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Sequential ^{1}H MR Spectroscopy(MRS) Studies of Kaolin-Induced Hydrocephalic Cat Brain*

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= Abstract =

Kaolin 유발 고양이 수두증 모델에서 양자 자기공명 분광상의 경시적 변화

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Objectives : The aim of this study is to evaluate the sequential metabolic changes in experimental hydrocephalus and the clinical applicability to the diagnosis and prognosis of hydrocephalus using proton MR spectroscopy.

Methods : Hydrocephalus was experimentally induced in 30 cats(2-3kg body weight) by injecting 1ml of sterile kaolin suspension(250mg/ml) into the cisterna magna. Proton MRS was performed with a 1.5 T MRI/MRS unit (Vision Plus, Siemens) at pre-treatment and at 1, 3, 7, 14, 21, and 28 days after the kaolin injection. PRESS(TR/TE=1500/270msec) technique was employed. The major metabolites which include *N*-acetyl aspartate (NAA), creatine(Cr), choline(Cho), and lactate(Lac) were quantitatively analyzed and the relative concentrations ratios were evaluated. Multislice T_2-weighted images were also obtained using fast spin echo sequence(TR/TE= 2500/96msec) to monitor the morphologic changes along with progression of hydrocephalus.

Results : Hydrocephalus was successfully induced in all 30 cats. Twenty five cats died within 3 days and one at the end of the second week. In all animals, the NAA/Cr ratios initially decreased during the acute stage. In 4 surviving cats, the NAA/Cr ratios initially decreased during the acute stage(<14 days) and then gradually increased to the pre-kaolin level as follows : pre-kaolin(1.49 ± 0.04), day 1(1.11 ± 0.07), day 7(1.17 ± 0.04), day 14(1.40 ± 0.03), day 21 (1.46 ± 0.06), day 28(1.43 ± 0.03). These levels were relatively well correlated with the symptomatologic improvement. Lactate peak, which reflects the evidence of ischemia, did not appear throughout the entire period except in one case which expired at the end of the second week.

Conclusions : The NAA/Cr ratio of the sequential proton MRS in kaolin-induced hydrocephalic cats reflects a metabolic aspect of the hydrocephalus at each stage. A decreased NAA level at the early stage is from both neuronal and axonal damage which may provide diagnostic information in the acute stage of hydrocephalus. In addition, the initial fall of NAA/Cr ratio and recovery in the late stage, when no lactate peak emerges, may suggest that the main insult of the parenchyma is not to the neuron itself but to the axon, which may be related to a good prognosis. However, emergence of the lactate peak and unrecoverable NAA/Cr at the end of the acute phase may be a poor prognostic factor. In the chronic stage, recovery of NAA/Cr ratio may provide a diagnostic clue for the differentiation between hydrocephalus and cortical atrophy.

KEY WORDS : Experimental hydrocephalus · Proton MR spectroscopy · NAA/Cr ratio.

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Introduction

Hydrocephalus is a relatively common disorder that may be difficult to treat satisfactorily because of uncertainty in diagnosis and complexity of shunt placement. Hydrocephalus implies an alteration in cerebrospinal fluid dynamics as a cause of increased ventricular volume ; ventriculomegaly is a more general term that covers ventricular enlargement from whatever the cause, including atrophy. Diagnosis and management are primarily based on signs and symptoms of increased intracranial pressure in combination with the assessment of ventricular size. In clinical practice, however, enlarged ventricles are not always associated with elevated ICP and are not typical symptoms and signs of hydrocephalus. And there are some conditions associated with normal- or low-pressure ventricular enlargement including low-pressure ventricular dilatation, cerebral atrophy, aging process and normal pressure hydrocephalus.

Cerebral damage in hydrocephalus presumably is caused by a combination of direct mechanical effects due to compression of periventricular brain, and cerebral ischemia as a consequence of reduced cerebral perfusion pressure and periventricular change in microvasculature. Hydrocephalus results in the progressive functional impairment of neuronal systems, often before there is any evidence of morphological injury. Histologically, cerebral damage in hydrocephalus has been proven to occur mainly in periventricular white matter, and the gray matter is relatively unaffected by increased intracranial pressure. There is controversy, however, regarding where and to what extent cerebral blood flow and energy status are affected[4)8)].

