기관고유연구사업 최종보고서

연수막전이 환자에서 Methotrexate 뇌실요부 관류항암법의 효능에 관한 임상 제1상 연구

Ventriculo-lumbar perfusion therapy for the treatment of leptomeningeal carcinomatosis: a pilot study with pharmacokinetic data

과제책임자 : 신상훈

국 립 암 센 터

1. 이　보고서는　국립암센터　기관고유연구
사업 최종보고서입니다.

2. 이　보고서　내용을　인용할　때에는　반드시
국립암센터　연구사업　결과임을　밝혀야
합니다.

(14 pont, 고딕체)

↑
6cm↓

--- 측면 ---

↑
5cm
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막이에서
요관암의
실요관암의에한상상제1상연구

연수
전자
환서
Methotrexate
뇌부류법효능관임제1상연구

국립암센터

↑
3cm
↓

제 출 문

국립암센터 원장 귀하

이 보고서를 기관고유연구사업 "연수막전이 환자에서 Methotrexate 뇌실요부 관류항암법의 효능에 관한 임상 제1상 연구" 과제의 최종보고서로 제출합니다.

2009. 12. 31

국 립 암 센 터

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목   차

< 요 약 문 >

연구분야(코드)			과제번호	0910250-1 (NCCCTS-09-387)
과 제 명		연수막전이 환자에서 Methotrexate 뇌실요부 관류항암법의 효능에 관한 임상 제1상 연구 및 뇌척수액 생물학적 특성 조사 준비와 고안		
연구기간/연구비 (천원)		2009년 1월 15일 ~ 2009년 12월 31일/ 연구비 20,000 (천원)		
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색인단어	국문	뇌척수액, 연수막전이, 항암치료, 뇌실, 관류		
	영문	cerebrospinal fluid, leptomenigeal, seeding, methtexate, ventricle, perfusion,		

◆ 연구목표

<최종목표>
- 연수막 전이 환자 치료율을 50%미만에서 80%로 향상
- 연수막 전이 환자에서 뇌실-관류치료법 및 분자생물학 기반 특이항체 치료법 확립

<당해연도목표>
- 연수막 전이 환자에서 Methtrexate 뇌실요부 관류 항암치료법의 독성 및 부작용 평가하며 목표점 (end point)는 ① 뇌실요부 관류치료에서의 Methotrexate의 최대허용 농도 (maximal tolerable dose: MTD) 결정 ② 기타 합병증 발생여부 관찰로 한다.

- 연수막 전이 환자의 뇌척수액에서 특정 유전자 발현 및 단백질특성을 연구하기 위한 방법의 고안으로 ① 뇌척수액 샘플의 1차 처리 및 보관법 확립 ② 뇌척수액 샘플에서 해당 유전자 및 단백질 확인 가능여부 확인

◆ 연구내용 및 방법

- 연수막 전이 환자에서의 Ventriculo-Lumbar Perfusion(VLP) Therapy
 : 환자 선정 기준은 본문 참조바람. 연수막 전이 환자에게 뇌실내 주입관을 삽입하고 이를 chemoport에 연결해 우측 전두부에 피하 고정함. 뇌척수액 경로에 단락이 없는 것이 확인되면 Day 7-9의 3일간 인공뇌척수액을 뇌실에 일정 속도로 주입하고 이를 요추부 배액 (lumbar drainage) 시스템으로 관류시키며 12시간 간격으로 총 6회 mehtotrexate를 주입함.
- Methotrexate level을 0, 1, 2, 3, 6 & 24 시간 간격으로 측정
- 예상가능한 부작용, 신경독성의 list (본문 참조)를 작성하여 이의 정도에 따라 dose-limiting toxicities를 정의하여 최대허용농도 (maximal tolerable dose)를 구함. 이를 위해 4단계의 dose-scaling up table을 작성함.
- 중간 자체평가에서 dose-scaling up을 30 mg에서 중단하고 continuous infusion method개발 하여 시행함

-Measurement scale of VLP therapy result

  1) CSF : 1. cytology negative conversion, 2.. tumor marker level

  2) pre-Tx neurologic Sx (cranial nerve deficit, L/E weakness, increased ICP):

      normalized/ improved/ stationary/ worsen

  3) Karnofski Performance Scale (KPS) change

- Measurement of VLP-related side effects

  1) VLP side effects: pre-MTX perfusion시기의 side effects관찰

  2) MTX concentration related side effects: CSF MTX 농도와 연동하여 관찰

 -뇌척수액 샘플

  1) 원심분리 후 supernatant와 pellet 각각에 대해서 프로테인 농도 측정

  2) Supernatant는 Multiple Affinity Removal Column (MARC)을 이용하여 target protein fraction을 추출 한 후 1-D SDS-PAGE 후 MALDI/TOF analysis

  3) Pellet에서는 DNA를 추출하여 EGFR mutaion, k-RAS activation, Her-2 expression등을 polymerase chain reaction (PCR)로 관찰한다.

◆ 연구성과

-정량적 성과

구분	달성치/목표치[1]	달성도(%)
SCI 논문 편수	1	50% (draft완성)
IF 합	3.3	
기타 성과	특허출원	100%

-정성적 성과

1) 뇌실요부관류치료의 약역동학 data를 확보하였다 (AUC, CL, t1/2 etc.)

2) pre-MTX artificial CSF perfusion을 통하여 VLP 자체의 합병증인 nausea/vomiting, confusion 및 sleep disturbance을 구분할 수 있었다.

3) CSF MTX 농도 측정을 통하여 encepalopathy는 MTX의 농도에 비례하며 평균 300 uM 이상의 농도에서 발생하였다.

4) Intraventricular injection에서 MTX의 dose scale-up은 첫 번째 증량인 30 mg에서 3/5에서 DLT가 발생하여 종료하였다.

5) 총 24명의 환자에서 pre-treatement symptoms에 따른 치료율을 구하였다.

6) 뇌실요부관류치료를 통한 연수막전이 환자의 생존율 증가 가능성 확인 (median = 249 days)

7) 뇌실요부관류치료의 부작용과 원인에 대한 분석을 통하여 continuous infusion method 개발

8) 뇌실요부관류치료의 합병증에 대한 대책을 고안하였다 (low perfusion rate, preventive antibiotics with closed dressing)

◆ 참여연구원 (최종연도 참여인원)	성 명	이승훈, 유헌, 곽호신, 유병철, 정유진
	주민등록번호	

※ 요약문의 총분량은 2page 이내로 제한함

Project Summary

Title of Project	Ventriculo-lumbar perfusion therapy for the treatment of leptomeningeal carcinomatosis: a pilot study with pharmacokinetic data
Key Words	Chemoport, Lumbar drainage, Perfusion, Leptomeningeal carcinomatosis, Intraventricular chemotherapy
Project Leader	Sang Hoon Shin
Associated Company	

Introduction: Conventional intraventricular methotrexate (MTX) via Ommaya reservoirs prolonged the patient survival only marginally and failed to improve morbidity from leptomeningeal carcinomatosis (LMC). We adopted ventriculo-lumbar perfusion (VLP) therapy for the purpose of improving LMCsymptoms and obtaining pharmacokinetic data to set up a sophisticated protocol.

