consortium *et al*., 2012). That is why cattle were acted as the control in this study.

Immunolight microscopy revealed strong AQP2 expression in the IMCD and OMCD of camel compared with the control. The results are in agreement with our expectation. AQP2 has been reported to occur in the principal cells along connecting tubule and collecting duct in mammalian kidneys, where it is localized to the apical plasma membranes and intracellular membrane vesicles (Nielsen *et al*.; Fushimi *et al*., 1993). AVP regulates the body's retention of water, increasing both the osmotic driving force for water reabsorption and the transcellular route for water transport,

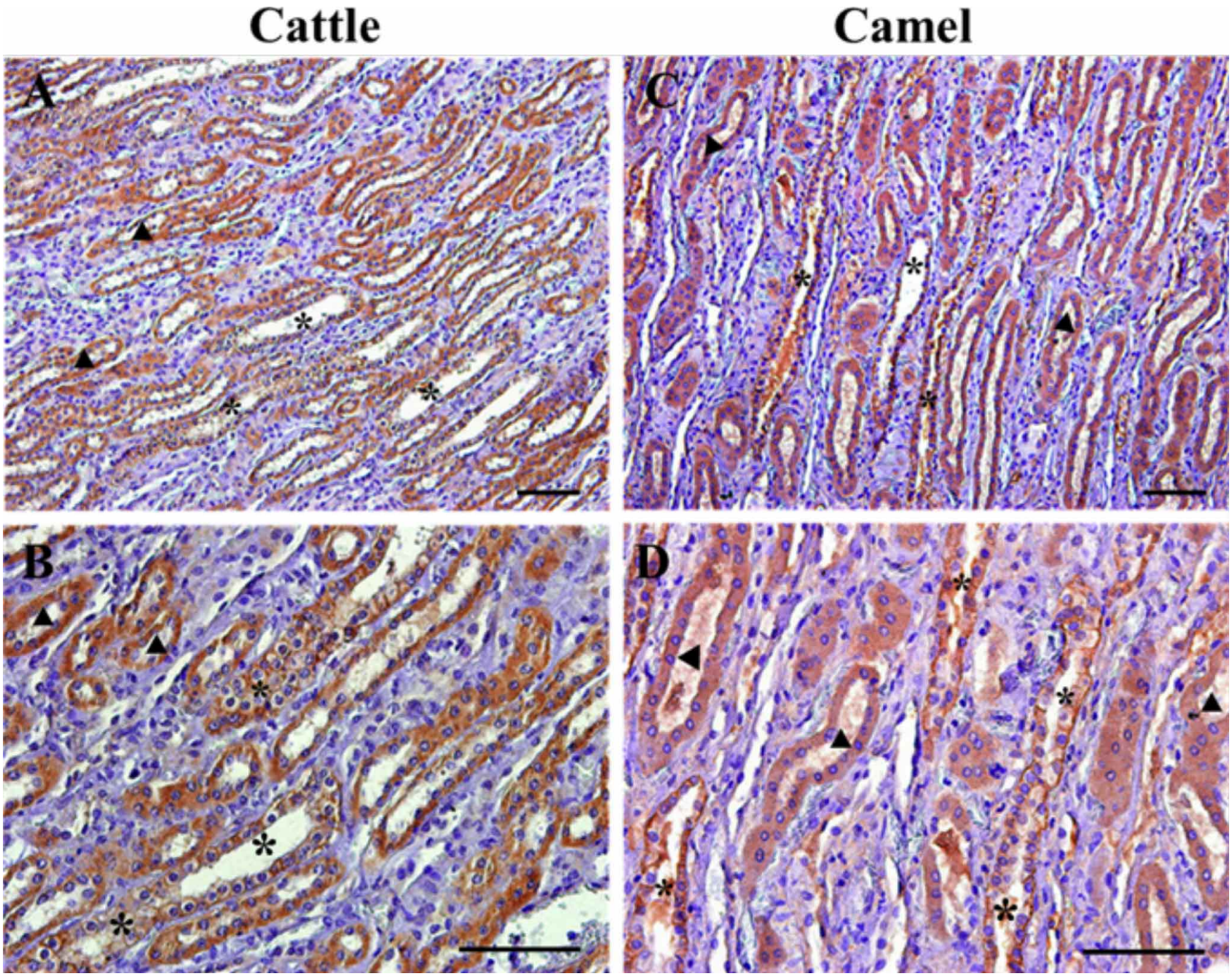

Fig. 2. AQP3 expression in the renal medulla of cattle (A-B) and camel (C-D). (Black triangle), Expression in the outer medullary collecting duct (IMCD); * , Expression in the inner medullary collecting duct (OMCD). Scale bar =100 mm.