Experimental hydrocephalus in animals has been a useful model to study both alterations in cerebrospinal fluid dynamics and impaired cerebral metabolism[11)13)]. One of the more reliable methods for the production of hydrocephalus has been the intracisternal injection of kaolin. The adjacent neuronal tissue or vasculature is not directly damaged by kaolin itself[3)8)].

Invasive methods of pressure monitoring carry the risk of intracranial bleeding and infection. Proton magnetic resonance spectroscopy(MRS) provides a non-invasive, potentially risk-free method with which to monitor an impaired energy metabolism and biochemical abnormalities of acute and chronic stages of disease.

We undertook the present study to determine the sequential metabolic changes in experimental hydrocephalus and the clinical applicability to the diagnosis and prognosis of each stage of hydrocephalus using proton MR spectroscopy at 1.5 tesla(T).

Materials and Methods

Hydrocephalus was experimentally induced in 30 sex unselected cats ranging in weight from 2 to 3kg. For induction of hydrocephalus, the kittens were anesthetized by intramuscular injection of ketamine(11 - 22mg/kg) and Rompun(Xylazine, 0.15mg/kg). The occipital scalp was shaved and soaked with potadine, and the animals were positioned with maximal head flexion. After the withdrawal of 1ml of CSF using 26-gauge scalp needle, 1ml of sterile kaolin suspension(250mg/ml) was injected into the cisterna magna during 30 to 45 minutes.

After induction of the anesthesia, as described above, ^{1}H MRS was performed with a 1.5 T MRI/MRS unit(Vision Plus, Siemens, Erlangen, Germany) at pre-treatment and at 1, 3, 7, 14, 21, and 28 days after the kaolin injection. PRESS (TR/TE=1500/270msec) technique with 1cm^{3} volume of interest was employed. The major metabolites, which include N-acetyl aspartate(NAA), creatine(Cr), choline(Cho), and lactate(Lac) were quantitatively analyzed and the relative concentration ratios were evaluated. Multislice T_2-weighted images also obtained using fast spin echo sequence (TR/TE=2500/96msec) to monitor the morphologic changes along with the progression of hydrocephalus.

During the first two weeks, animals were supplied with 5% D/S solution intravenously to prevent starvation and dehydration. The surviving cats were capable of eating spontaneously.

Results

Hydrocephalus was successfully induced in all 30 cats. Upon recovering from the anesthesia, the animals were capable of moving all extremities and remained in an upright position in their cage. The following day, the cats appeared apathetic and refused to eat. They were found lying on their sides in their cages, unable to stand, with spasticity of all extremities. Twenty-five cats died within 3 days and one died at the end of the second week. All cats surviving this period usually remained free of any sub-

