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Identification of NKCC1 and Aquaporin 1 in Blood Vessels of Human Dura and Chronic Subdural Hematomas. A New Target for Bumetanide?

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Abstract

Chronic Subdural Hematoma (CSH) may be challenging to manage due to neurological complications and frequent recurrence. The pathophysiology of fluid collection in CSHs is incompletely understood and the role of water and cation cotransporters in the movement of fluid from dural capillary/venous complexes into the dural border zone and hematomas is unknown.

In the present study, normal dura was collected at autopsy from 9 fetal, 6 neonatal or infant, 11 adult and 16 aged patients. CSHs were collected from 15 surgical specimens. Na-K-2Cl cotransporter (NKCC1) and aquaporin 1 (AQP1) were evaluated using immunohistochemistry.

In the normal dura, NKCC1 immunoreactivity was extensive in neonatal, infant and adult capillaries. NKCC1 was not detected in arterioles and large venules in the fetal and adult dura or in unlined channels at any age. AQP1 was extensive in capillaries in all fetal, infant, aged and 82% of adult dural samples. In venules, AQP1 was extensive in most immature and adults. In CSHs, NKCC1 and AQP1 were extensive in capillaries and sinusoids in the outer dural membrane of each case and exhibited an overlapping distribution.

This study shows that NKCC1 and AQP1 are present on endothelial cells in the developing and adult dura and likely participate in fluid and ion movement from dural and hematoma capillaries into CSHs. Potential pharmacological interventions using NKCC1 inhibitors such as bumetanide and AQP1 inhibitors, along with other agents inhibiting angiogenic factors, should augment current management options and reduce the morbidity and mortality of CSHs.

Keywords: NKCC1; Aquaporin 1; Chronic subdural hematoma; Dura

Introduction

Chronic Subdural Hematomas (CSH) is commonly encountered in neurosurgical practice and may be challenging to manage due to neurological complications and frequent recurrence [1-3]. The pathogenesis of fluid collection in CSHs is incompletely understood [4-9]. Moreover, the role of water and cation cotransporters in dural capillary/venous networks management of interstitial fluid and cerebrospinal fluid is unknown. Capillary ingrowth from the dura to the outer membrane contributes to the formation of CSH [5,6,8,9] yet the function of these macrocapillaries in fluid accumulation is incompletely characterized. Leak of these capillaries in the outer membrane has been hypothesized as a mechanism contributing to the expansion of CSHs but the role of cation chloride channels has not been studied and investigation of aquaporin channels is preliminary.

Abnormalities in cation/chloride homeostasis have been attributed to the pathophysiology of cerebral edema, subdural hygromas and arachnoid cyst formation [10-15] and may contribute to fluid collection in CSHs. However the presence of the cation chloride channel neutral Na-K-2Cl cotransporter (NKCC1) and water channels such as aquaporin 1 (AQP1) have not been evaluated in normal dura or in CSHs.

NKCC1 is a membrane bound cation chloride cotransporter encoded by the SLC12 gene family [11,12]. It mediates chloride influx in several tissues including the brain. NKCC1 is located at the luminal surface and moves sodium and chloride into endothelial cells. In some physiological and disease processes, sodium/potassium ATPase pumps move sodium and associated chloride channels release chloride into the extracellular space. This is coupled with concomitant egress of water via various aquaporin channels from the endothelial cells into the extracellular space.

Aquaporins include a family of 19 water channels that allow the transfer of water and small molecules along the cell membrane. Of these, aquaporins 1, 4 and 9 have been identified in the mammalian brain. Aquaporin 1 (AQP1) has been found in choroid plexus epithelium and tumors of the choroid plexus. Aquaporin 4 is located primarily on astrocyte endfeet lining capillaries [16]. Recently, we have found AQP1 in the arachnoid, meningiomas and the dural sites of meningioma invasion [17].

In the present study we evaluated the expression of AQP1 and NKCC1 in the fetal, infant, adult and aged dura and compared this to expression in vascular ingrowth in CSHs.