causing the kidneys to concentrate the urine (Bisset & Chowdrey). The transcellular route of regulation mainly occurs via modulating cell surface expression of AQP2. AVP binds to the vasopressin type-2 receptor (V2R), present in the basolateral membrane of renal collecting duct principal cells and connecting tubule cells (Fenton *et al*., 2007; Mutig *et al*., 2007), inducing a signaling cascade, involving Gs protein mediated activation of adenylate cyclase, a rise in intracellular cAMP, activation of protein kinases, and redistribution of AQP2 water channels from intracellular vesicles to the apical membrane (Kuwahara *et al*., 1995; Katsura *et al*., 1997). The overall process results in a concentration of the urine.

In adult male rat kidneys, AQP2 expression at the apical cell membrane of OMCD and IMCD was mainly found in the dDAVP-infused rat after 6 days, whereas in control rat, expression of AQP2 tended to be localized to the intracellular membrane vesicles (Poulsen *et al*.). Comparing with the control, we observed strong AQP2 expression at the apical cell membrane in the IMCD and OMCD of camels, it may be result of long-term V2R stimulation, which was consistent with the high water permeability of collecting duct, thereby camels can produce highly concentrated urine. However, the specific function and mechanisms of this observation is not clear and this issue should be investigated further.

It has been suggested that AQP3 and AQP4 are localized in the basolateral membrane of principal cells of the collecting duct and will normally produce a net flow of water into the interstitium (Ecelbarger *et al*., 1995; Terris *et al*., 1995; Coleman *et al*., 2000; Holmes, 2012). Moreover, AQP3 and AQP4 were thought to represent an additional