Material and Methods: Basic techniques of VLP therapy are thesame with previously reported. Briefly, pre-warmed artificial CSF is infused via pre-installed intraventricular subcutaneous reservoir. In the intraventricular injection protocol, methotrexate (MTX) of different doses (12, 20 and 30 mg) was injected to the ventricle on constant artificial CSF perfusion per 12 hours for 3 consecutive days. For continuous infusion protocol, 20 or 12 mg of MTX in 500 ml of artificial CSF was infused at 20 ml or 40 ml per hour, respectively. The MTX level from the lumbar drainage CSF was checked at various time intervals after the injectionwith daily serum MTX level for leucovorin rescue. The intracranial pressure (ICP) and LMS-related symptoms were monitoredthroughout and 2 weeks after the treatment. The side effects were assessed in terms of Common Terminology Criteria for Adverse Events (CTCAE, version 3.0).

Result: Twenty cytologically proven LMC patients who had increased ICP or LMC related symptoms were enrolled and 34 sessions of VLP therapy was applied. Among 26 incidence of pretreatment increased ICP (> 15 cm H2O), seventeen (65%) patients showed normalized ICP and improved in seven (27%). Among 11 patients had altered mentality from hydrocephalus, 6 (54%) showed improvement including one had normalized symptoms. Six patients (37%) out of 16 patients suffered from cauda equine involvement improved. All patients suffered from nausea/vomiting and sleep disturbance with VLP before MTX injection But grade 3 side effects occurred only in 7 (21%) and 12 (35%) patients, respectively and not happened in low (20 ml/hour) perfusion rate protocol. Eleven (32%) cases of confusion were noticed and six of them showed encephalopathy including delirium, whose peak MTX concentration > 300 uM.

Conclusion: The apparent VLP side effects appeared to be related to either the peak MTX concentration or the perfusion rate. The considerable rates of improvement of LMS-related symptoms encourage us to launch a sophisticated phase 1 clinical trial.

※ 연구목표, 연구방법, 연구성과를 영문으로 요약하여 2쪽이내의 분량으로 작성

1. 연구의 최종목표

- 연수막 전이 환자에서 Methtrexate 뇌실요부 관류 항암치료법의 독성 및 부작용 평가하며 목표점 (end point)는 ① 뇌실요부 관류치료에서의 Methotrexate의 최대 허용 농도 (maximal tolerable dose: MTD) 결정 ② 기타 합병증 발생여부 관찰로 한다.

- 연수막 전이 환자의 뇌척수액에서 특정 유전자 발현 및 단백질특성을 연구하기 위한 방법의 고안으로 ① 뇌척수액 샘플의 1차 처리 및 보관법 확립 ② 뇌척수액 샘플에서 해당 유전자 및 단백질 확인 가능여부 확인

2. 연구의 내용 및 결과

[1] Material and Methods

All patients were agreed to be enrolled to the experimental protocol, which was approved by Institutional Review Board of National Cancer Center Korea.

Patient population

From October, 2008 to August 2009, twenty cytologically proven leptomeningeal carcinomatosis (LMC) patients who had LMC-related symptoms were enrolled and 34 sessions of VLP therapy was applied. Twelve patients were male and eight patients were female. Mean age of the patients was 52 years-old (range 37-63). Seventeen patients had non-small cell lung cancer. The others were small cell lung cancer, ovarian cancer, and nasopharyngeal carcinoma, each. Eleven patients were unable to 'care for self' at the time of the installation (6 patients were of KPS 60 and 5 patients were KPS 50). Mean KPS was 63.5 (range 50 -80).

Eligibility criteria

Considering that this pilot study, strict proven CSF cytology was required (if clinically suspicious, repeat 3 times of CSF cytology at least 1 week interval before clarifying it negative). To be included in this protocol, patients must have one of two following conditions; 1) increase ICP measured at the reservoir is > 15 cm H2O regardless of ventricular enlargement on image, 2) Cauda equina symptoms other than mass symptom. To ensure the CSF flow on perfusion, wholespine MRI was performed in all patients before the VLP therapy. If any suspicious intradural metastasis obstructing CSF flow was detected, radioisotope citernography was performed. In case of CSF pathway block, the VLP therapy was deferred and patient was referred to radiation of involved segment. Following exclusion criteria was thoroughly examined; 1) brain space-occupying lesion, which could possibly cause herniation, 2) hemorrhagic metastases. 3) grade 3 leucopenia (WBC < 2,000 or ANC < 1000/mm^3)or thrombocytopenia (< 50 K), 4) more than stupor mentality that can disable to check the side effects of VLP therapy.

VLP installation

We used Chemoport for stable VLP instead of conventional Ommaya reservoir. Small size Celsite®, ST205, 6.5 French, (B. Braun, Boulogne Cedex, France) is used as a Chemoport, which was originally invented for intermittent or continuous venous infusions. Basic techniques of intraventricular Chemoport placement are the same with well established method of stereotactic Ommaya reservoir installation[1] except engraving of skull bone to settle down the Chemoport. In brief, a hockey-stick incision is centered at Kocher's point. After a burrhole trephination on Kocher's point, the outer layer of skull posterior to the burrhole was engraved in the shape of Chemoport to set the Chemoport down and reduce the height of whole device over the scalp closure. A ventricular catheter is inserted via planned trajectory and depth assisted by stereotactic system. After confirmation of ventricular access by CSF flow, the catheter is then connected to the reservoir and the reservoir is secured to periosteal apron. The VLP via Chemoport was delayed until at least 5 days after the installation to prevent possible wound dehiscence from the leakage of chemotherapeutic agent. The 21 gauge Hoover needle was inserted to the Chemoport chamber and maintained its position without any supportive device except closed dressing. Artificial CSF was connected to the plastic tube of Hoover needle and perfused via warming kit (37.5℃) at the designated rate after confirmation of steady CSF flow thorough lumbar drainage (Figure 1). The formula of artificial CSF is 2 ml of 7% $NaHCO_3$ and 0.5 ml of 50% glucose in 500 ml of lactated Ringer's solution[13].
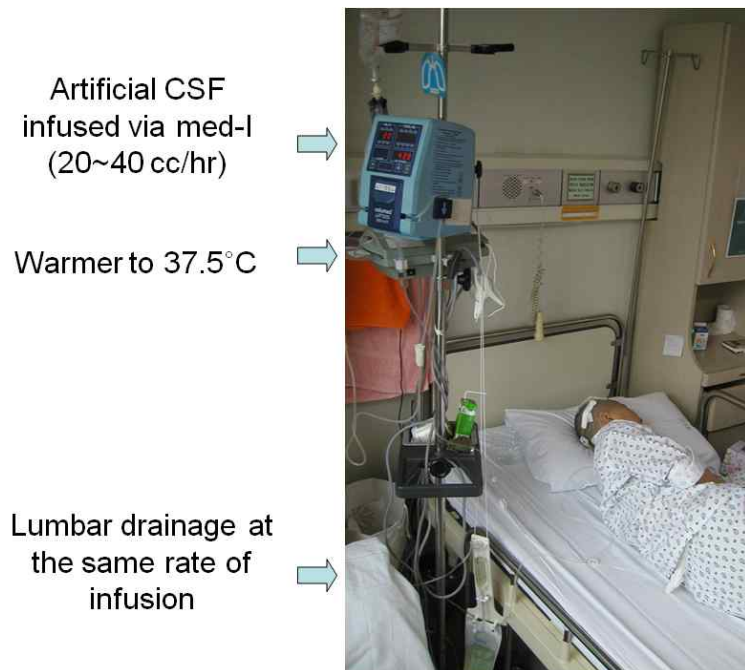