Materials and Methods

Dural samples were collected postmortem from 9 fetal, 6 neonatal or infant (mean age=26 days), 11 adults (26-60, mean age=48 years), and 16 elderly (65 or older, mean age=75 years) autopsies performed between 2010 to 2012 from the University of Rochester Medical Center. These were mid-hemisphere parasagittal samples lateral to

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the superior sagittal sinus, fixed in formalin and paraffin embedded. Fifteen formalin-fixed paraffin embedded archival chronic subdural hematomas evacuated between 2002 and 2013 were also identified and studied (Table 1). Use of remnant excised tissue and autopsy material was approved by the University of Rochester Institutional Review Board with prior autopsy/surgical consent.

Immunohistochemistry

Each case was analyzed with a rabbit polyclonal antibody to human AQP1, (Abcam Inc., Cambridge, MA) and goat polyclonal antibody to NKCC1 (Santa Cruz, Santa Cruz CA) with MAC4 universal HRP-polymer (Biocare, Concord, CA) or Goat on Rodent HRP-polymer kit (Biocare) with Diaminobenzidene (DAB) chromagen and hematoxylin counterstain (Biocare). Antibody to CD31 (DAKO, Carpenteria CA) immunohistochemistry was used to characterize blood vessels. For antigen retrieval, tissue sections were incubated in a thermoresistant chamber with Reveal Decloaker (Biocare Medical, Concord CA) at 120-123°C and pressure of 20-24 psi. According to manufacturers specifications. Immunoreactivity in the cytoplasm was assessed and graded as 0 if there was no distinct immunoreactivity, 1+ if 1 to 25% of capillaries, arterioles and venules were immunoreactive, 2+ if over 25% were immunoreactive.

Results

NKCC1 and AQP1 in the developing and adult dura

NKCC1 was detected in endothelial cells in capillaries in all of the fetal, infant and adult dural samples and was 2+ in the majority of cases (Figure 1 and Table 2). In the aged dura, NKCC1 immunoreactivity was 1+ in the majority of capillaries and was not detected in 2 of 16 cases. NKCC1 was not detected in venules in the fetal and adult dura and was only detected in 17% of infant and 19% of aged dural samples. Definite NKCC1 was not seen in dural unlined channels (Table 2). Most samples had no or only a rare arteriole and consequently NKCC1 could not be assessed. NKCC1 immunoreactivity was extensive in trigeminal nerve twigs in a dural sample that contained nerve twigs.

As illustrated in (Figure 1) and summarized in (Table 2) , AQP1 was 2+ in capillaries in all of the fetal, infant and aged dural samples and was 2+ in 82% of adults. In venules, AQP1 was 2+ in all of the fetal and infant duras, 2+ in 55% of adult and 50% of aged duras but not in unlined vascular channels or larger diameter veins in the fetal, neonatal or aged dura. AQP1 was seen in endothelium in a rare adult arteriole but most samples had no or few arterioles. Significant AQP1 was not detected in arterioles and was not seen in dural fibroblasts.

	Age gender	Site	Clinical history
1	82F	Rt. hemisphere	na
2	82M	Convexity	na
3	67F	na	na
4	61 M	Lt hemisphere	na
5	69 F	Convexity	Shunt for NPH
6	79 F	Rt. hemisphere	Trauma
7	86 M	Convexity	No trauma
8	na	na	na
9	na	na	na
10	66 F	Rt. hemisphere	Trauma
11	na	na	na
12	87 M	Bilateral	Trauma
13	72 M	Lt. hemisphere	Trauma
14	67 M	Lt. hemisphere	Stroke, hemodyalysis
15	38 M	Rt. hemisphere	Trauma

na=not available, NPH=normal pressure hydrocephalus

Table 1: Clinical characteristics of chronic subdural hematomas.

