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Comparative Study of Periocular Fat Tissue Concentrations Following Retrobulbar, Intravenous, and Combined Retrobulbar-Intravenous Injection of Liposomal Amphotericin B in Rats

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Abstract

Background: Recently, retrobulbar injection of liposomal amphotericin B has been explored as an alternative treatment of rhino-orbital-cerebral mucormycosis.

Objectives: This study aims to measure amphotericin B concentration in the periocular fat tissue following intravenous, retrobulbar, and combined intravenous and retrobulbar injections.

Methods: In this study, 45 rats were divided into 15 groups, receiving either intravenous, retrobulbar, or combined intravenous and retrobulbar injections. Three groups received the same dose of liposomal amphotericin B. Rats were sacrificed at 4-, 6-, and 24-hours post-injection and the periocular fat tissue was analyzed for amphotericin B concentration using HPLC.

Results: Results showed that amphotericin B concentrations after intravenous injection of 10 mg/kg were 0.0001, 0.1154, and 0.0693 µg/mL at 4, 6, and 24 hours, respectively; for 15 mg/kg, the concentrations were 0.0339, 0.3534, and 0.4209 µg/mL. Retrobulbar injection resulted in concentrations of 8.8965, 9.8124, and 9.4156 µg/mL. Combined injections (10 mg/kg IV + 0.25 mg/kg retrobulbar) yielded concentrations of 8.8401, 7.8869, and 8.6409 µg/mL, while the combined 15 mg/kg IV + 0.25 mg/kg retrobulbar yielded 8.1940, 8.5277, and 9.0889 µg/mL.

Conclusions: The findings indicate that retrobulbar injection of liposomal amphotericin B achieves suitable drug concentrations in periocular tissue, suggesting that for rhino-orbital-cerebral mucormycosis, retrobulbar injection alone may be sufficient, potentially eliminating the need for intravenous administration.

Keywords: Amphotericin B, Periocular Fat Tissue, Retrobulbar Injection, Intravenous Injection

1. Background

Invasive fungal infections are a major cause of morbidity and mortality in immunocompromised individuals, such as AIDS patients, transplant recipients, or those undergoing immunosuppressive chemotherapy (1). Amphotericin B, one of the oldest and most effective antifungal drugs, plays a crucial role in treating these infections (2, 3).

Mucormycosis is a severe and potentially fatal fungal infection, with rhino-orbital-cerebral mucormycosis being the most common form (4, 5). While typically seen in immunocompromised patients, it can also affect healthy individuals (6). The global incidence of rhino-orbital-cerebral mucormycosis has risen significantly, especially during the Covid-19 pandemic (7). Managing this disease involves sinus debridement using endoscopic methods, systemic antifungal treatment, and controlling immunosuppression (7). Orbital involvement presents a significant challenge, with

treatment strategies including orbital exenteration, conservative orbital debridement with or without amphotericin B lavage, and retrobulbar injection of amphotericin B (8).

Amphotericin B, the first important commercial antifungal drug, has been in use for over 50 years (9). Despite newer agents like azoles, this polyene macrolide remains vital in treating systemic fungal infections (10). Given its proven efficacy and the increasing need for antifungal treatments, further investigation into amphotericin B is justified (11). However, its use is limited by dose-dependent side effects, particularly nephrotoxicity (12). To improve its safety, new formulations of amphotericin B have been developed to reduce renal toxicity (2, 13).

Amphotericin B-deoxycholate is a broad-spectrum polyene antifungal agent that has been the gold standard for antifungal therapy for decades, despite a high incidence of infusion-related side effects and nephrotoxicity (14). While still used, newer drugs like lipid formulations of amphotericin B, azoles (e.g., voriconazole), and echinocandins (e.g., caspofungin and micafungin) have often replaced it as first-line treatments (15).

A study of 217 clinical isolates of *Mucorales* from January 2001 to February 2007 at the United States Fungal Testing Laboratory found that the minimum inhibitory concentration (MIC_{90}) for amphotericin B varied between 0.5 and 1 μg/mL, regardless of the mucormycosis type (rhino-orbital, rhinocerebral, pulmonary, or cutaneous) (16).

A case report described a 55-year-old diabetic man with left proptosis, headache, maxillary sinus pain, and diplopia. Biopsy results indicated mucormycosis, showing wide, irregular nonporous hyphae (17). Despite intravenous antifungals and endoscopic sinus debridement, his condition did not improve until he received a retrobulbar injection of amphotericin B deoxycholate, leading to disease resolution without exacerbation. This case highlighted the effectiveness of retrobulbar injection as an adjunctive treatment (17).

