We previously reported a new effective 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 coccyeal 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 first and the second coccygeal spinous processes, ligamentum flavum, and epidural fat were sequentially removed; and the underlying dura was exposed. After a small slit in the dura mater and 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 confirm correct localization.

**Received** 8 February 2012  **Returned for modification** 5 July 2012  **Accepted** 22 July 2012  **Published ahead of print** 30 July 2012  
Address correspondence to Tian Ming Lu, lutianming@139.com.  
Copyright © 2012, American Society for Microbiology. All Rights Reserved.  
doi:10.1128/AAC.00304-12
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 concen-

**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
tration in the CSF in the continuous intrathecal AMB group between 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 determined by clinical observations. There were no adverse drug reactions, including neurological impairments, in the continuous intrathecal AMB group. Two rabbits in the conventional intrathecal injection group suffered fever and convulsion, and one had paralysis 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 treatment 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 recurrence. 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 administration, 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 intrathecal injection is regarded as a kind of bolus injection, which can quickly increase the drug concentration levels and induce severe neurological impairments (1). Also, intraventricular injection of AMB (4 mg/day), reported by De Socio et al. in 2003, could increase the concentration level of AMB significantly, but it decreased 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 toxicity, it had been reported that intravenous administration of AmBisome 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 provide 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 continuous 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 neoformans is considered susceptible to AMB at a concentration reaching 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 intravenously 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 fluconazole therapy (400 mg/day orally) for 6 months. All of the patients were cured without relapse after 2 years of follow-up visits, 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 percutaneous 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
to the coccygeal 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.

ACKNOWLEDGMENT

The Science and Technology Planning Project of Guangdong Province, People’s Republic of China, is acknowledged for funding (grant no. 2007B031511011 and 2009B030801208).

REFERENCES

13. Reference deleted.