Cryptococcal meningitis (CM), caused by Cryptococcus neoformans or Cryptococcus gattii varieties, is a life-threatening opportunistic fungal infection in both human immunodeficiency virus (HIV)-positive and HIV-negative patients. The key management principles include therapy for CM with antifungal agents, as well as control of the underlying disease (15). The challenges in the treatment of CM correlate with its long course of disease, high relapse rate, and unsatisfactory therapeutic efficacy. The 3-month mortality rate during management of acute CM is approximately 20% despite access to advanced medical care (3). Moreover, the advent of the azoles has not led to cure. Emphasis was placed on the combined use of antifungal agents such as polyenes, fluconazole in new guidelines for the management of CM (15, 21). Amphotericin B (AMB), a major member of the polyenes, remains a first-line option for empirical therapy in the treatment of CM (15). However, its poor penetration into cerebrospinal fluid (CSF) limits its effect against CM (14). Many clinicians prefer intrathecal AMB as a part of the CM treatment regimen in order to clear infection more completely and rapidly. Conventional intrathecal AMB might increase the drug concentration in CSF and improve therapeutic efficacy, but the toxicities associated with AMB given intrathecally have limited its clinical applications (9, 18). Taking account of this, it is necessary to investigate new therapeutic options in the treatment of CM.

We previously reported a new therapy, continuous intrathecal amphotericin B (AMB), for the treatment of cryptococcal meningitis, which had fewer side effects and complications than conventional intrathecal AMB. In this study, the pharmacokinetics of continuous intrathecal administration and conventional intrathecal AMB were compared in rabbits, providing a pharmacokinetic basis for the use of continuous intrathecal AMB therapy. The AMB concentration in the cerebrospinal fluid (CSF), sampled via an inserted cisterna magna catheter, was determined by a liquid chromatography-tandem mass spectrometry assay. The results revealed significant pharmacokinetic differences between the two groups. In the continuous intrathecal group (0.15 mg/kg/24 h), the concentration of AMB peaked 7.01 µg/ml at 4 h and then decreased to a stable level of 1.0 to 1.34 µg/ml, with no neurological impairments, while in the conventional intrathecal group (0.015 mg/kg), the drug concentration reached a peak of 3.41 µg/ml at 1 h and then decreased progressively, with fever and neurological impairments, including convulsion and paralysis. The pharmacokinetic results indicated that the continuous intrathecal AMB is a more effective and safe therapy than the conventional intrathecal AMB, with comparatively rational pharmacokinetics and fewer neurological impairments.

MATERIALS AND METHODS

Animals. New Zealand White rabbits (weight, 2.0 to 2.5 kg) were obtained from the Laboratory Animal Center of Southern Medical University and maintained under clean conditions. All animals had access to appropriate food and water. On the day of surgery, animals were anesthetized with an intravenous injection of pentobarbital sodium (30 mg/kg). The study was carried out according to the ethical guidelines of the International Association for the Study of Pain (26) and was approved by the local animal research ethics committee.

Intrathecal catheterization. We produced a new efficient method of intrathecal catheterization via the coccygeal vertebra for continuous intrathecal administration without spinal cord damage. A 20-cm-long catheter was prepared before implantation. It was made of two parts glued together, a distal PE-10 part (15 cm) and a proximal PE-50 part (1.5 cm) (Fig. 1). The catheter can be closely connected with the needle of the microsyringe (250 µl) that was used for drug administration. The dead space of the catheter lumen is about 10 µl.

Under sterile conditions, a 4-cm longitudinal skin incision was made between the first and the second coccygeal spinous processes. Muscles were detached; the fascia and the underlying dura were sequentially removed; and the arachnoid membrane at the Cy1-Cy2 coccygeal vertebra, the prepared catheter was inserted about 15 cm into the subarachnoid space at the level of the L1 lumbar vertebra. The correct intrathecal localization was confirmed by backflow of CSF. Subsequently, the catheter was fixed by sutures with the surrounding muscles. The incision was then carefully sutured, and the outer end of the catheter was blocked by a removable nylon stylet. All animals were checked the next day after intrathecal catheterization to

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find if there were any adverse drug reactions and neurological abnormalities such as fever, weight loss, convulsion, or paralysis.