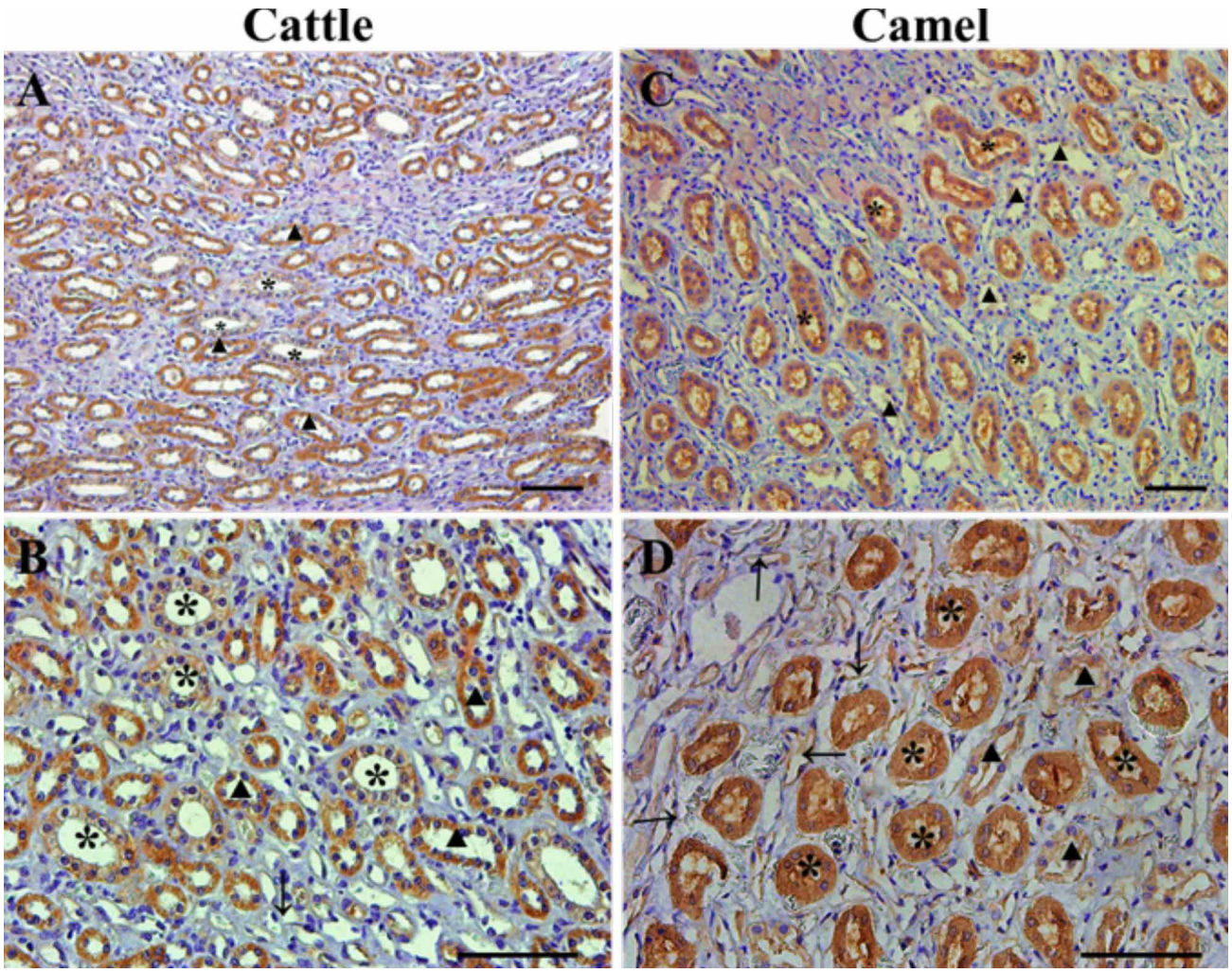

Fig. 3. AQP4 expression in the renal medulla of cattle (A-B) and camel (C-D). (Arrow), AQP4 expression in the outer medullary collecting duct (IMCD); *, AQP4 expression in the inner medullary collecting duct (OMCD). Ø, AQP4 expression in the endothelium of capillary network and vasa recta. Scale bar =100 mm.

exit pathway for water reabsorbed via AQP2 (Ecelbarger *et al*.; Mobasheri *et al*., 2007). There is no evidence for short-term regulation of AQP3 by V2R-induced trafficking. However, prolonged increased AVP levels, as seen during water deprivation or AVP infusion, increase AQP3 protein and mRNA levels both in cortex and medulla (Terris *et al*., 1996; Kortenoeven *et al*., 2013; Poulsen *et al*.). AQP3 has been shown to be important for the urine concentrating mechanism, as AQP3 knockout mice have an increased urine volume, lower urine osmolality and a reduced osmotic water permeability of the basolateral membrane of the collecting duct (Ma *et al*., 2000). It has been found no increase in renal AQP4 abundance in response to 5 days of dDAVP administration in rats (Terris *et al*., 1996). 8 days of dDAVP administration however up-regulated the abundance of AQP4 in OMCD (Poulsen *et al*.). In AVP-deficient rats, long-term administration with dDAVP increases AQP4 protein abundance in the outer and the inner medullary collecting duct, suggesting a regulation of AQP4 by AVP (Murillo-Carretero *et al*., 1999). AQP3/AQP4 knockout mice have a greater impairment of urinary-concentrating ability than the AQP3 single-knockout mice (Ma *et al*.). Therefore, both AQP3 and AQP4 were indeed regulated in abundance by AVP in long-term (≥8 days) in rats. We speculated whether there was a innate regulatory mechanism in water reabsorption for camels as above Namely, more AVP has been secreted in the hypothalamus since long-term adaptation to drought, than AVP combined with V2R and induced a series of signaling cascade; eventually lead to upregulation of aquaporin proteins in renal inner medulla and outer medulla, thereby enhancing water reabsorption and effectively reserving water of body.

In this study, we observed moderate AQP3 expression in the apical cell membrane in the OMCD of camel, whereas strong expression of AQP3 was observed in the IMCD compared with the control. Comparing with AQP3 expression in the OMCD and IMCD, however, the result of AQP4 expression was opposite. It was observed strong and moderate expression of AQP3 in the OMCD and IMCD of camel, respectively. This result of observation in this paper is basically consistent with our speculation, however, further research on specific mechanisms is still needed.