Fig. 1. Medical illustration depicting Ventriculo-lumbar perfusion therapy

Treatment protocol

Two different methotrexate infusion methods were adopted. In intraventricular side mode, artificial CSF was perfused overnight at the flow rate of 40 ml/hour before 20 mg of MTX injection q 12 hours for 3 days. The perfusion was maintained 12 hours after the last injection for clearing MTX. In patients showing more than grade 3 of nausea/vomiting or confusion, the flow rate of 20 ml per hour was

applied and MTX dose was reduced to 12 mg q 12 hours. Also, in this mode, MTX dose of 30 mg was tried to assess peak concentration of maximal tolerable dose. Another method was continuous infusion mode. For continuous infusion protocol, 24 mg of MTX in 500 ml of artificial CSF was infused at the different perfusion rates (20 ml or 40 ml per hour). The MTX level from the lumbar drainage CSF was checked at various time intervals after the injection with daily serum MTX level for leucovorin rescue. An anti-5HT3 receptor agent was given 1 hour before the injection to suppress chemotherapy-induced nausea and vomiting. The protocol was applied once more under IRB control if the patients still have inclusion criteria LMC symptoms. The scheme of the protocol is illustrated in figure 2.
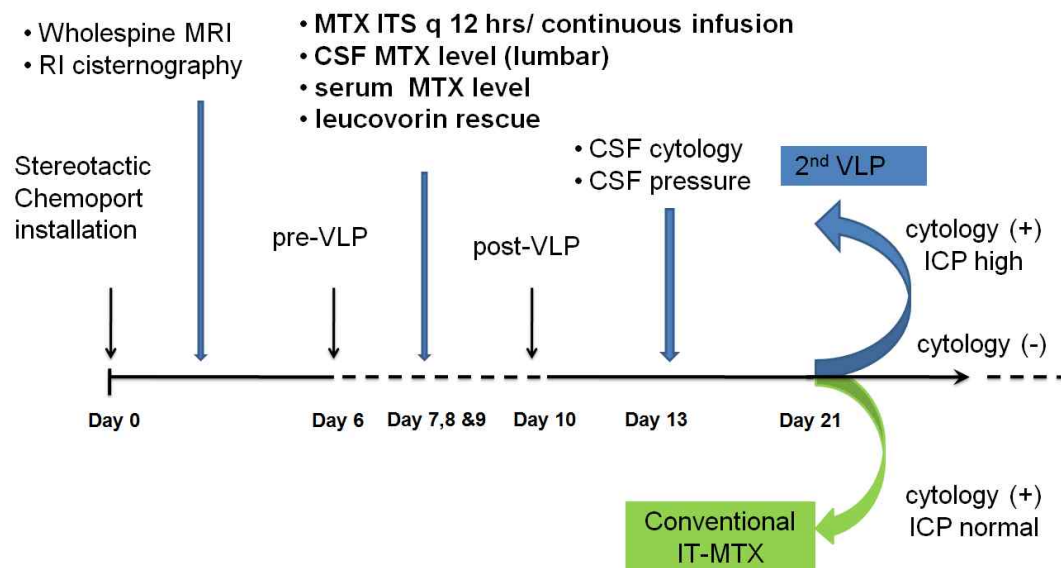

Fig. 2. Schematic illustration of ventriculo-lumbar perfusion therapy schedule

Protocol regulation

The initial pilot study was opened for 40 ml per hour and 20mg of MTX q 12 hours protocol as the same method of Nakagawa et al. to gather pharmacokinetic data of up to 10 patients. After acquire of preliminary data, the phase I clinical study of different mode of injection and perfusion rates was planned as the end point of more than 30% occurrence of CTCAE grade 3 or more side effects (as more than 2 occurrences in 6 patients).

Response evaluation

 Post-treatment ICP were evaluated on 3 days after the cessation of VLP to avoid immediate effect of VLP on CSF flow. The CSF cytology, pre-treatment symptoms and signs were checked at 10 days after the therapy (2 weeks after the start of VLP).

Toxicity evaluation

The side effects from VLP were closely monitored and checked prospectively according to the Case

Report Form. The lists of expected side effects were sleep disturbance, nausea, vomiting, confusion, seizure, speech impairment and encephalopathy including delirium and agitation. The side effects were assessed in terms of Common Terminology Criteria for Adverse Events (CTCAE, version 3.0) [18] and counted as an occurrence when the symptoms were the same or more than grade 3

Statistical methods

The incidence of side effects and the rate of clinical symptom improvement were also verified their significance using Fisher's exact test and the peak MTX concentration between the groups were compared by Student t-test using SPSS software (version 12.0, Chicago,IL).