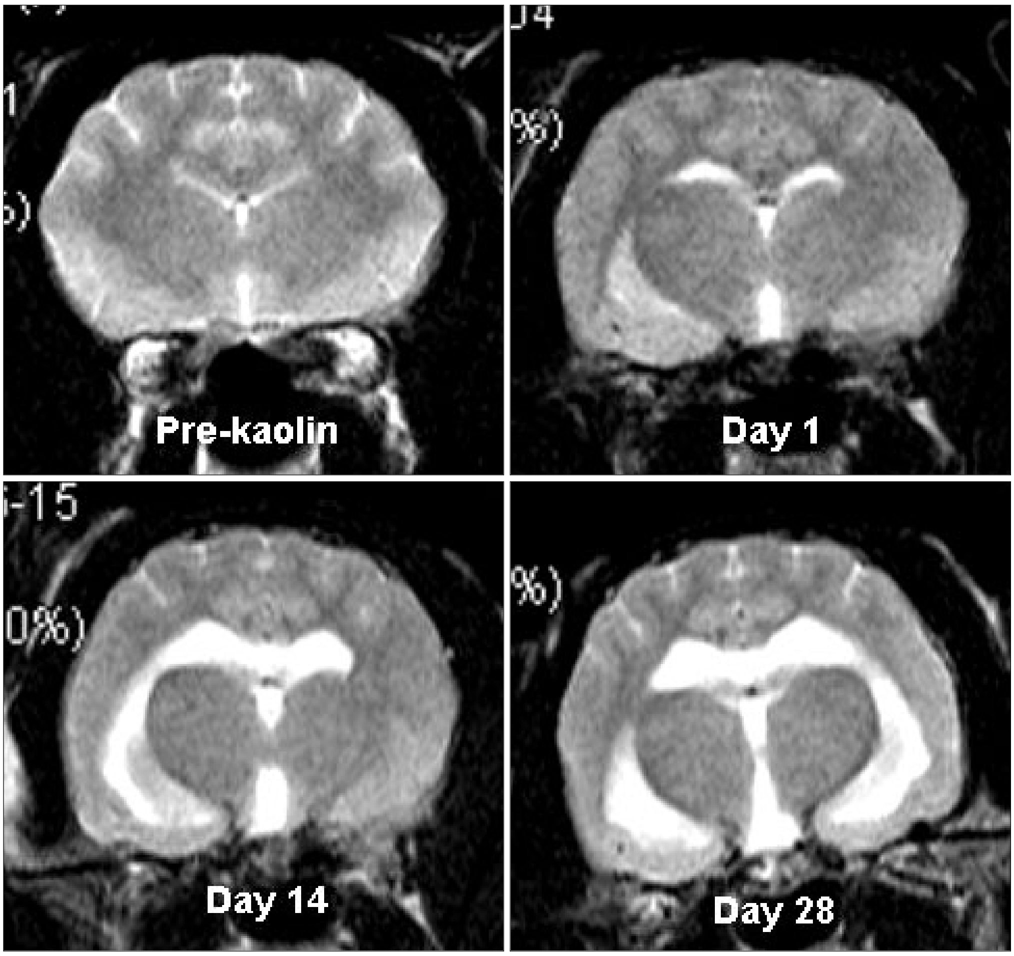

Fig. 1. Sequential changes on T_2-weighted images showing remarkable attenuation of the cerebral mantle, dilatation of the ventricles, and periventricular edema as time passes.

Table 1. NAA/Cr ratios in all surviving cats

	Pre-Tr	1day	3days	1wk	2wk	3wk	4wk
Cat 1	1.37	0.99	1.01	1.25	1.35	1.37	1.38
Cat 2	1.56	1.33	1.33	1.19	1.42	1.56	1.44
Cat 3	1.54	1.01	1.26	1.07	1.36	1.34	1.43
Cat 4	1.52	1.12	1.05	1.16	1.48	1.58	1.50
Mean	1.49±0.04	1.11±0.07	1.16±0.07	1.17±0.04	1.40±0.03	1.46±0.06	1.43±0.03

sequent gross neurological deficits. Symptoms gradually improved, and the 4 surviving cats fully recovered by the 14th day.

T_2-weighted MR imaging to analyze the temporal changes in kitten model of hydrocephalus showed that enlargement of the lateral ventricles occurred within 1 day of injection of kaolin and progressed for 2 weeks to severe ventriculomegaly, which is associated with thinning of the cerebral cortex(Fig. 1).

The NAA/Cr ratios in ^{1}H MRS of all cats decreased at the early stage. In all surviving cats, the NAA/Cr ratios initially decreased during the acute stage(<14 days) and then gradually increased to the pre-kaolin level afterwards as follows(Fig. 2, 3, Table 1) : pre-kaolin(1.49±0.04), day 1(1.11±0.07), day 3(1.16±0.07), day 7(1.17±0.04), day 14(1.40±0.03), day 21(1.46±0.06), and day 28(1.43±0.03). This data was relatively well correlated with the symptomatologic improvement. Lactate peak, which reflects the presence of ischemia, did not appear throughout the entire period except in one case which expired at the end of the second week(Fig. 2). The NAA/Cho ratios and Cho/Cr ratios were not constant, but fluctuated during the