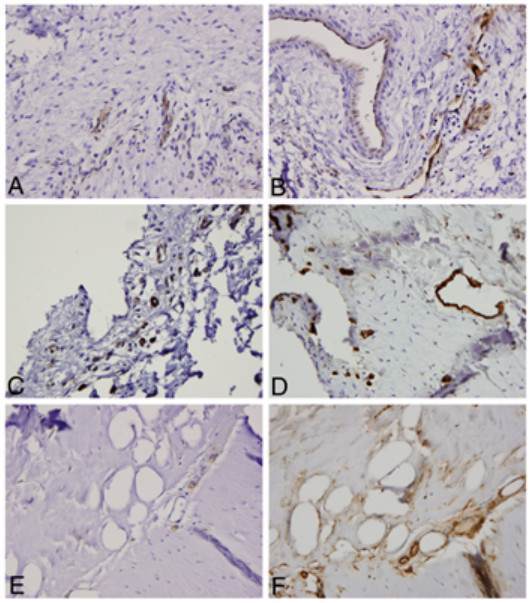

Figure 1: NKCC1 and AQ1 in developing and adult dura. (A) NKCC1 immunoreactivity (brown) in capillaries of a 21 wk fetal dura. (B) AQ1 immunoreactivity in capillaries of a 21 wk fetal dura. (C) NKCC1 immunoreactivity in capillaries of newborn dura. (D) AQ1 immunoreactivity in capillaries of newborn dura. (E) NKCC1 immunoreactivity in capillaries in 38 year old dura. (F) AQ1 immunoreactivity in capillaries of 38 year old's dura (Hematoxylin counterstain and diaminobenzidine chromagen; original magnification 400x).

	NKCC1 in capillaries	NKCC1 in venules	AQP1 in capillaries	AQP1 in venules
Fetal dura	1+ in 2/9	0	2+ 9/9	2+ in 9/9
	2+ in 7/9			
Infant dura	1+ in 1/6	0 in 5/6	2+ in 6/6	2+ in 6/6
	2+ in 5/6	1+ in 1/6		
Adult dura	1+ in 4/11	0 in 11/11	0 in 1/11	0 in 4/11
	2+ in 7/11		1+ in 1/11	1+ in 1/11
			2+ in 9/11	2+ in 6/11
Aged dura	0 in 2/16	0 in 13/16	2+ in 16/16	0 in 4/16
	1+ in 9/16	1+ in 3/16		1+ in 4/16
	2+ in 5/16			2+ in 8/16
Chronic subdural hematomas	1+ in 1/14	n.a.	1+ in 5/15	n.a.
	2+ in 14/15		2+ in 10/15	

n.a.=not applicable

Table 2: Summary of NKCC1 and AQP1 in normal dural blood vessels and chronic subdural hematoma capillaries.

NKCC1 and AQP1 in chronic subdural hematomas

NKCC1 immunoreactivity was extensive in endothelial cells in macrocapillaries, sinusoids and capillaries in the outer subdural membrane of each CSH (Table 1 and Figure 2). Extensive AQP1 was also found in endothelial cells in macrocapillaries and capillaries in the outer subdural membrane. The distribution was identical to the distribution of NKCC1. No difference was seen in the extent or distribution of the immunoreactivity in different patient ages or causes of the hematoma.

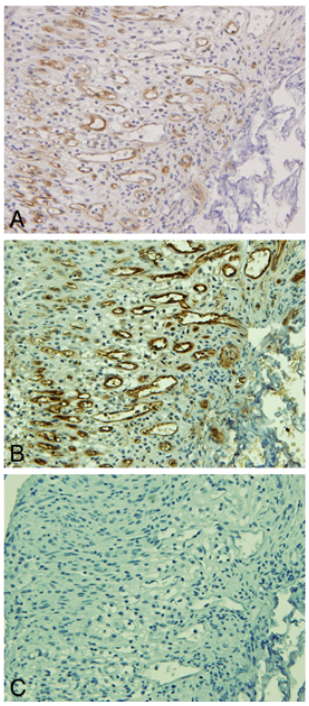

Figure 2: NKCC1 and AQ1 in chronic subdural hematomas. (A) NKCC1 immunoreactivity is extensive in capillaries and sinusoids infiltrating the chronic subdural hematoma. (B) AQ1 immunoreactivity in capillaries of the same subdural hematoma as in (A). (C) Control (Hematoxylin counterstain and diaminobenzidine chromagen; original magnification 400x).