[2] Results

LMC related symptoms and signs

Pre-treatment LMC symptoms and signs were checked at the entry of VLP therapy and the response was summarized in Table 1. Increased ICP was the most common LMC symptoms as seventeen (85%) out of twenty patients suffered from. Eleven patients got normalized ICP after the 1st VLP trial. Four patients showed improvement (>5cm H_2O drop of ICP) and became normalized after the 2nd therapy. The other two patients had no improvement after the 1st VLP and dropped from the protocol. However, 2 patients who once got normalized ICP experienced re-bout of ICP and had ventriculo-peritoneal shunt in 2 and 3 months later, respectively. Thus, the actuarial control rate of ICP was 88% (15 out of 17 patients) at the end of the VLP therapy and 76% in terms of long-term observation. Nine patients suffered from altered mentality during the course of therapy. Four patients presented with disorientation and difficulty in communication at the 1st VLP therapy. One of these patients got orientation with fully communicable mentality after the therapy. The second patient worsened symptom. The other two patients showed improvement in terms of disorientation and but not normalized in spite of the 2nd therapy. Five patients revealed altered mentality at the entry of 2nd VLP therapy. Two patients showed remarkable improvement as they could recognized family and tell patient's own name but remained not fully communicable. The other 3 patients showed no discernible improvement. In counts of patients, one (11%) showed normalization of altered mentality, 4 (44%) patients improved, 3 patients remained stable and one got worsened. Among patients complained of walking or voiding difficulty, thirteen patients were proven to have involvement of cauda equine by both neurologic examination and electromyography. One (11%) patient became free of urinary catheterization and could walk without assistance after the 1st VLP therapy. Five (38%) patients showed improved gait and urinary control but still needed intermittent urinary catheterization at the end of therapy. Four patients remained stable and the other four patients got worsened and resulted in paraplegia at the time of response evaluation.

In counts of total 34 VLP therapy trials, there was no significant difference of improving rate according to the mode of therapy or the figure was too small to be statistically examined their significance.

Table 1. Clinical results of ventriculo-lumbar perfusion therapy according to the mode of injection and the infusion rate.

Pre-treatment symptoms Results	Intraventricular injection q 12 hours (N=24)	Continuous infusion (N=10)	Total (N=34)
Increased ICP	19	7	26
normalized	13	4	17(65%)
improved	4	3	7(27%)
stable	2		2(8%)
worsen	-		-
Altered mentality	6	5	11
normalized	-	1	1(9%)
improved	3	2	5(45%)
stable	2	2	4(36%)
worsen	1	0	1(9%)
Cauda equina symptom	11	5	16
normalized	1	-	1(6%)
improved	4	1	5(31%)
stable	2	3	5(31%)
worsen	4	1	5(31%)

* Detailed mode of administration is described in Materials and Method section
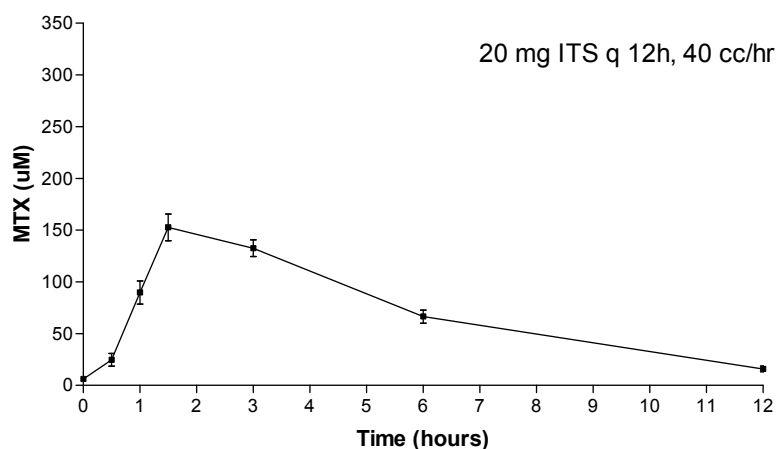
Pharmacokinetic data

To obtain the distribution of MTX through the subarachnoid space, CSF samples from lumbar drainage were collected at various time intervals. For the intraventricular injection protocol, the MTX concentration was checked at 0, 0.5, 1, 1.5, 3, 6 and 12 hours after the MTX injection. In continuous infusion, the MTX concentration was checked at 0, 1, 3, 6, 9 and 12 hours at the first day of infusion and checked daily until 24 hours after the MTX infusion. In the intraventricular injection protocol of 40 ml per hour perfusion, the peak CSF concentration was observed at 1.7 hours and 2.3 hours after the injection at 20 mg and 30 mg injection respectively, while the peak was delayed to 3.0 hours at 20 ml per hour perfusion rate (Table 2). The peak CSF concentrations and the area under the CSF concentration curve (AUC_{inf})were in proportion to the MTX dose given. The peak concentrations were 248.7 $\pm$ 137.5 uM at 20 mg of MTX and 355.2 $\pm$ 180.4 uM at 30 mg of MTX in 40 ml per hour perfusion (Fig 3. A and B) and 114.5 $\pm$ 43.5 uM at 12 mg of MTX in 20 ml per hours protocol (Fig 3. C). The calculated AUC_{inf} were 842.5 $\pm$ 243.5 umole*h/L at 20 mg of MTX and 1284.6$\pm$ 87.4 5 umole*h/L at 30 mg of MTX in 40 ml per hour perfusion and 643.1 $\pm$ 54.9 5 umole*h/L at 12 mg of MTX in 20 ml per hours protocol (Table 2).

At 12 hours after intraventricular injection, the MTX was cleared to mean 15.9 uM and 11.8 uM in 20

mg and 30 mg of MTX of 40 ml per hour perfusion, respectively and 14.3 uM in 12 mg of MTX of 20 ml per hour perfusion. Estimated clearance of MTX (CL, L/hour) is nearly the same in 40 ml per hour perfusion protocol as 0.057 ± 0.018 for 20 mg of MTX and 0.052 ± 0.3 in 30 mg of MTX. In 20 ml per hour perfusion protocol, clearance was reduced to 0.041 ± 0.004. The clearance rates in term of elimination half-life (t1/2) were 2.0 and 2.7 hour in 20 mg and 30 mg of 40 ml per hour perfusion and that was 2.6 hour in 20 ml per hour perfusion.