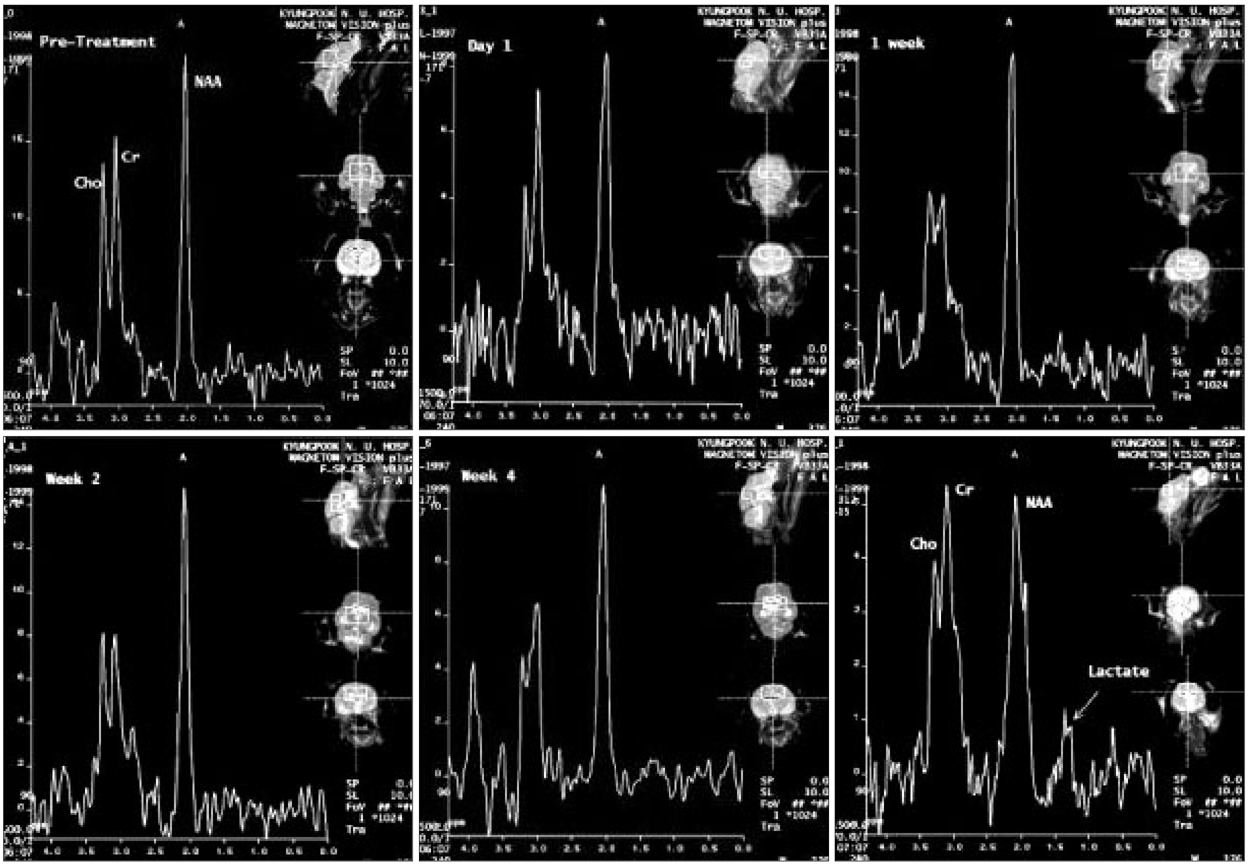

Fig. 2. The pictures are sequential ^{1}H MRS findings in which the voxel is positioned to the periventricular white matter. The last figure demmilrating the case with the lactate peak.

