A new less invasive therapy for hydrocephalus using hepatocyte growth factor

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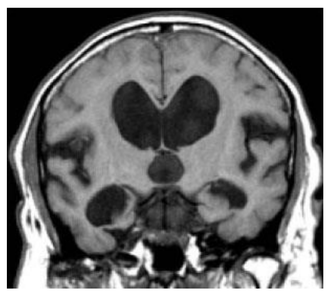
Background

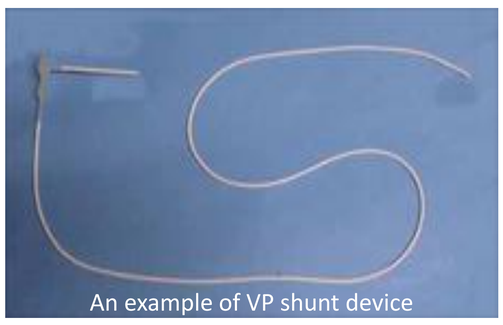
Clinical features of Normal Pressure Hydrocephalus (NPH)

1. Expanding ventricular dilatation following SAH or meningitis.
2. Incidence of idiopathic NPH is 1.4% in elder population.
3. No obstruction at the outlets of each ventricle.
4. It's called 'normal pressure hydrocephalus (NPH)', because the brain pressure is not always high.
5. Typical symptoms: urinary incontinence, gait disturbance, dementia.
6. Symptoms are recovered with ventriculo-peritoneal (VP) shunt.


T1 weighted MRI

Disadvantage of VP shunt

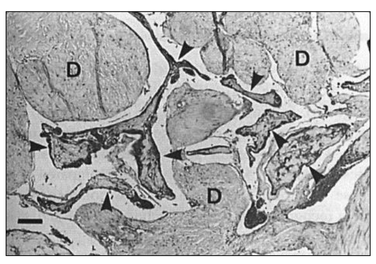
1. The artificial device must be implanted in the body permanently under general anesthesia.
2. It sometimes accompanies various complications; Infection, Obstruction, Disconnection, Over-drainage, Migration of tube, Relatively shortening of the peritoneal tube according to the body growth etc…
3. Patients need periodic medical care to prevent complications.


An example of VP shunt device

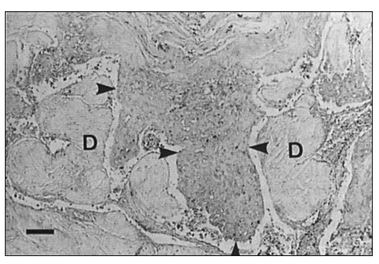
Histology of NPH

SAH induces proliferation of leptomeningeal cells and deposition of extracellular matrices in the arachnoid granulations and subarachnoid space. (Motohashi O, *1995*) Focal fibrosis accompanied by incomplete obliteration of subarachnoid space, suggesting a stasis of cerebrospinal fluid (CSF). (Akai K, *1987*) These observations indicate that the pressure gradient in the subarachnoid space would be important to generate ventricular dilatation.

Immunostaining for cytokeratin D means dura mater. Arrow head indicates leptomeningeal tissue.



Control



NPH

Elevated transforming growth factor ß1 (TGF-ß1) levels in CSF of NPH patients

SAH:

Platelets store lots of TGF-ß1, which is released at the ictus in CSF. We compared the TGF-ß1 levels in CSF after SAH between patient suffering hydrocephalus later or not. Graph shows time course of TGF-ß1 levels in CSF of patients with and without VP shunt following SAH. The TGF-ß1 levels of the VP shunt group were statistically higher than those of the group without VP shunt. (Kitazawa K & Tada T, 1994)

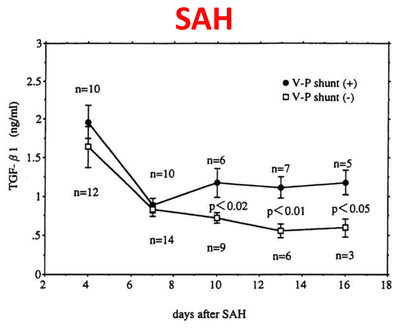
Meningitis:

TGF-ß1 levels in CSF were significantly higher in children with bacterial meningitis as opposed to those with aseptic meningitis*, or control subjects*. (Huang C, 1997)

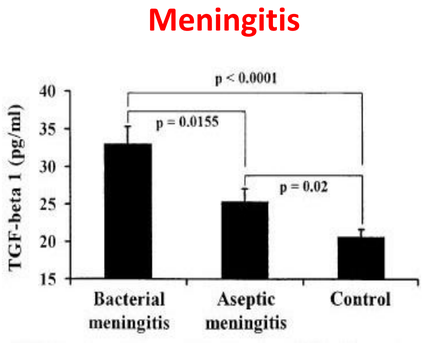
Idiopathic :

