ORIGINAL ARTICLE

Eur. J. Anat. 19 (2): 125-129 (2015)

Expression of aquaporin-1 in the choroid plexus in communicating and non-communicating hydrocephalic rats

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SUMMARY

Hydrocephalus is an accumulation of cerebrospinal fluid (CSF) with dilatation of brain ventricles which can be either communicating or non-communicating. Multiple pathophysiological mechanisms underlie the appearance of hydrocephalus, which has many different causes including birth defects, brain hemorrhage, infection, meningitis, tumor, or head injury. The choroid plexuses (ChP) are circumventricular structures closely related to the above-mentioned pathophysiological mechanisms of the CSF, and aquaporin-1 (AQP1) is the water channel directly implicated in CSF production. Our studies with hydrocephalic rats revealed an increase and redistribution of AQP1 in the ChP, with AQP1 being expressed not only in the cell apical pole, but also in the cell basal pole and in the stroma. The immunohistochemical changes observed in both communicating and non-communicating hydrocephalus suggest a variation in the efficiency of the cells of the ChP, where AQP1 could perform both CSF production and reabsorption in order to delay ventricular dilatation.

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Key words: Aquaporin-1 – Hydrocephalus – Choroid plexus

INTRODUCTION

Hydrocephalus is characterized by a cerebrospinal fluid (CSF) increase, with an enlargement of brain ventricular cavities and a progressive damage to surrounding tissue. In this disorder, the mechanism of brain tissue injury appears to be a combination of mechanical injury due to brain distortion, ischemic damage due to impaired blood flow, and an accumulation of toxic waste products in the CSF due to impaired flushing of CSF from the ventricles (Del Bigio, 1993). Hydrocephalus is a result of multiple pathophysiological mechanisms, with many different causes including birth defects, brain hemorrhage, infection, meningitis, tumor, or head injury (Oi et al., 1999). Kaolin has been widely used to induce hydrocephalus in several developmental stages of experimental animals including neonates and adults. This model of experimental induction was chosen because it is efficient and inexpensive (Khan et al., 2006; Tashiro, 1997). On the other hand, some of the brain structures involved in the etiology and pathophysiology of hydrocephalus are the circumventricular organs such as the choroid plexus (ChP).

The choroid plexuses (ChP) are highly vascularized tissues suspended from each of the cerebral

Submitted: 16 September, 2014. *Accepted:* 2 December, 2014.

ventricles. These specialized organs have two major functions: to act as a diffusion barrier between the blood and the CSF (the localization of the blood-CSF barrier) and to secrete CSF that fills the ventricles and the subarachnoid spaces. The choroid plexuses comprise a central stroma with numerous blood vessels covered by a single layer of specialized ependymal cells (the choroid epithelium) resting on a thick basement membrane. The choroid plexuses are part of the circumventricular organs and, like all blood vessels in this part of the brain, the vessels in choroid plexuses are fenestrated and the tight junction strands linking adjacent endothelial cells are discontinuous (Carmona-Calero et al., 2012; González-Marrero et al., 2012).

Aquaporin 1 (AQP1), located in the apical pole of the ChP cells, makes a substantial contribution to CSF production, and is a potential therapeutic target in the management of CSF circulation disorders (Owler et al., 2010). Although apical AQP1 is well placed to contribute significantly to CSF production, it is not clear how the passage of water across the basolateral membrane is facilitated. There are reports of AQP1 being identified in the basolateral membrane of the choroid plexus epithelium, and it is relatively scarce. Several mechanisms may be involved in AQP1 regulation. Firstly, the AQP1 function may be altered by various compounds or biological processes. Secondly, since the AQP1 function is dependent on its location in the apical membrane of the ChP, AQP1 may be regulated by changes in its location within the cell (Praetorius, 2007). Therefore, hydrocephalus is the main disorder of the central nervous system associated with defective CSF turnover, and AQP1 and AQP4 are water channels located in the areas described above associated with the elimination of cerebral edema (Castañeyra-Ruiz et al., 2013; Amiry-Moghaddam et al., 2004; Shen et al., 2006).