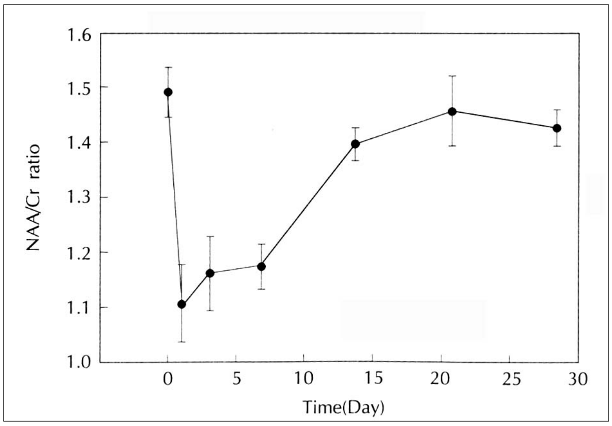

Fig. 3. Changes of NAA/Cr ratios in all surviving cats.

entire experimental period(Fig. 4, Table 2, 3).

Discussion

Hydrocephalus is excessive accumulation of CSF within the ventricles which may be associated with elevation in intracranial pressure. This is accompanied by enlargement of the ventricles, and of the head itself if calvarial sutures are open. Delayed diagnosis and treatment of progressive hydrocephalus may result in a variety of neurological deficits, including intellectual impairment, learning disabilities, epilepsy, developmental delay and poor visual acuity. These residual deficits suggest that the irreversible neuronal injury occurs during the hydrocephalic process. And there are many cases in which the hydrocephalus slowly progresses as the increase in resistance to CSF drainage is only slightly elevated beyond the degree that is physiologically tolerated by a given patient, these patients will not show the tell tale signs of acute hydrocephalus. In these cases, MRI would show enlargement of ventricles, but the T_2-weighted image would not show periventricular hyperintensity and indicates that the water content of the subependymal region is not detectably abnormal. CSF pressure above physiological levels may produce progressive parenchymal damage over a prolonged period of time[2].

Possible mechanisms of neuronal injury in hydroce-

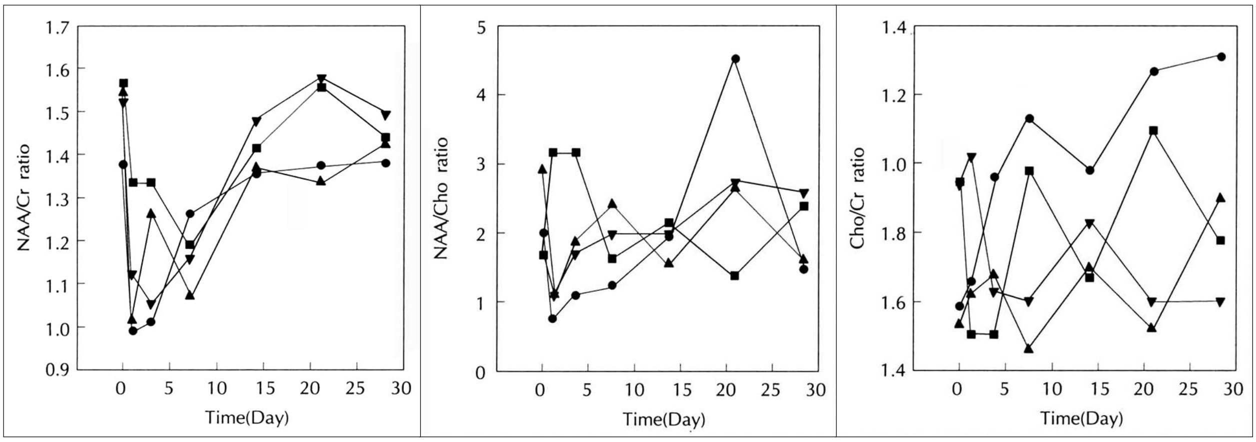

Fig. 4. Changes of metabolites ratios in all surviving cats.