Discussion

Expression of NKCC1 and AQP1 in endothelial cells of dural capillaries, arterioles and venules has not been described. The present study suggests widespread prevalence of these channels in vascular structures in the normal developing and adult dura. NKCC1 and AQP1 were also widely distributed in the neovascularization of the outer membrane of CSHs. These findings suggest that capillaries and sinusoids in CSH are functionally similar to those in the "normal" overlying dura at least in terms of NKCC1 and AQP1 expression. The formation of chronic subdural hematomas involves extensive ingrowth of these capillaries and sinusoids from the zone of dural border cells [18,19]. These capillaries/sinusoids are thought to be the source of hemorrhage and possibly fluid leakage [18]. Consequently, the widespread distribution of NKCC1 and AQP1 in endothelial cells in CSHs also raises the possibility that cation chloride and water channels may participate in cation, chloride and fluid movement from capillaries into the parenchyma of CSHs.

Current studies suggest the expansion of CSH is largely secondary to hemorrhage [1] and possibly increased fibrinolysis [5,20] along with production of angiogenic and/or inflammatory cytokines [21,22]. Movement of fluid into the CSH due to an osmotic gradient [19] created by protein from degenerating cells and macrophages has also been proposed although not established by animal studies. Nonetheless, fluid in CSHs is neither blood, pure serum nor cerebrospinal fluid [7,19]. Findings presented here raise the possibility that some expansion is due to water movement from capillaries via AQP1. Similar capillary leakage has been implicated in other forms of cerebral edema including epilepsy [12].

NKCC1 was widely distributed in capillaries in the immature and adult dura and capillaries and sinusoids in CSHs. In contrast, NKCC1 was less prevalent in capillaries in the dura in the elderly. These findings are consistent with neovascularization of the CSH and raise the possibility that, at least in the elderly, pharmacologic agents blocking cation chloride and aquaporin channels might have a differential effect on the CSH compared to normal tissue.

In many areas, the dura contains a complex venous and capillary network along with unlined fluid channels [13,23-25]. Arterioles are less prevalent and were not present in many of our samples and consequently could not be adequately evaluated. Nonetheless, in a rare sample with a prominent arteriole, AQP1 was detected suggesting it is also on endothelium of arterioles.

The extensive network of unlined channels in the dura may contribute to the development of subdural hygromas and hematomas [13,23-25]. However, their role in expansion of existing CSHs has yet to be established. In the present study they were not found to have NKCC1 or AQP1 channels but were found adjacent to or in proximity to numerous dural capillaries. Mack et al. [14] have hypothesized that the channels may contain cerebrospinal fluid and contribute to the formation of subdural hygromas and hematomas. The role of these channels, their functional association to capillaries feeding CSH and role as a conduit of fluid into "leaky" capillaries warrants further analysis.

Identification of NKCC1 on endothelium of feeding blood vessels and in CSHs has potential therapeutic implications since ion and fluid outflow from these channels might be blocked by NKCC1 inhibitors such as bumetanide. Bumetanide is a highly specific small molecule NKCC1 inhibitor. It is already approved by the United States Food and Drug Administration for treatment of epilepsy and also brain trauma associated with interstitial edema from capillary leakage [26-28]. Bumetanide is effective at low concentrations, shows complete penetrance of the blood brain barrier and has been shown to be safe in multiple clinical studies and current clinical experience [27,28]. Potential pharmacological interventions using bumetaninde and AQP1 inhibitors along with other agents inhibiting angiogenic factors may augment current management options (e.g. surgical intervention) and reduce the morbidity and mortality associated with CSH.

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