The overall level of AQP1 and AQP4 protein may be regulated in response to various physiological or pathophysiological parameters. In terms of the overall level of AQP1 and AQP4 protein, there is some evidence that regulation of AQP1 in the ChP does occur (Owler et al., 2010; Praetorius 2007; Castañeyra-Ruiz et al., 2013; Amiry- Moghaddam et al., 2004; Shenet al., 2006). Therefore, the aims of the present work are to examine AQP1 expression in the ChP in communicating hydrocephalus and non-communicating hydrocephalus, and to study AQP1 participation in the physiopathology of the water channels in CSF production in the ventricular enlargement occurring in the two different types of hydrocephalus.

MATERIAL AND METHODS

We used the brains of 15 male Wistar-Kyoto (WKY) rats, sacrificed at 30 weeks of age, which were devised into three groups: a) eight rats (WKY) used as control group, b) four kaolin-induced hydrocephalic WKY rats used as the non-communicating hydrocephalus group, and c) three Wistar-Kyoto rats, which spontaneously developed a tetra ventricular hydrocephalus, used as the communicating hydrocephalus group. Kaolin hydrocephalus was induced in male Wistar-Kyoto rats by administration of 0.1 ml Kaolin (250 mg/ml) into the cistern magna at 10th week of life and 20 weeks later (30 weeks of age) before sacrifice. All experiments were conducted according to the European Directive of 24 November 1986 (86/609/EEC) and subsequent Royal Decree RD 1201/2005 of 10 October 2005 for the maintenance and use of laboratory animals, which were approved by the Committee of Animal Use for Research at the University of La Laguna. The number of animals used, as well as stress and suffering of these subjects during handling and experimentation, were minimized.

Rats from each group were fixed by intracardiac perfusion with Bouin's fluid, dehydrated and embedded in paraffin under standard conditions. The brains were cut into four serial coronal sections. One of the serial coronal sections was stained by the Klüver-Barrera method.

The section containing the ChP was cut from the paraffin-embedded blocks and attached to silanised slides, deparaffinized, hydrated and washed with Tris-buffer saline. After pretreatment for the enhancement of labeling, the sections were blocked with 3% hydrogen peroxide and then incubated with the primary antibody overnight inside a humid chamber. Aquaporin-1 (AQP1) at 1:1000 (Ab9566 Abcam Cambridge, UK) was used as the primary antibody. Immunohistochemistry required a pre-treatment of 15 minutes boiling in citrate buffer, pH 6.0. After rinsing, the slides were incubated with the biotinylated secondary antibody (VECTOR, diluents universal). The immunoreaction was developed in a solution of diaminobenzidine (DAB; Sigma) and 0.003% hydrogen, washed and dehydrated with ethanol series, rinsed with xylene, and mounted in Eukitt. Omission of the incubation in the primary antibody was used as a control.

Three rostrocaudal levels of the immunohistochemistry slides were converted into digital images using a LEICA DMRB photomicroscope with a LEICA DC 300 F camera (Germany). Image analysis was completed in Image J (v. 1.43 u, NIH, Bethesda, MD, USA).The 'Mean Gray Value' was measured from the selected nuclei for all stained tissue and membranes. This value gives the average stain intensity in gray-scale units for all threshold pixels. A single-factor analysis of variance (ANOVA) and post Hoc test Turkey was used for the immunohistochemistry statistical study, which was conducted using IBM SPSS statistic 19 software.