AVP combining with V2R induced a series of signaling cascade, which resulted in the redistribution of AQP2 from intracellular vesicles to the apical membrane (Flamion & Spring, 1990; Fenton *et al.*). Driven by the transcellular osmotic gradient, water then will enter principal cells through AQP2 and enters the blood via AQP3 and AQP4 water channels, which are expressed in the basolateral membrane, resulting in concentrated urine (Ishibashi *et al*., 1997; Ma *et al*.; Kortenoeven *et al*.; Kortenoeven & Fenton). Interestingly, we observed AQP4 expression in the endothelium of capillary network and vasa recta in camels, whereas very weak/absent expression of AQP4 was observed in the control. It is suggested that this observation maybe explain water entering the blood via AQP4 water channels in the endothelium. On the long term, AVP also increased AQP2 expression via phosphorylation of the cAMP responsive element binding protein (CREB), which stimulates transcription from the AQP2 promoter (Kortenoeven *et al*.; Kortenoeven & Fenton). We have been known that diabetes insipidus (DI) is characterized by an impaired renal water reabsorption, leading to polyuria and consequently, polydipsia (Kortenoeven & Fenton). In central DI, the production of AVP is impaired (Lolait *et al*., 1992). In model of central DI, there is decreased expression of AQP2 in the AVP deficient Brattleboro rat, which can be reversed by chronic AVP infusion, suggesting that patients lacking AVP are likely to have decreased AQP2 expression that can be treated by AVP (DiGiovanni *et al*., 1994). As AVP regulates other aquaporin proteins involved in urine concentration, such as AQP3 and AQP4 in connecting tubules and collecting ducts (Kortenoeven & Fenton). Administration of the synthetic AVP homolog dDAVP is usually able to drastically decrease urine output in central DI patients, suggesting that AVP or dDAVP stimulation induces regulation of aquaporin proteins in renal medulla and cortex of DI patients.

In conclusion, comparing with AQP2, AQP3 and AQP4 expression in the OMCD and IMCD at cattle (the control), we showed that strong AQP2 expression at the apical cell membrane in both the IMCD and OMCD of camels; strong expression of AQP3 was observed in the IMCD, and strong expression of AQP4 was observed in the OMCD of camel compared with the control. To adapt to the harsh conditions of hot and arid deserts or semi-deserts, camels tolerate a high dietary intake of salt and can produce highly concentrated urine. AQPs may play a key role in body water balance, thereby maintaining water homeostasis. Our findings may also improve our understanding of special water metabolism mechanisms that enable camels to survive in extreme environments. In addition, it is important to study other AQPs in the camel kidney to better understand the exact functions in water reabsorption. Further studies could focus on the molecular functions of V2R and AVP, combining with AQPs to investigate the special physiology of the camel.

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WANG, J.; YANG, M.; WANG, Z.; WU, J.; CHEN, J.; YANG, W. & WANG, J. Expresión de acuaporina 2, acuaporina 3 y acuaporina 4 en la médula renal del camello bactriano (*Camelus bactrianus*). *Int. J. Morphol., 36(1)*:303-309, 2018.

RESUMEN: Las acuaporinas (AQP) son miembros de las proteínas de transporte que desempeñan un papel importante en la reabsorción de agua del líquido tubular renal para concentrar la orina. Estudiamos la expresión de AQP2, AQP3 y AQP4 en la médula renal del camello bactriano (*Camelus bactrianus*) usando tinción inmunohistoquímica en secciones de parafina. La médula renal del bovino (*Bos taurus*) se usó como control. En comparación con el control, se observó una fuerte expresión de AQP2 en la membrana plasmática apical y vesículas intracelulares tanto en el conducto colector medular externo (CCME) como en el conducto colector medular interno (CCMI) del camello. Se observó una fuerte expresión de AQP3 en la membrana plasmática basolateral del CCMI del camello. También se observó una expresión fuerte de AQP4 en la membrana plasmática basolateral en el CCME de camello. Además, se detectó una expresión moderada de AQP4 en el endotelio de los capilares en la región medular de los camellos, mientras que en el endotelio de los capilares del bovino se detectó una expresión muy débil. Concluimos que la expresión de AQP2, AQP3 y AQP4 en el riñón de camello mostró algunas diferencias con el bovino en el transporte trans-epitelial de agua renal. El estudio podría mejorar nuestra comprensión de los mecanismos especiales del metabolismo del agua que permiten a los camellos sobrevivir en ambientes extremos.

PALABRAS CLAVE: Camello bactriano (*Camelus bactrianus*); AQP2; AQP3; AQP4; Medula renal.

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