**Methylene blue intrathecal injection.** To confirm that the catheter was properly inserted into the spinal subarachnoid space, dissection was performed after methylene blue infusion via the inserted catheter the next day after catheterization. Five rabbits underwent the continuous infusion of methylene blue solution (1%, 100 μl/kg) for 4 h with an infusion pump via the inserted catheter and then were perfused via the aorta with 10 U/ml heparin saline and subsequently with 10% paraformaldehyde in phosphate-buffered saline (PBS). The distribution of the dye in the spinal cord and brain was assessed by autopsy.

**SEM.** To verify the catheter position exactly, morphological observations were performed with scanning electron microscopy (SEM) in five rabbits the next day after catheterization. The rabbits were perfused via the aorta with 10 U/ml heparin saline and subsequently with 10% paraformaldehyde in PBS. A smooth resection surface of the tissue was made by using a rotating shaft equipped with special high-frequency knives. SEM was performed on dissected specimens of the vertebrae, with the spinal cords and catheter remaining in position according to established procedures (23).

**Cisterna magna catheterization.** A method of CSF sampling via an inserted cisterna magna catheter through the atlanto-occipital membrane was adapted from that described by Huang et al. in 1996 (8). Ten rabbits were operated for cisterna magna catheter insertion after intrathecal catheterization and then individually housed for AMB administration the next day after the operation.

**Intrathecal administration and sampling of CSF.** The 10 rabbits were randomly assigned to two groups (n = 5 each): group 1 was for the continuous intrathecal AMB, and group 2 was for the conventional intrathecal AMB. AMB deoxycholate (Sigma-Aldrich, St. Louis, MO) was reconstituted per the manufacturer’s instructions and further diluted to the desired concentration in sterile water to be given in 0.2 to 0.25 ml. Rabbits received either continuous intrathecal AMB (0.15 mg/kg/day) for 72 h in group 1 or conventional intrathecal AMB (0.015 mg/kg) for 30 min in group 2 with an infusion pump (SynchroMed model RWD 204; RWD Life Science Co., Shenzhen, China). CSF (50 μl) was taken at 1, 2, 3, 4, 5, 6, 8, 12, 24, 28, 32, 40, 48, 56, 64, and 72 h after the start of drug administration for all individual rabbits. All samples were stored at −80°C until used for the assay of AMB concentration.

**Determination of AMB concentration.** Concentrations of AMB in CSF samples were determined by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay (10) in the Hygiene Detection Center of Southern Medical University. An API 2000 (Applied Biosystems Corporation) triple-quadrupole mass spectrometer was used to assess AMB with electrospray interfaced to an Agilent Zorbax Extend C18 analytic column (150 by 4.6 mm; 5 μm). The lower detection limit of this assay was 0.005 μg/ml.

**RESULTS**

**Distribution of methylene blue.** By dissecting the rabbits injected with methylene blue, we found that all of the catheters were placed into the spinal subarachnoid space; three of them were located at the ventral side and two at the dorsal side. The tips of all catheters were located at the level of the first lumbar vertebra. As can be seen in Fig. 2, the surface of the spinal cord and the brain was dyed blue. The darkest parts were located at the spinal cord near the tip of the catheter, and staining was progressively paler farther from the tip of the catheter. The base of the brain was also dyed, but the surface of the cerebellum and hemispheres looked pale. This may indicate the gradient of the dye concentration in the CSF, which may correlate with the gradient of the AMB concentration in the CSF after continuous intrathecal infusion.

**SEM findings.** With macrography after laminectomy, it can be seen that the tips of all catheters were located in the subarachnoid space. SEM confirmed that the catheters were located in the subarachnoid space without any damage in the spinal cord. There was no clot or other exudates around the catheter (Fig. 3).