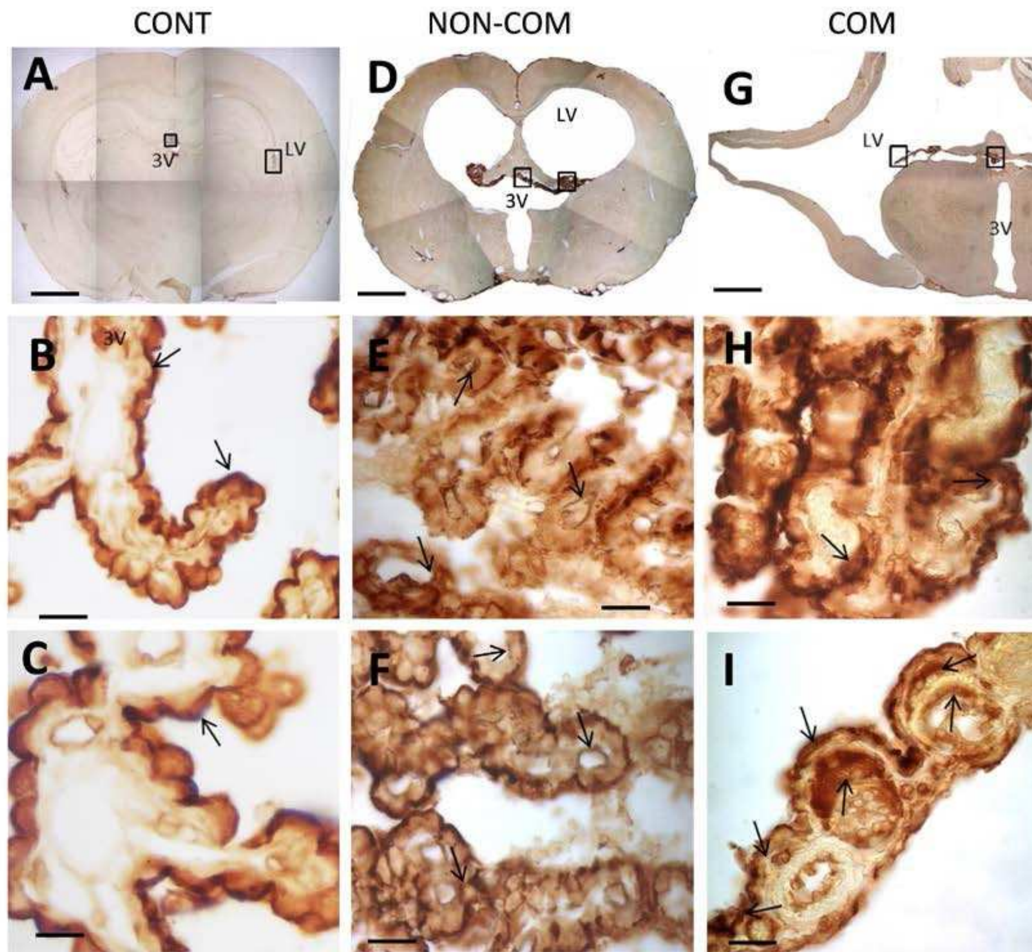

Fig. 1. Coronal section of the rat brains showing the choroid plexus (ChP) marked with anti- aquaporin 1 (AQP1). (A,B,C) control rats; (D,E,F) non-communicating hydrocephalus; (G,H,I) communicating hydrocephalus. Bars: 600 µm in (A,D); 200 µm in (G); 60 µm in (B,C); and 40 µm in (E,F,H,I). AQP1, aquaporin-1; CONT, control; COM, communicating hydrocephalus; LV, lateral ventricle; 3V, third ventricle; NON-COM, non-communicating hydrocephalus; ChP, choroid plexus; Arrow, AQP1 localization.

RESULTS

AQP1 immunoreaction was mainly observed in the apical pole of epithelial cells of ChP in control rats (Fig. 1 A, B, C). Redistribution (arrow) of AQP1 immunoreaction was observed in non-communicating hydrocephalus (Fig. D, E, and F), in such a way that AQP1 was also found in the basal poles of the choroid plexus cells and in the basal membrane of endothelial cells. Communicating hydrocephalus showed (Fig. 1 G) an increase in vascular size and also a relocation of AQP1 in the stromal part of the ChP and in perivascular spaces (Fig. 1 H, I, arrows). The reaction intensity of AQP1 in the stromal part and in the ChP of the hydrocephalic rats was higher than in the control group (Fig. 2). The result was similar in the third and fourth ventricles.

DISCUSSION

Aquaporins (AQPs) are membrane proteins that facilitate water and small solute movement in tissues. Hydrocephalus is the main disorder of the central nervous system associated with defective

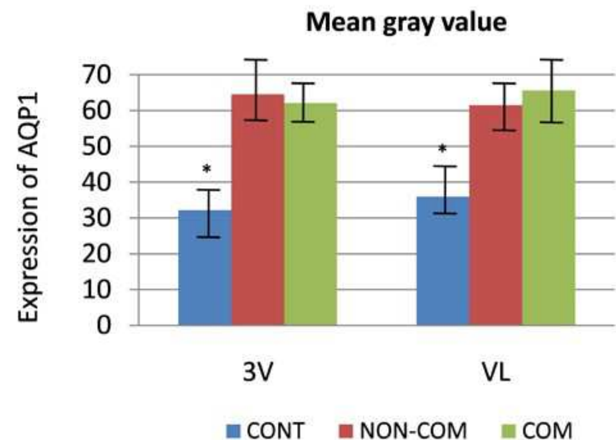

Fig. 2. Histogram of aquaporin-1 in choroid plexus measured by densitometry in control rats (CONT), in non-communicating hydrocephalus (NON-COM) and in communicating hydrocephalus (COM). * significantly different from control and hydrocephalus. LV, lateral ventricle; 3V, third ventricle.