Table 2. NAA/Cho ratios in all surviving cats

	Pre-Tr	1day	3days	1wk	2wk	3wk	4wk
Cat 1	1.99	0.68	1.06	1.23	1.95	4.45	1.45
Cat 2	1.68	3.15	3.14	1.58	2.11	1.36	2.32
Cat 3	2.94	1.09	1.88	2.37	1.51	2.59	1.61
Cat 4	1.67	1.09	1.67	1.97	1.96	2.64	2.53
Mean	2.07±0.30	1.50±0.56	1.94±0.44	1.79±0.25	1.88±0.13	2.76±0.41	1.98±0.26

Table 3. Cho/Cr ratios in all surviving cats

	Pre-Tr	1day	3days	1wk	2wk	3wk	4wk
Cat 1	0.58	0.65	0.95	1.12	0.97	1.28	1.32
Cat 2	0.93	0.49	0.49	0.97	0.67	1.10	0.77
Cat 3	0.52	0.62	0.67	0.45	0.70	0.51	0.89
Cat 4	0.92	1.02	0.62	0.59	0.83	0.59	0.59
Mean	0.74±0.10	0.70±0.11	0.68±0.10	0.78±0.16	0.79±0.07	0.87±0.19	0.89±0.12

phalus include direct compression, chronic ischemia, and metabolic derangement with anaerobic glycolysis. Obstruction of CSF outflow causes a decrease in brain compliance. Subsequent enlargement of the ventricles tears the ependyma and distorts and compresses the microvasculature. The fine aspiny or sparsely spiny dendrites of diffusely projected intrinsic interneurons may be most sensitive to mechanical distortion, and the axonal transport of neurotransmitters through thin and mostly unmyelinated axons may be easily affected[5)7)16)]. Axonal and secondary myelin damage occur through a combination of mechanical and ischemic effects. The tissue injury stimulates an astroglial reaction. The effect on cortex may be due to retrograde damage secondary to axonal injury. Direct damage may occur if ventriculomegaly is severe or if cerebral blood flow is significantly impaired. The cerebral blood flow in gray matter is in the normal range or only moderately reduced which reflects that ischemia may not have a prominent role in neuronal injury of the hydrocephalic brain[6)]. A combination of slow physical distortion and ischemia likely contributes to the axonal injury in hydrocephalus. Hydrocephalus results in the progressive functional impairment of neuronal systems, often before there is any evidence of morphological injury. Histologically, cerebral damage in hydrocephalus has been proven to occur mainly in the periventricular white matter, and the gray matter is relatively unaffected by increased intracranial pressure. There is controversy, however, regarding where and to what extent cerebral blood flow and energy status are affected[4)8)17)].

The model of kaolin-induced hydrocephalus was first used by Bering and Sato[1)]. Intracisternal injection of kaolin induces a marked fibrosis of the cisterna magna and basal cisterns, and obstruction of the outlets of the fourth ventricle[9]. The effects of intracisternal kaolin are apparent within 48 hours of the injection by mechanically occluding the foramina of the fourth ventricle, and the intraventricular pressure in cats may increase by as much as 10-fold. The intraventricular pressure increases during the acute phase of hydrocephalus, which is during the first few days after the kaolin injection. In acute hydrocephalus, the increase in intraventricular pressure is the result of the increased resistance to CSF absorption. Approximately 2 to 3 weeks after the kaolin injection, the intraventricular pressure of cats returned to within a normal range which is called chronic hydrocephalus. The return of intraventricular pressure to a normal range is associated with an increase in CSF absorption capacity[10].

In the literature review[10)11)13-15], we found many clinical articles pertaining to the flow study, but in terms of symptomatology, the parenchymal injury is our major concern. ^{1}H MRS is known to be a useful method to demonstrate the metabolic status of the brain parenchyma. Major metabolites detected were N-acetyl aspartate(NAA), a neuronal marker, creatine(Cr), which is bioenergetic metabolites, choline(Cho) that is released during membrane disruption, and lactate(Lac), which accumulates in response to tissue damage and associated anaerobic metabolism[12]. In our results of 30 cats, decreased NAA/Cr level at the early stage may be from both neuronal and axonal damage which may provide diagnostic information in the acute stage of hydrocephalus. The NAA/Cr recovery in all cats surviving the acute period may represent that an acute parenchymal insult is a transient deterioration of axon transportation mostly in the white matters apart from direct neuronal damage. We expect no permanent or irreversible damage to the neuron per se. But in one case the lactate peak did not recovered, which means permanent damage to the neurons caused ischemic injury. A decreased NAA/Cr phase without lactate peak in acute hydrocephalus and recovering NAA/Cr in the chronic stage may represent a transient condition of axonal damage, and a good recovery of the brain parenchyma. Emergence of lactate peak and unrecoverable NAA/Cr at the end of the acute phase may be a definitely poor prognostic factor. In the chronic stage, recovery of NAA/Cr ratio may provide a diagnostic clue for the differentiation between hydrocephalus and cortical atrophy in which the NAA/Cr ratio will fall.