A prospective, interventional study conducted on 82 post-COVID-19 rhino-orbital-cerebral mucormycosis (ROCM) patients from May to July 2021 evaluated the effectiveness of daily 1 mL liposomal amphotericin B injections over three doses (18). Despite other treatments like orbital debridement and exenteration for moderate and severe cases, 72% of patients showed symptomatic improvement without serious side effects (18). This study demonstrated that transcutaneous retrobulbar injection of amphotericin B (TRAMB) is an effective and safe treatment for mild to moderate ROCM and serves as a useful adjunct in severe cases (18).

A review suggested that retrobulbar injection of amphotericin B is a relatively safe and protective measure against orbital enhancement in patients with COVID-19-associated orbital mucormycosis (CAM), potentially preserving vision (19). Given the unclear impact of orbital enhancement on patient survival, retrobulbar injection is a viable alternative intervention (19).

In another study, more than three injections of liposomal amphotericin B were given to three patients, two of whom showed significant improvement after the initial series, and one improved with each subsequent injection (20). Compared to historical controls, patients with invasive rhino-orbital fungal sinusitis treated with a modified therapeutic ladder algorithm, including TRAMB, had a lower risk of deformity without an increase in mortality risk (20).

2. Objectives

Against this background, this study examines the concentration of amphotericin B in periocular fat tissue in rats following intravenous or retrobulbar injection of liposomal amphotericin B.

3. Methods

3.1. Animals

Adult male Wistar rats weighing 250 - 300 g were used. The animals acclimatized to their new environment for one week. They were housed in a fully controlled setting in Plexiglass cages (temperature: 22 ± 2°C, humidity: 50 - 60%, and a 12-hour light-dark cycle) with free access to standard food and water. Amphotericin B liposomal was purchased from Exir Nano Sina, Iran, and ketamine and xylazine were purchased from Merck, Germany.

The study protocol was ethically approved by the ethics committee of IR.SBMU.AEC.1401.072.

The animals were randomized into fifteen groups of four rats each. Following anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg), all rats except those in groups 7 - 9 received silastic catheters in the femoral vein before starting the treatment. The dose and method of liposomal amphotericin B injection for each group are shown in Table 1. Additionally, rats in groups 7 - 15 received a 0.25 mg/kg bilateral retrobulbar injection of liposomal amphotericin B.

According to the study plan, 4, 6, and 24 hours after receiving liposomal amphotericin B, the rats were

Table 1. Amount and Concentration of Liposomal Amphotericin B in Tissue Across Different Groups of Rats

Groups	Intravenous Dose (mg/kg)	Retrobulbar Dose (mg/kg)	Sampling Time (Hours After the End of the Infusion)	Concentration in Tissue (µg/mL) (Mean ± SD)
1	10	-	4	0.0001 ± 0.00012
2	10	-	6	0.1154 ± 0.15031
3	10	-	24	0.0693 ± 0.11997
4	15	-	4	0.0339 ± 0.05872
5	15	-	6	0.3534 ± 0.16300
6	15	-	24	0.4209 ± 0.18848
7	-	0.25	4	8.8965 ± 0.21155
8	-	0.25	6	9.8124 ± 1.38202
9	-	0.25	24	9.4156 ± 0.60750
10	10	0.25	4	8.8401 ± 0.80826
11	10	0.25	6	7.8869 ± 0.93015
12	10	0.25	24	8.6409 ± 1.56958
13	15	0.25	4	8.1940 ± 0.27718
14	15	0.25	6	8.5277 ± 1.11503
15	15	0.25	24	9.0889 ± 0 41087

euthanized, and the retrobulbar tissue was completely extracted to measure the concentration of amphotericin B.

3.2. Tissue Extraction Procedures

After removing the retrobulbar tissue, 2 mL of methanol was added, and the tissue was minced in a micro-homogenizer cup (Sorvall). The cup was placed on ice, and the mixture was homogenized at high speed for three 30-second periods. The homogenate was then transferred to a Falcon tube with two 1.5 mL methanol rinses and placed in a shaking water bath (1.5 mL each) at 50°C for 15 minutes. The extraction mixture was centrifuged at 12,000 RPM for 10 minutes, and the clear supernatant was collected. Finally, the collected liquid was filtered using a filtration syringe and the concentration of amphotericin B was measured using a UV spectrophotometer at a wavelength of 408 nm (HP UV-Vis Spectrometer) (21).