CSF turnover (Amiry-Moghaddam et al., 2004; Shenet al., 2006). Most of these studies on AQP1 and hydrocephalus are based on animal models, such as congenital hydrocephalic rats (H-Tx rats), kaolin-induced hydrocephalus in rats or AQP1 knockout mice (Kalani et al., 2012). Human studies are based on a few observations in cases of hydrocephalus associated with choroid plexus hyperplasia or tumors choroid plexus. Other studies show a down-regulation of AQP1 expression in hydrocephalus suggesting a reduction of CSF production that acts to lower intracranial pressure (Rash et al., 1998; Dominguez-Pinos et al., 2005; Sival et al., 2011; Blocher et al., 2011). However, another study was unable to identify any changes, while a study in human, Mao et al. (2006) indicates a particularly strong expression in choroid plexus tumors with hydrocephalus. Currently, the expression patterns of AQP1 in hydrocephalus are not clear in the literature. Therefore, the results relating to the expression of AQP1 in hydrocephalus, or its contribution to the production of extra cellular fluid and to the regulation of intracranial pressure should be interpreted with caution, and used only as an indicator of the function of AQP1 in hydrocephalus.

On the other hand, a study by Amiry-Moghaddam and Ottersen (2003) detecting AQP1 and AQP4 in CSF determined that the mean concentration of AQP1 in CSF was significantly higher in patients with bacterial meningitis (BM). However, biphasic AQP1 expression in the ChP with increased AQPs 1 and 4 at the brain-fluid interfaces may indicate compensatory mechanisms to regulate choroidal cerebrospinal fluid secretion and increase parenchymal fluid absorption in high-pressure hydrocephalus (Paul et al., 2011; Wang et al., 2011).

A study on the AQP1 expression in the ChP in AQP1-deficient mice following kaolin- induced hydrocephalus the total choroidal AQP1 protein was not significantly altered in hydrocephalus, but the 50% of AQP1 protein was redistributed from the apical membrane to intracellular vesicles, and the ventricular size in AQP1-deficient mice was smaller than in wild-type mice, both at baseline and following hydrocephalus (Wang et al., 2011). The reduced plasma membrane AQP1 localization following kaolin-induced hydrocephalus, which involves endocytosis, may be a compensatory mechanism to reduce CSF secretion (Wang et al., 2011). Nonetheless, the results here showed that in control rats AQP1 was typically located in the apical pole of the choroid plexus cell, but that the AQP1 was increased and redistributed in both types of hydrocephalus rats regarding the control. The AQP1 redistribution corresponded to the change in the polarity locations; AQP1 was found in both poles of the ChP cells in hydrocephalic rats as occurs in the fetal period when the ChP function could be the production and the reabsorption of CSF. Therefore, this change of polarity of AQP1 in chronic hydrocephalus may take place so that AQP1 performs both the production and the reabsorption function to delay ventricular dilatation. One should bear in mind that the rats studied here were 30 weeks old and the hydrocephalus can be considered as being chronic, and perhaps, at this age, the compensatory or dampening effect of hydrocephalus has taken place.

Conclusion: taking into account that AQP1 performs a water channel function in the CSF production, in the CSF processes, and that chronic hydrocephalus could induce reabsorption of water to decrease CSF volume, it be can concluded that the AQP1 expression in communicating hydrocephalus is altered in the ChP, where a redistribution of its cellular localization is observed, and where it is expressed in both the apical and basal poles of the ChP cells and in perivascular spaces. The AQP1 immunohistochemical changes observed in the communicating and non-communicating hydrocephalus suggest a decrease in the efficacy of the ChP cells.

ACKNOWLEDGEMENTS

This study was supported by the research projects from: “Fundación Canaria Instituto de Investigacion y Ciencias de Puerto del Rosario” (INIPRO) Project no. 01/10 and “Universidad de la Laguna” (ULL) Project no. 2013/0001341.

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