**Pharmacokinetics of AMB.** As shown in Fig. 4, there were significant differences between the two groups. In the continuous intrathecal AMB group, the concentration of AMB peaked at 7.01 μg/ml at 4 h and then decreased to a stable level of 1.0 to 1.34 μg/ml after 24 h. In the conventional intrathecal injection group, the AMB concentration reached a peak of 3.41 at 1 h and then decreased progressively to a concentration of 0.31 μg/ml at 72 h. As shown by the results of methylene blue staining, since the highest AMB concentration should locate at the lumbar subarachnoid space near the tip of the catheter, the determined AMB concentration in the CSF sampled from cisterna magna might be lower than that from the lumbar subarachnoid space. Because the AMB was administered continuously, the gradient of the AMB concent-

![Fig 1](http://aac.asm.org/Downloaded from http://aac.asm.org on September 9, 2017 by guest)

**FIG 1** Schematic illustration of the construction of the intrathecal catheter. The inner PE10 tube was inserted 3.5 cm into the outer PE30 tube, with 1.5 cm of the outer tube protruding for connecting the microinjector. The protruding 15-cm inner tube was used for intrathecal catheterization.

![Fig 2](http://aac.asm.org/Downloaded from http://aac.asm.org on September 9, 2017 by guest)

**FIG 2** Photographs of the methylene blue staining of the spinal cord and the brain after continuous intrathecal administration of methylene blue. The tip of the catheter was located at the ventral spinal cord at the level of the first lumbar vertebra (arrow), where the darkest-dyed part was located.

![Fig 3](http://aac.asm.org/Downloaded from http://aac.asm.org on September 9, 2017 by guest)

**FIG 3** SEM of the spinal cord with the catheter in position. The catheter was inserted into the spinal subarachnoid space.
tation in the CSF in the continuous intrathecal AMB group be-
tween the lumbar subarachnoid space and cisterna magna might
be less than that in the conventional intrathecal injection group.

**Adverse drug reactions.** The comparative toxicity was deter-
mined by clinical observations. There were no adverse drug reac-
tions, including neurological impairments, in the continuous in-
trathecal AMB group. Two rabbits in the conventional intrathecal
injection group suffered fever and convulsion, and one had paral-
sis in the right rear limb. All of these adverse drug reactions
emerged as soon as intrathecal AMB injection finished, so they
might be associated with the peak levels of the AMB concentration
in the lumbar subarachnoid space. There was no significant
weight loss in either group.

**DISCUSSION**

AMB remains a first-line option for empirical therapy in the treat-
ment of CM, especially for primary therapy, and in recent years its
MIC has been found to range from 0.03 to 1 mg/liter (17). The
therapeutic effect of AMB is dependent on both concentration
and time, which implies that the concentration of AMB in CSF
must remain higher than the MIC for long enough to achieve
clinical improvement or cure, reduce resistance, and prevent re-
occurrence. However, its poor penetration into CSF, ranging from
undetectable to no more than 4% of serum concentrations (14),
limits its effect against CM. The results from our previous studies,
indicating that the concentration of AMB in CSF was undetectable
after 4 h of intravenous administration before intrathecal admin-
istration, intensified the thought that intravenous therapy with
AMB is not potentially a curative treatment (5).

Intrathecal injection has been postulated to increase the AMB
concentration in CSF effectively (25). The injection time course is
far shorter than one cycle time of CSF circulation, and hence in-
trathecal injection is regarded as a kind of bolus injection, which
can quickly increase the drug concentration levels and induce se-
vere neurological impairments (1). Also, intraventricular injec-
tion of AMB (4 mg/day), reported by De Socio et al. in 2003, could
increase the concentration level of AMB significantly, but it de-
creased rapidly from 100.5 mg/liter to 1.75 mg/liter in 17 h (2).

Although the use of liposomal AMB can greatly reduce the toxic-
ity, it had been reported that intravenous administration of Am-
Bisome at 3 mg/kg/day did not result in significant elevation of the
AMB concentration in CSF, and it was no more effective than
AMB (7).