In the field of MRS, it has long been accepted that the decreased NAA/Cr ratio means permanent neuronal damage with poor prognosis, however, in our study, we observed a recovery of decreased level of NAA/Cr which challenges previous belief.

We believe that early diagnosis and shunt installation prevent possible ongoing insult of permanent ischemic injury by compromised blood flow in compressed brain parenchyma. Therefore, early diagnosis and intervention of hydrocephalus is the very important.

We expected that the Cho/Cr ratio may increase during the hydrocephalic process due to demyelinization, but the data fluctuated, which mean further study is necessary.

Conclusion

The NAA/Cr ratio of the sequential proton MRS in kaolin-induced hydrocephalic cats reflects a metabolic aspect of the hydrocephalus at each stage. A decreased NAA/Cr level at the early stage is from both neuronal and axonal damage which may provide diagnostic information in the acute stage of hydrocephalus. In addition, initial fall of the NAA/Cr ratio and recovery in the late stage, when no lactate peak emerges, may suggest that the main insult of the parenchyma is not to the neuron itself but to the axon, which may be a good prognosis. Emergence of lactate peak and unrecoverable NAA/Cr level at the end of the acute phase may be a definite poor prognostic factor. In the chronic stage, recovery of NAA/Cr ratio may provide a diagnostic clue for the differentiation between hydrocephalus and cortical atrophy in which the NAA/Cr ratio will fall.

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Kaolin 유발 고양이 수두증 모델에서 양자 자기공명 분광상의 경시적 변화

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= 국문초록 =

목 적 :

실험적으로 유발된 고양이 수두증 모델을 통하여 양자 자기공명 분광상의 수두증에서의 임상적 적응과 경시적 뇌대사산물의 변화를 규명하는데 목적이 있다.

대상 및 방법 :

30마리의 고양이에서 뇌대조내에 kaolin을 주입후 수두증을 유발한후 양자 자기공명 분광상을 이용하여 kaolin 주입전, 주입후 1, 3, 7, 14, 21, 28일째에 주요 대사산물인 N-acetyl aspartate(NAA), creatine(Cr), choline (Cho), lactate(Lac)의 분광상을 분석하였다.

결 과 :

30마리의 고양이에서 모두 수두증이 유발되었고, 급성기에 NAA/Cr비가 감소하는 소견보였으며, 1마리에서 lactate peak이 관찰되었다. 만성기(>14 days)까지 생존한 4마리의 고양이에서 NAA/Cr비의 증가가 관찰되었으며 이는 임상적 증상의 회복과 상응하는 결과를 나타내었다.

결 론 :

Kaolin 유발 수두증모델에서 양자 자기공명 분광상은 급성기의 진단적 정보의 제공과 수두증에서의 뇌대사 및 생화학적 결과를 반영해 준다. 그리고, 급성기에서 lactate peak없이 NAA/Cr비의 감소와 만성기의 NAA/Cr비의 회복은 수두증으로인한 뇌실질의 손상이 신경세포 자체보다는 축색손상을 의미한다. 급성기말까지 초기 NAA/Cr비 감소가 회복없이 lactate peak이 동반되는 경우는 불량한 예후인자가 된다. 만성기의 NAA/Cr비의 정상화는 NAA/Cr비의 감소를 보이는 뇌피질위축의 경우와의 감별진단에 실마리를 제공한다.

중심 단어 : 실험적 수두증 · 양자 자기공명 분광상 · NAA/Cr비.