In continuous infusion protocol, MTX level reached steady state after 9 hours of the infusion (Fig. 4). The steady-state CSF concentration was as 49.6 ± 7.1 uM in 0.96 mg/hour (24 mg of MTX in 500 ml at 20 ml per hour infusion) and 50.8 ± 7.1 uM at 1.92 mg/hour (20 mg of MTX in 500 ml at 40 ml per hour infusion) protocol (Table 2). Estimated CL was 0.043 ± 0.006 for 0.96 mg/ hour 20 ml perfusion and 0.078 ± 0.013 for 1.92 mg/ hour 40 ml perfusion protocol.
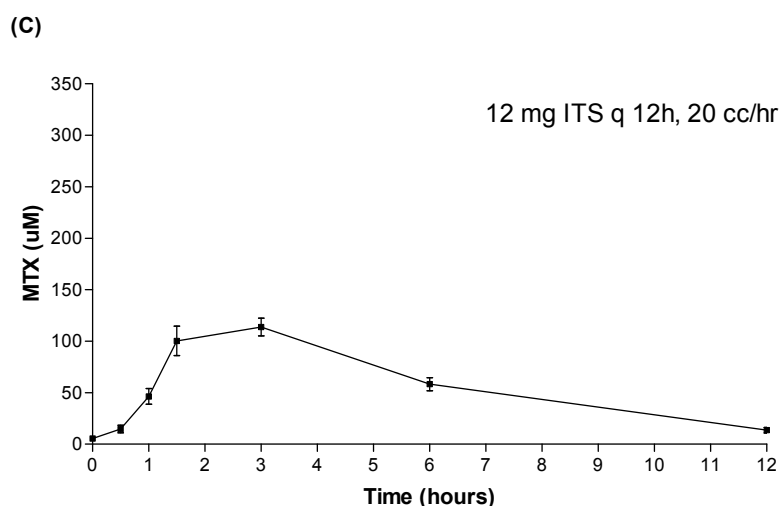
(A)

(B)

(C)
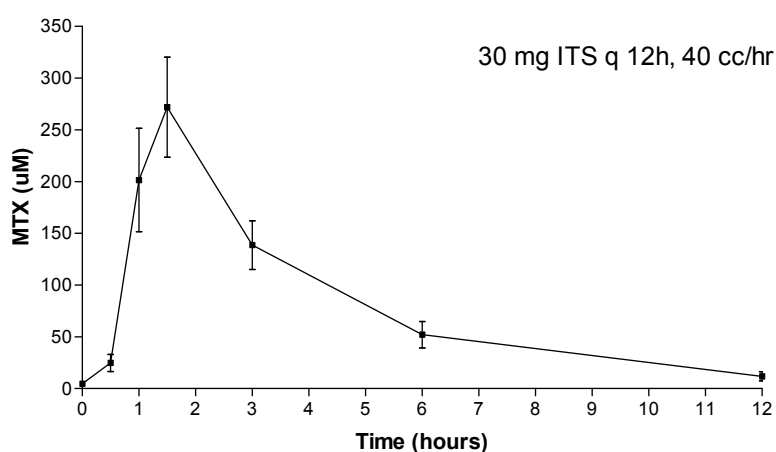
Fig. 3. CSF pharmacokinetic data of Ventriculo-lumbar perfusion therapy of intraventricular shooting of methotrexate per 12 hours. Dose schedule are 40 ml/hour with 20 mg (A) and 30 mg (B)), and 20 ml/ hour with 12 mg of methotrexate (C). *Each bar represents standard error means (SEM) of observed concentrations.

Table 2. Pharmacokinetic results of ventriculo-lumbar perfusion therapy in intraventricular injection mode with different doses and infusion rates

MTX dose	Perfusion rate	AUC_{inf}(umole *h/L)	C_{max} (umole/L)	T_{max}*(hour)	CL (L/h)	Vd_{ss}(L)	$t_{1/2}$(hour)
12 mg	20 cc/hr	643.1±54.9	114.5±43.5	3.0 (1.5~6.0)	0.041±0.004	0.21±0.08	2.6±0.7
20 mg	40 cc/hr	842.5±243.5	248.7±137.5	1.7 (1.0~3.0)	0.057±0.018	0.21±0.10	2.0±0.9
30 mg	40 cc/hr	1284.6±87.4	355.2±180.4	2.3 (1.5~3.0)	0.052±0.3	0.23±0.14	2.7±1.5

*T_{max} value indicates the median (range) and the other values indicate mean± standard deviation.
Abbreviations: AUC_{inf}, area under the CSF concentration curve until infinite; C_{max}, peak CSF concentration; CL,clearance; C_{ss}, steady-state plasma concentration; T_{max},time to peak CSF concentration; $t_{1/2}$, terminal elimination half-life; Vd_{ss},volume of distribution at steady state
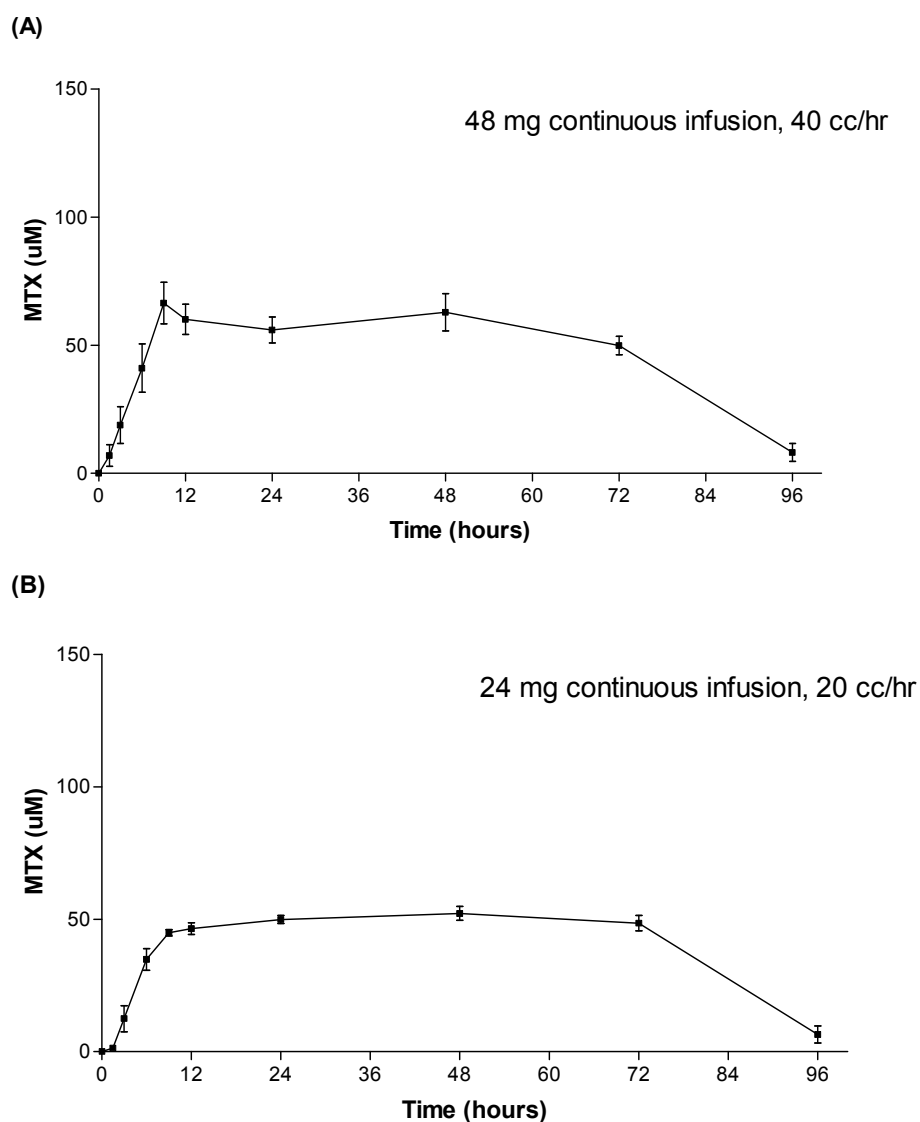
(A)