3.3. Statistical Analysis

All studies were repeated three times, and the values are expressed as mean ± standard deviation. One-way ANOVA and TUKEY tests were used for statistical analysis of the results. SPSS software was used for the ANOVA test design.

4. Results

The results of intravenous, retrobulbar, and combined intravenous and retrobulbar injections are presented in Table 1 and Figure 1.

4.1. Intravenous Injection

For intravenous injection of 10 mg/kg, the tissue concentrations at 4-, 6-, and 24-hours post-injection were 0.0001, 0.1154, and 0.0693 µg/mL, respectively. With an intravenous injection of 15 mg/kg, the tissue concentrations of amphotericin B were 0.0339, 0.3534, and 0.4209 µg/mL at 4, 6, and 24 hours, respectively.

4.2. Retrobulbar Injection

In group 7, which received a retrobulbar injection of 0.25 mg/kg, the amphotericin B concentration in periocular fat was 8.8965 µg/mL at 4 hours. For the same dosage, the tissue concentration reached 9.8124 µg/mL at 6 hours, and in group 9, it was 9.4156 µg/mL at 24 hours post-injection.

4.3. Intravenous plus Retrobulbar Injection

In groups 10 to 15, where rats received both retrobulbar and intravenous injections, tissue concentrations were influenced by the intravenous dose. In groups 10, 11, and 12, the tissue concentrations at 4, 6, and 24 hours were 8.8401, 7.8869, and 8.6409 µg/mL, respectively. For groups 13 to 15, which received an intravenous injection of 15 mg/kg, the concentrations at 4, 6, and 24 hours were 8.1940, 8.5277, and 9.0889 µg/mL, respectively.

To compare what is known, at the $P < 0.05$ significance level, there is a significant difference in

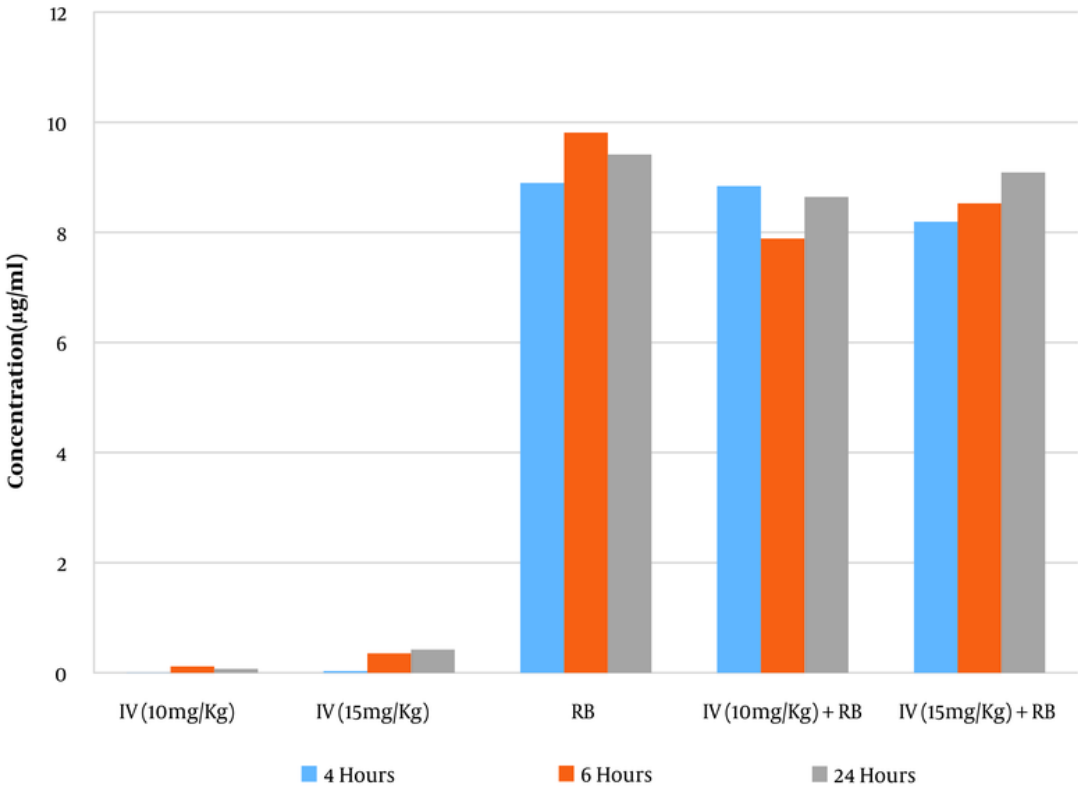

Figure 1. Trend of amphotericin B concentration changes in periocular fat tissue following different injection methods (IV: Intravenous injection; RB: Retrobulbar injection [0.25 mg/kg])

tissue amphotericin concentration between intravenous injection alone, intravenous injection plus retrobulbar, and retrobulbar. However, no significant difference is observed between retrobulbar injection alone and retrobulbar plus intravenous injection.