CSF samples from idiopathic NPH showed a highly significant increase in TGF-ß1. (Li X, 2007)

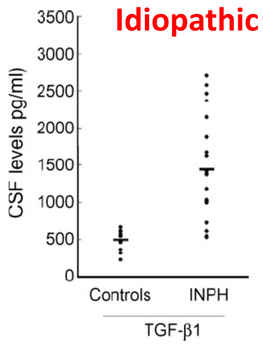
SAH



Meningitis



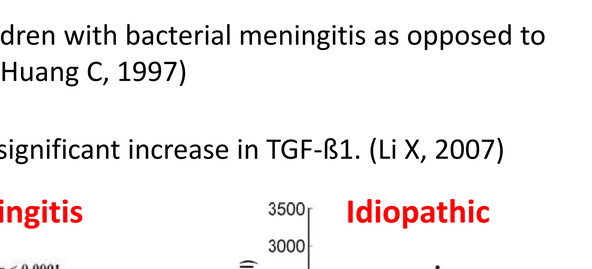
Idiopathic



Experiment 1

Inducing hydrocephalus in mice with human recombinant TGF-ß1

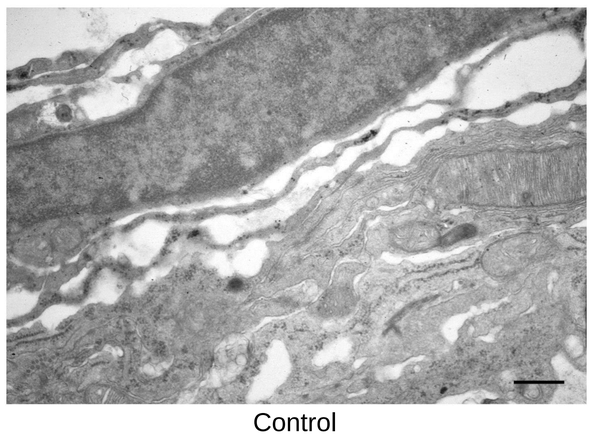
C57BL/6 pup mice were injected 600 ng of human recombinant (hr) TGF-ß1 into the subcranium under anesthesia. The ventricular system started to dilate within 3 weeks after the inoculation. Right photograph shows the ventricular dilatation on the coronal section of a TGF-ß1-injected mouse, in which hr TGF-ß1 had been injected 3 weeks earlier. Control mouse was injected with the solvent only.



Coronal section of mouse brain

Histology of mouse hydrocephalus

Proliferation of cells was observed in the meninges at 3 weeks after TGF-ß1 administration. The accumulated cells were positive for anti-fibroblast monoclonal antibody.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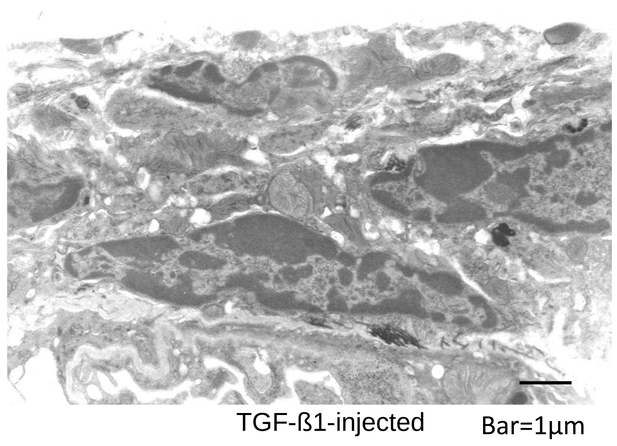

TGF-ß1-injected

Bar=1µm

Experiment 2

Treating mouse hydrocephalus with human recombinant hepatocyte growth factor (HGF)

Before Experiment 2, we prepared hydrocephalic mice, which were confirmed ventricular dilatation and disturbed spatial learning with MRI and Morris water maze. These hydrocephalic mice were administrated with 30µg of hr HGF for 1 or 2 weeks, or the solvent only, then their ventricular size and spatial memory were compared between them, again.

Ventricular size

The relative ventricular size (a/b) of hydrocephalic mice administrated hr HGF decreased from 10.7±2.5% before HGF administration to 7.4±2.1% at 4 weeks after the beginning of HGF administration.