Continuous intrathecal infusion with an implanted pump has
been utilized widely in the treatment of patients with severe pain
and disability (12, 17). Continuous intrathecal infusion can pro-
vide a rational pharmacokinetics with stable and effective drug
concentrations and contribute to a better clinical efficacy and less
neurological impairment. We have previously reported a contin-
uous intrathecal therapy with a percutaneous lumbar catheter in
the treatment of CM and purulent meningitis (5, 11, 24). With the
therapy of continuous intrathecal AMB, the AMB concentration
in CSF can be significantly elevated and maintained at a stable and
effective level by bypassing the blood-brain barrier, with fewer
side effects and complications (5). Currently, *Cryptococcus neofor-
mans* is considered susceptible to AMB at a concentration reach-
ing the MIC of 0.03 to 1 mg/liter (4, 20). The patients were first
treated by routine therapy (AMB at 0.7 to 1.0 mg/kg/day intrave-
nously plus flucytosine at 100 mg/kg/day orally) combined with
conventional intrathecal injection of AMB (0.5 to 1.0 mg/day)
twice per week for 1 month. As none of them improved clinically,
we prepared the continuous intrathecal AMB to substitute for the
conventional intrathecal AMB for 2 to 4 weeks, followed by oral
flucconazole therapy (400 mg/day orally) for 6 months. All of the
patients were cured without relapse after 2 years of follow-up vis-
its, which confirmed that continuous intrathecal AMB is effective
even in the event of treatment failure with routine therapy (5, 11).
Compared to continuous intrathecal infusion with an implanted
pump, the therapy of continuous intrathecal infusion with a per-
cutaneous lumbar catheter has some advantages, such as better
cost-effectiveness and ease of handling, which has led it to emerge
as a potential solution for infectious, neoplastic, and other severe
meningitis varieties.

The rabbit model that we created in this study permits better
investigation of the pharmacokinetics of continuous intrathecal
AMB. We changed the route of insertion from the cisterna magna

![FIG 4](http://aac.asm.org/)
(23) to the coccyeal route, which made the surgery less invasive and significantly decreased the risk of complications such as spinal cord or spinal nerve root damage. A lumbar approach was also adopted for catheterization (19, 22); however, spinal cord or cauda equina damage was still inevitable. That was because the spinal cords of rodents were extended into sacral vertebra. A modified catheter was also used in order to reduce injury (16), but the procedure was quite complicated and time-consuming. Our results suggested that this method has a good stability, good repeatability, and high probability of success. Moreover, no signs of spinal cord and spinal nerve root damage were found by neurological observation.

The results of this study revealed that continuous intrathecal administration can provide a relatively rational pharmacokinetics compared with conventional intrathecal injection, in accord with our previous clinical study (5). With the continuous intrathecal administration, the AMB concentration maintained a stable level of 1.0 to 1.34 µg/ml above MIC after the peak at 4 h, without any neurological impairment, while in the conventional intrathecal injection group, the AMB concentration decreased progressively after the peak at 1 h, with obvious neurological impairments. The neurological impairments that presented in the conventional injection group might be a result of the drug depot around the tip of the catheter, an inevitable phenomenon in the bolus injection, in the lumbar cisterna after the intrathecal injection. In the single-compartment pharmacokinetic model, the drug should accumulate in the CSF and rise until a steady-state condition is reached (6). Nevertheless, the AMB concentration in the continuous intrathecal AMB group revealed an unexpected pattern, just like the single-dose administration pattern. The cause of this remains unknown. It has nothing to do with the speed of drug infusion, as this remains constant during the administration. Also, it is unlikely to be associated with the combination of the protein, for there is very little protein in CSF. Therefore, the possibility is restricted to the CSF circulation or upregulation in secretion or absorption. The CSF secretion from the choroid plexus is in the upstream of the CSF circulation, which is impossible to be affected by intrathecal administration; therefore, we assume that the decrease in AMB concentration after the peak at 4 h might be attributed to the upregulation in CSF absorption caused by the stimulation of AMB on some unknown receptors in the sections of CSF absorption. Furthermore, just about 4 h is needed for CSF to circulate from the lumbar cistern to the superior sagittal sinus or the olfactory bulb, where some unknown receptors might reside.

The results of this study also indicate that continuous intrathecal AMB is more effective and safe than the conventional intrathecal injection by providing rational pharmacokinetics. We sought to use dosages of AMB in rabbits that are relevant to those given intrathecally to humans. The continuous intrathecal AMB dose of 0.15 mg/kg/day closely mimics the 4 mg/day for a 60- to 70-kg human (11), and the conventional injection dose of 0.015 mg/kg is close to a dose of 1 mg for a 70-kg human (1). Patients who underwent continuous intrathecal AMB treatment of 4 mg/day showed none of the neurological impairments described above (5). Further clinical studies on the pharmacokinetics, safety, and efficacy of continuous intrathecal AMB need to be carried out before its widespread clinical application can be realized.

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