(B)

Fig. 4. CSF pharmacokinetic data of Ventriculo-lumbar perfusion at 40 ml/hour (A) and 20 ml/hour (B) with continuous infusion of 24 mg of methotrexate in 500 ml of distilled water

Table 3. Pharmacokinetic results of ventriculo-lumbar perfusion therapy in continuous infusion mode with different doses and infusion rates

MTX dose	Perfusion rate	C_{ss}(umole/L)	CL (L/h)
0.96 mg/hour	20 cc/hr	49.6±7.1	0.043±0.006
1.92 mg/hour	40 cc/hr	50.8±7.1	0.078±0.013

Abbreviations; CL, clearance; C_{ss}, steady-state plasma concentration

VLP related side effects

All the side effects observed at each VLP trials according to the different protocols were summarized in Table 4. We could observe VLP-related symptoms in patients with intraventricular side protocol as they got pre- and post-MTX perfusion for 12 hours. All patients suffered from a varying degree of sleep disturbance and nausea/ vomiting. However, more than grade 3 severity side effects occurred as twelve (35%) incidence of serious sleep disturbance, 10 (29%) cases of nausea and vomiting and 11 (32%) cases of confusion was observed only in 40 ml per hour (high flow) protocol. Among 24 of high flow protocol patients, the incidence of side effects was 50% for sleep disturbance, 42% for nausea and vomiting and 46% for confusion. All these side effects are temporary and got normalized within a few days after the therapy. In contrast, these thought-to-be "VLP-related" side effects occurred only as mild as grade 1 or 2, which neither require specific treatment nor disturb average daily life in the 10 events of 20 ml per hour (low flow) protocol. Speech impairment that disabled the patient for communication occurred only in 5 (15%) cases of 40 ml per hour perfusion protocol, in two of which patients couldn't speak at all. Delirium, hallucination and agitation were recorded as a manifestation of encephalopathy, which was observed in 7 (21%) cases. These side effects didn't happen in low flow intraventricular side and continuous infusion protocols (Table 2). The peak MTX concentration of these occurrences were mean 334 uM ($\pm$ 64, standard error means) which was significantly higher than those who didn't show these side effects (172 $\pm$ 21 uM) (p = 0.004, t-test).

No mucositis occurred except moderate diarrhea in one patient with leucovorin rescue administration when serum MTX level exceeded 0.15 uM. Three patients (15%) got CSF infection by gram positive cocci during the course of therapy. One patient revealed CSF leukocytosis and fever at the 2nd day and the other two patients at the 3rd day. The therapy was immediately stopped upon the report gram positive cocci in the CSF sample and patients underwent removal of the reservoir for infection control. All 3 patients had extraventricular drainage during the course of antibiotics therapy because they have relied on EVD of their ICP control before the entry into the protocol. As stated, we didn't use prophylactic antibiotics but applied clean closed dressing for EVD and the VLP therapy. They all cleaned of CSF infection and needed the intraventricular reservoir revision for ICP control or further therapeutic option.

Table 4. The observed side effects of ventriculo-lumbar perfusion therapy according to the mode of injection and the infusion rate.

	Intraventricular shot q 12 hours			Continuous infusion		Total
MTX dose	30mg	20 mg	12 mg	0.96 mg/hr	1.92 mg/hr	(N=34)
Side effects	(N=4)	(N=14)	(N=6)	(N=6)	(N=4)	
Sleep disturbance	[a]3	[a]5	-	[a]4	-	12(35%)
Nausea/Vomiting	[a]3	3	-	1	-	7(21%)
Confusion	[a]3	[a]5	-	[a]3	-	11(32%)
Seizure	-	-	1	-	-	1(3%)
Speech impairment	[a]2	1	-	2	-	5(15%)
Encephalopathy	[a]2	4	-	-	-	6(18%)

* Side effects are referenced to CTCAE ver 3.0 and are counted as occurrence when the side effects were of or more than grade 3.

¶ : The side effects that occurred more than 1/3 of patients of each group.

Overall survival

Nine patients were died during the observation period and thus, 60% of patients were censored and the data is not appropriate for survival analysis. However, apparent median overall survival (from the diagnosis of LMC) of all 20 patients was 10.0 months (249 days).

3. 연구결과 고찰 및 결론

Problems in intrathecal chemotherapy for LMC

The concept of chemotherapeutic agent delivery directly to ventricle was brought by Rubin et al. in the context of reducing the absorption of the drug into systemic blood compartment by the virtue of blood-brain barrier while achieve effective concentration to control brain tumor or CNS leukemia[24]. The intraventricular chemotherapy for LMC has been the most effective single modality, which prolongs patient's survival, among therapeutic modalities including radiation and systemic chemotherapy. However, the prognosis is still poor about 6 months and in spite of improvement of drug delivery via subcutaneous intraventricular reservoir, the development of late neurotoxicity hinders intensive drug administration [6,8,19,23,33]. The reasons for treatment failure are ineffective distribution of drug to target space and significant neurotoxicity, which increase in proportion to the number of intrathecal chemotherapy. Although there is no randomized study about the relationship between the number of intrathecal therapy and delayed neurotoxicity, it has been constantly suggested that MTX may accumulated in brain and cause late neurotoxicities[4,5]. The relevance of intraventricular chemotherapy was once denied by two randomized studies as they concluded that the addition of intra-CSF chemotherapy increase the neurotoxicity while the survival benefit remained the same with systemic chemotherapy plus radiation control group[6,7]. However, it was well-known that combined radiation to systemic or intraventricular MTX remarkably increased leukoencepalopathy up to 30% in LMC patients and also in lymphoma patients[4,13,22]. Thus, the former 2 studies didn't represent the risk of intraventricular chemotherapy for neurotoxicity as a single therapeutic modality. The author's insisting additional risk of neurotoxicity from combining radiation suggested the prior radiation might disturb normal CSF flow, by which the MTX clearance preventing brain accumulation was ensured. The concept of reducing CSF drug concentration to avoid neurotoxicity was tried by Blyer et al. in 1978[5]. They randomly applied two different regimen of intrathecal MTX to leukemic patients. One was conventional 12 mg/m^2 twice weekly schedule and the other was 1mg every 12hours for 3 days.The lumbar CSF sampling revealed the mean cumulative doses of 173 ± 64 mg/ m^2 for conventional one and 66 ± 41 mg/m^2 for so-called "concentration x time" schedule. The neurotoxicity occurred in 7 of 10 patients in the 12mg/m^2 group and one of 8 patients in the "concentration x time" group while the remission rates were remained the same. And even more, the maintenance time of effective CSF concentration (>5 x 10^{-7}M) was more prolonged in "concentration x time" regimen up to 72 hours than conventional one, which the concentration felt below the guideline within 48 hours. In their patients, cranial irradiation prior to MTX showed no difference to the incidence of neurotoxicity.