Administrated Materials	n	Duration of infusion (Days)	Ventricular Rate of Coronal Section		P- value	Abnormal Fluid Collection
			Pre administration	Post administration*		
0.1% BSA-PBS	7	7	13.8 ± 5.0 %	13.8 ± 4.8 %	0.996	Subcutaneous fluid collection (3)**
Human recombinant HGF	5	7	18.5 ± 0.6 %	15.3 ± 2.9 %	0.085	(-)
Human recombinant HGF	5	14	10.7 ± 2.5 %	7.4 ± 2.1 %	0.013	(-)

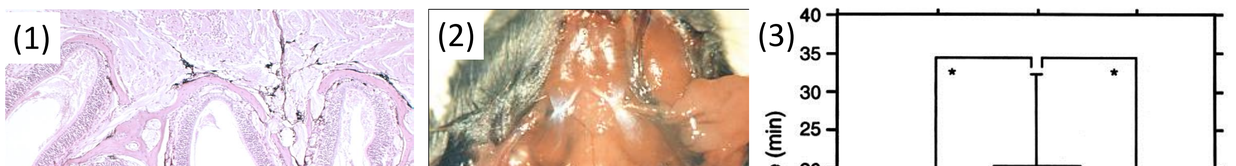
* : T2 weighted image of magnetic resonamce were taken after 4 weeks after the start of hr HGF administration

** : Subcutaneous fluid collection above the burr hole was found in 3 sham-operated hydrocephalic mice

Morris water maze

Quadrant A is the quadrant in which the platform was placed, and the remaining quadrants (B, C, D) were labeled in a clockwise fashion. The time the mice spent swimming in each quadrant was measured as the score.

a) The hydrocephalic mice receiving only the solvent continued to show disturbed spatial memory.
b) Hydrocephalic mice receiving 30 µg of hr HGF for a week, spent the majority of the time in the trained quadrant [p=0.03], which reflects recovery of spatial memory.



Ink passage time

Although the microstructure of human subarachnoid membrane is very similar to mouse lepto-meningeal layer, the CSF drainage route is completely different between them. While human CSF mainly drains to superior sagittal sinus through arachnoid granules, mouse CSF drains to nasal lymphatic ducts through olfactory groove (1). Using this difference, we examined the velocity of CSF flow in the leptomeningeal layer of hydrocephalic mice.

Black ink (10µl) was injected into the lateral ventricle of examined mice, after the deep cervical lymph nodes were exposed (2). The time from ink injection to positive lymph nodes staining were recorded as the ink passage time. It was significantly longer in the hydrocephalic mice receiving hr TGF-ß1. By contrast, the ink injection time had normalized in the hydrocephalic mice receiving a 7-day course of hr HGF (3).

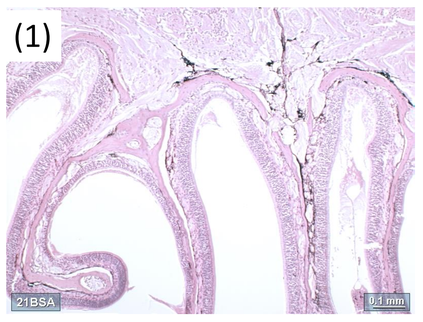
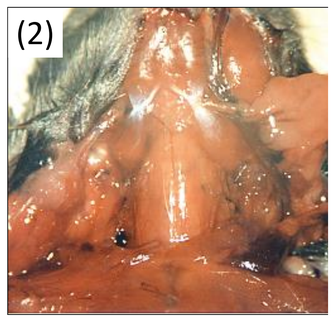
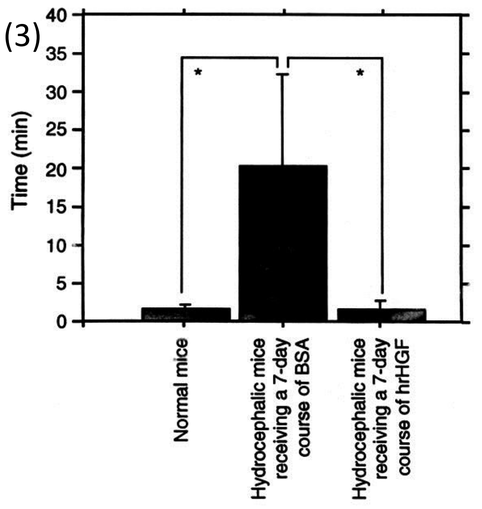


Photo indicating the channels from the basal intrameningeal CSF space to nasal lymphatics



Black ink indicating mouse lymphatic drainage route of CSF



Conclusion

1. Although VP shunt is only one method to treat NPH at present, the device must be implanted in the body permanently.
2. TGF-ß1 is a key factor generating NPH after SAH or meningitis, it's also elevated in CSF of idiopathic NPH.
3. Experimental hydrocephalus can be induced in mice with intraventricular administration of hr TGF-ß1.
4. Subarachnoid fibrosis is common feature between human and mouse hydrocephalus.
5. The symptoms of mouse hydrocephalus can be improved by intraventricular administration of hr HGF.
6. Hr HGF has a potential to become a new therapeutic material to treat NPH without VP shunt.