5. Discussion

The MIC of amphotericin B for mucormycosis is between 0.5 to 1 mg/mL (22). To effectively treat this condition, the drug concentration needs to reach this level within the target tissue. Liposomal amphotericin B has a volume of distribution ranging from 0.2 to 1.6 L/kg, indicating an appropriate distribution compared to its deoxycholate form. When administered at 2 mg/kg, the blood concentration reaches 22.9 µg/mL, significantly higher than the MIC_{90} (23). However, with an intravenous injection of 10 mg/kg (the maximum recommended dose for mucormycosis), the tissue concentration within the first 4 hours is negligible, almost zero (24). Even after 6 hours, the tissue concentration does not reach the effective MIC_{90}, nor does it achieve therapeutic levels within 24 hours. The increase in tissue concentration from the first to third intravenous injections (10 mg/kg) was not significant.

An injection of 15 mg/kg (1.5 times the maximum recommended dose of liposomal amphotericin B) does not achieve effective fungicidal levels for mucormycosis treatment. This dose can lead to significant toxicity and complications like renal failure, hypokalemia, hypomagnesemia, metabolic syndrome, and nephrogenic diabetes insipidus-related polyuria, without producing adequate fungicidal levels in the fat tissue (25). Within the first 4 hours of injection, tissue concentration is minimal, and after 6 hours, it remains below the therapeutic threshold. Even after 24 hours, the tissue concentration is insufficient to reach the MIC_{90} of amphotericin B.

In contrast, retrobulbar injection of a very low dose (0.25 mg/kg) of liposomal amphotericin B achieves more than 8 times the MIC_{90} within 4 hours of administration, equivalent to one-twentieth of the minimum recommended dose (5 mg/kg) for mucormycosis treatment (24). Tissue concentration remains consistently high, with no significant decrease or increase up to 24 hours post-injection.

Combined intravenous injection (10 mg/kg) and retrobulbar injection (0.25 mg/kg) also result in tissue concentrations exceeding 8 times the MIC_{90} within 4 hours, particularly in fat tissue around the eyes. This combined approach yields a higher and more significant concentration compared to a single intravenous injection but is not notably different from the concentration achieved with solo retrobulbar injection.

Similarly, a combination of intravenous injection (15 mg/kg) and retrobulbar injection (0.25 mg/kg) leads to tissue concentrations more than 8 times the MIC_{90} within 4 hours, maintaining consistent levels at 6 and 24 hours without significant change. Despite producing a higher concentration than intravenous injection alone, this combination does not significantly differ from retrobulbar injection alone in terms of tissue concentration.

5.1. Conclusions

Liposomal amphotericin B is recommended for treating mucormycosis, with a maximum dose of 10 mg/kg. This laboratory study showed that while intravenous injection of liposomal amphotericin B at the maximum recommended dose or 1.5 times its MIC_{90} can cause many adverse effects, it effectively treats various fungi that do not cause rhino-orbital-cerebral mucormycosis. Additionally, the study demonstrated that a much lower dose of liposomal amphotericin B administered via retrobulbar injection can achieve a suitable concentration for treating this disease. Combining intravenous and retrobulbar injections does not significantly alter amphotericin B tissue concentration around the eye, suggesting that treatment could be limited to retrobulbar injection alone. Further human studies are recommended to confirm these findings.

Footnotes

Authors' Contribution: M. Sh. and H. E. conceived and designed the evaluation and drafted the manuscript. A. B. and S. M. S. participated in designing the evaluation, performed parts of the statistical analysis, and helped to draft the manuscript. H. E. and A. B. re-evaluated the data, revised the manuscript and performed the statistical analysis, and revised the manuscript. Sh. S., M. A., and A. H. collected the data, interpreted them, and revised the manuscript. Final approval of the version to be published: M. Sh, A. B., S. M. S., Sh. S., M. A., A. H., and H. E.

Conflict of Interests Statement: The authors declared no conflict of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to the relevant data is included in the article as much as possible and other data will be sent upon request.

Ethical Approval: Research Ethics Committees of Laboratory Animals - Shahid Beheshti University of Medical Sciences (IR.SBMU.AEC.1401.072 on 22.01.2023).

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