High dose intravenous MTX was suggested as one of effective treatment modality for LMC patients theoretically reducing neurotoxicity from large volume of distribution and rapid clearance. Based on the pharmacokinetic data, Tetef et al. insisted that the target CSF MTX concentration (> 1 uM) could be achieved safely by high-dose intravenous MTX at a dose of 1-7.5 g/m2 for 24 hours infusion[30]. However, the clinical result of their scheme in LMC patients was not followed and the duration of maintaining effective concentration is short to intraventricular drug delivery.

The neurologic response rate of LMC by intrathecal chemotherapy was less than 30%, although improved results has been reported in combination with radiation therapy to the involved field[15,19,23,26,31]. Failure of neurologic treatment is attributed to limited number of intrathecal therapy and ineffective drug distribution to subarachnoid space. According to the pharmcokinetic data of intrathecally injected drugs and CSF flow between compartments, VLP therapy can provide more effective concentration of intrathecally-injected drug to suabarachnoid space in addition to reducing the neurotoxicity by increasing drug clearance[2,3]. Nakagawa et al., who introduce the VLP using artificial CSF, expected the delivery of effective MTX concentration to lumbar subarachnoid space via enforced CSF perfusion. Although they didn't measure the local CSF MTX concentration, they could achieve improved neurologic response rate of 50% as 3 out of 6 bedridden patients became ambulatory[21]. In our study, the response rate of cauda equina symptoms was 38% as 6 out of 16 patients became ambulatory or free of indwelling urinary catheter.

The importance of CSF flow in the intraventricular treatment

Radioisotope CSF flow studies revealed that 40-70% of LMC patients have CSF flow disturbances with or without hydrocephalus[9,17] and the figures were higher than those reported as clinical incidence of hydrocephalus in LMC patients. Grossman et al. suggested that these CSF flow disturbances may be the one of reasons for treatment failure and treatment-related neurotoxicity. In detail, their survival data revealed that among 3 types of CSF flow abnormalities, type I, ventricular outlet obstruction posed the worst survival of median 28 days against either other abnormalities or normal findings. In this type of abnormality, the injected chemotherapeutic agent didn't distribute to the subarachnoid space, where the drug supposed to act, and caused neurotoxicity from transependymal penetration into the brain parenchyma.

In our series, most of patients with increased ICP could not undergo conventional outpatient-based intraventricular chemotherapy due to intractable headache, nausea and vomiting. And as in the illustrative case 1, intraventricular injection to these CSF flow-disrupted patients frequently causes acute encephalopathy and the MTX remained in the ventricle could cause delayed neurotoxicity.

Nakagwa et al. described that their VLP protocol caused so frequent side effects that further study for analyzing the causes and re-structuring the protocol to reduce the unwanted complications[21]. The most frequent side effects were nausea and vomiting as 12 of 13 patients suffered at their perfusion rate of 50 – 60 ml per hour. In our study, it was obvious that these side effects were caused by enforced CSF perfusion itself and to be related to the perfusion rate. Although, all patients complained nausea sense within hours of beginning the perfusion, serious vomiting, which necessitate parenteral fluid administration, occurred not in 20 ml per hour perfusion but only in 40 ml per hour perfusion group.

And nausea/ vomiting and confusion accompanying sleep disturbance started to be observed from occurred overnight pre-perfusion before MTX intraventricular injection. Thus, we could postulate the rapid enforced CSF perfusion caused these side effects. The incidence of abnormal CSF flow rate in disease such as Alzheimer dementia and normal pressure hydrocephalus was reported[16,20]. However, the effect of enforced CSF flow on neurological functions has yet to be found. In our intraventricular injection protocol, over-night pretreatment perfusion was performed to ensure steady CSF perfusion flow. Thus, we could observe the pure effects of artificial CSF perfusion. All patients experienced mild to moderate sleep disturbance and nausea sense during this period and the confusion and vomiting were only observed in 40 ml per hour perfusion group.

In our study, one of remarkable therapeutic effect is improvement of increased ICP as seventeen patients showed normalization and seven patients improved more than 5 cm H_2O. The increased ICP from disturbed CSF flow has been indicated one of unfavorable prognostic factors by many authors[11,14,17,28]. Wasserstrom et al. reported the improvement of increased ICP by direct measurement of ICP (> 160 mm H_2O) and 15 out of 64 (23%) increased ICP was improved after radiation plus intraventricular chemotherapy[33]. The re-establishment rates of disturbed CSF flow in radioisotope studies were higher than clinical response rate. Grossman reported the improved CSF flow in 4 out of 11 patients with intraventricular therapy plus radiation to involved neuraxis[17]. Chamberlain et al. reported the re-establishment of CSF flow of 50% by wholebrain irradiation and 40% by spinal involved field radiation[9].

Suggestions from our study

The importance of CSF flow on the intraventricular-injected drug distribution was emphasized by many in vivo models. Blasberg et al. performed sophisticated experimental study by gathering in vivo data of CSF methotrexate concentration from different mode of administration and computing the distribution of the drug using compartment model[3]. The diffusion of intraventricular injected drugs largely depends on CSF bulk flow between the compartments. Diffusion itself does not contribute significantly because the diffusion distances between ventricular and lumbar compartments are too long to be reached by diffusion itself.

In their model, calculated elimination half-life ($t_{1/2}$) in normal CSF flow was 6~8 hours. We measured the half-life of 2.0 - 2.7 hours, which are three to four times increased elimination rate. MRI phase study calculated the normal CSF production rate of 0.3 ~ 0.47 ml/min, which is equal to 18 ~ 28 ml/hour and the same value with already known CSF bulk clearance of 17 ~ 22 ml/ hour[20,27]. Our perfusion rate of 20 ml per hour looked apparently the same value of normal CSF flow rate. However, the rate is not the real CSF bulk flow but a rate of drainage. As we could see the calculated CSF clearance (CL) in intraventricular injection protocol, the CL of 20 ml per hour (4l ml per hour) is double and those of 40 ml per hour (57 and 52 ml per hour) is three times of normal CSF clearance. Thus, we could say our method of VLP effectively increased the CSF clearance of methotrexate. Although our results of improved clearance have not been proved to be effective for reducing neurotoxicity, theoretical benefit is obvious.

The pharmacokinetic benefit of intraventricular injection over intravenous administration came from the longer duration of effective concentration and the little systemic side effects. To prolong the duration of 'above-the-effective concentration'', the methotrexate should be infused in a continuous 24 hours mode.

Intraventricular injection thorough subcutaneous reservoir provides a convenient and reliable route for CSF drug delivery. However, this injection cause unwanted high peak intraventricular methotrexate concentration, which could penetrate into the brain, and uneven distribution in case of CSF flow disruption. The theoretical benefit of continuous intraventricular infusion of methotrexate to achieve steady-state was proved in primate model (*Macaca mulatta*) by Balis et al[2]. They directly sampled the CSF from 4th ventricle, basal cistern and lumbar sac via installed reservoir. What they observed is CSF methotrexate was continuously transported out thorough meninges and only 20 – 25% of injected methotrexate reached the lumbar sac. Thus, continuous intraventricular infusion could provide more stable CSF concentration than intraventricular injection and achieve more effective steady-state concentration (Css) than intravenous infusion with absolutely small amount of the drug. Our study is the first report of in vivo observation of Css of intraventricular continuous infusion method.

4. 연구성과 및 목표달성도

(1) 연구성과
가. 국내 및 국제 전문학술지 논문 게재 및 신청

논문명	저자 (저자구분[1])	저널명(I.F.)	Year; Vol(No):Page	구분[2]	지원과제번호[3]
Chemoport as a more durable and accessiblereplacement of Ommaya reservoir for the treatment of leptomeningeal carcinomatosis	곽호신외 7명	draft 회람중			
Ventriculo-lumbar perfusion therapy for the treatment of leptomeningeal carcinomatosis: a pilot study with pharmacokinetic data	곽호신외 7명	draft 회람중			

1) 저자구분 : 교신, 제1, 공동
2) 구분 : 국내, 국내 SCI, 국내 SCIE, 국외, 국외SCI, 국외SCIE 등
3) 지원과제번호(Acknowledgement)

나. 국내 및 국제 학술대회 논문 발표

논문명	저자	학술대회명	지역[1]	지원과제번호
Ventriculo-lumbar perfusion therapy for leptomeningeal seeding: a pilot study with pharmacokinetic data	곽호신 외 6명	제19차 대한뇌종양학회 정기학술 대회	대전	
Ventriculo-lumbar perfusion therapy for the treatment of leptomeningeal carcinomatosis: a pilot study with pharmacokinetic data	곽호신 외 7명	Society for Neuro-Oncollgy	New Orleans, USA	

1) 지역 : 국내, 국외

다. 산업재산권

구분[1]	특허명	출원인	출원국	출원번호
발명특허	Burrhole type chemoport		대한민국	

1) 구분 : 발명특허, 실용신안, 의장등록 등

라. 저 서

저서명	저자	발행기관(발행국, 도시)	쪽수	Chapter 제목, 쪽수 (공저일 경우)

마. 연구성과의 정부정책 기여

보고서명	정부정책	기여내용

바. 기타연구성과

(2) 목표달성도

가. 연구목표의 달성도

최종목표	연차별목표	달성내용	달성도(%)	
			연차	최종
- 연수막 전이 환자 치료율을 50%미만에서 80%로 향상 - 연수막 전이 환자에서 뇌실-관류치료법 및 분자생물학 기반 특이항체 치료법 확립	- 연수막 전이 환자에서 Methtrexate 뇌실요부 관류 항암치료법의 독성 및 부작용 평가하며 목표점 (end point)는 ① 뇌실요부관류치료에서의 Methotrexate의 최대허용 농도 (maximal tolerable dose: MTD) 결정 ② 기타 합병증 발생여부 관찰로 한다. - 연수막 전이 환자의 뇌척수액에서 특정 유전자 발현 및 단백질특성을 연구하기 위한 방법의 고안	- 뇌실요부관류치료의 약역동학 data 확보 - 뇌실요부관류치료를 통한 연수막 전이 증상의 치료율 획득 - 뇌실요부관류치료를 통한 연수막 전이 환자의 생존율 증가 가능성 확인 - 뇌실요부관류치료의 부작용과 원인에 대한 분석을 통하여 continuous infusion method 개발 - 뇌실요부관류치료의 합병증에 대한 대책마련 (low perfusion rate, preventive antibiotics with closed dressing)	80%	30%

나. 평가의 착안점에 따른 목표달성도에 대한 자체평가

평가의 착안점	자 체 평 가
Side Effects of Intrathecal MTX and dose-limiting toxicities	- pre-MTX artificial CSF perfusion을 통하여 VLP 자체의 합병증인 nausea/vomiting, confusion 및 sleep disturbance을 구분할 수 있었다. - CSF MTX 농도 측정을 통하여 encepalopathy는 MTX의 농도에 비례하며 평균 300 uM 이상의 농도에서 발생하였다.
Dose escalation	- intraventricular injection에서 MTX의 dose scale-up은 첫 번째 증량인 30 mg에서 3/5에서 DLT가 발생하여 종료하였다
Measurement of VLP therapy result	총 24명의 환자에서 pre-treatement symptoms에 따른 치료율을 구하였다.

5. 연구결과의 활용계획

(1) 연구종료 2년후 예상 연구성과

구　　분	건　　수	비　　　　　고
학술지 논문 게재	2	- Chemoport를 LMS에 이용한 논문: J Neurosurg (IF: 2.6) - VLP pilot study 논문: J Clin Oncol (IF: 9.6)
산업재산권 등록	1	- VLP의 continuous infusion method: 한국, 미국, 일본 등
기　　타		

(2) 연구성과의 활용계획

- 연수막 전이환자에서 뇌실요추관류 치료의 안전 perfusion rate와 MTX 농도가 얻어졌으며 이를 바탕으로 기존의 intraventricular injection method와 연구자가 고안하여 개발한 continuous infusion method의 randomized prospective phase I study가 필요함.

- 뇌실요추관류에서의 pharmacokinetic data가 얻어졌으므로 methotrexate외에 cytosine arabinoside (Ara-C) 및 hydrocortisone 병합 치료에 대한 pilot study 가 필요함

- 최근의 연구동향에 맞추어 연수막전이 된 암세포에 대한 분자생물학적인 연구를 바탕으로 Tarceva나 Herceptin등 molecular targeted drug 또는 antibody를 이용한 관류치료에 대한 in vitro data확보가 요망됨.

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