Journal of Biotechnology & Biomaterials

Research Article Open Access

The Use of Intranasal Versus Intravenous Route to Limit Adverse Effects of Amphotericin B Administration

Naveed Ahmed Khan[1]*, Ruqaiyyah Siddiqui[1] and Timothy Yu Yee Ong[2]

[1]*Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, University City, Sharjah, United Arab Emirates*
[2]*Department of Biological Sciences, School of Science and Technology, Sunway University, Bandar Sunway, Malaysia*

Abstract

Infection due to *Naegleria fowleriis* devastating and results in death within days, with more than a 90% mortality rate. The current mode of treatment is via intravenous therapy and is not very effective. For the first time, we tested the modality of the intranasal route to treat this deadly brain infection versus the intravenous route, post-infection *in vivo*. We compared the adverse effects of Amphotericin B administration, through blood biochemistry, liver, kidney and brain histopathological evidence of toxicities in both scenarios. The findings clearly depicted that intranasal administration of Amphotericin B, in comparison to the intravenous route significantly limited the adverse side effects *in vivo*. As *N. fowleri* exhibits unequivocal affinity to the olfactory bulb and frontal lobe in the central nervous system and the fact that parasite portal of entry is via nose, intranasal administration of therapy may be able to directly reach amoebae bypassing the blood-brain barrier selectivity and achieve the minimum inhibitory concentration at the target site. These findings are very encouraging and could lead to the development of a much needed new mode of therapy for this distressing infection. This work is pioneering, and could be a key step in the rationale development of therapeutic interventions against brain-eating amoebae infections by highlighting the use of the intranasal route as a modality to treat this normally fatal infection.

Keywords: Amphotericin B; Adverse effects; Naegleria *fowleri;* Primary amoebic meningoencephalitis; Brain-eating amoebae

Introduction

Primary amoebic meningoencephalitis (PAM) caused by *Naegleria fowleri* is a devastating infection that results in death within days with more than a 90% mortality rate. Amphotericin B has been used widely in the treatment of PAM but shows limited efficacy to target the parasite residing within the central nervous system and there can be side effects including nephrotoxicity, hepatotoxicity etc. In part, this is due to application of the drug intravenously at high dose to reach the effective concentration at the target site, however this results in the distribution of the drug to unwanted tissues. The fact that the portal of entry of *N. fowleri* in the host is through the olfactory neuroepithelium, accessing the roof of the nasal cavity and then traversing through cribriform plate to ultimately reaching the olfactory bulb and brain, it makes sense that intranasal route should be utilized to treat the infection as well, and should offer advantage in the delivery of drug at an effective concentration to kill the parasite. Furthermore, we postulated thatthe use of the intranasal route, in comparison to the intravenous route may limit side effects to the host's tissues. In this study, for the first time, we tested intranasal (IN) and intravenous (IV) route and compared the adverse effects of Amphotericin bad ministration, through blood biochemistry, liver, kidney and brain histopathological evidence of toxicities *in vivo* post-infection with *N. fowleri*. To our comprehension, this is the first study implemented to explore the modality of the intranasal route to treat infection due to *N. fowleri*.

Results and Discussion

In the control group and the IN group, weight changes and motor incoordination was not observed, however, IV group showed a gradual decline in weight. The electrolytes, creatinine level and uric acid were examined for renal function while liver enzymes, aspartate transferase; alanine transferase; gamma-glutamyl transferase and liver synthetic functions (total protein, albumin and globulin) were evaluated for liver function [1]. The elevation of liver enzymes was more profound and marked in the IV group as opposed to the IN group, indicating that the severity of liver toxicity is significant in IV treatment of Amphotericin B compared to IN treatment. The mice in IN group and control group showed intact renal tubules especially in the area of proximal tubule with no sign of glomerular ischemia or atrophy which are the hallmarks of Amphotericin induced-nephrotoxicity. The liver architecture was devoid of signs of hepatic centrilobular degeneration, multifocal hepatocellular necrosis or macrophage vacuolation which are usually seen in Amphotericin B- induced hepatotoxicity. The liver tissues from IV group however showed hepatocyte necrosis with shredding into hepatic central vein. The portal triad consisting of portal venules, bile ducts and hepatic arteries was distorted due to malalignment of liver architecture resulting from hepatocyte necrosis. The laboratory blood biochemistry of liver and kidney functions correlated well with the findings of histopathological evidence of nephrotoxicity and hepatotoxicity [2]. The examination of brain of all groups was carried out in olfactory bulb, olfactory cortex, medulla-midbrain borders and cerebellum. The examination revealed mild neutrophilic inflammation as expected of PAM or necrosis in the Virchow-Robin space (perivascular area) for IV group. Haemorrhage was also not observable in the aforementioned brain regions. The necrosis and gangliosis were not observable in IN groups. The IV group in contrast had more widespread of neutrophilic infiltration in the borders of medulla and midbrain as well as olfactory cortices and inflammation evident by foci

***Corresponding author:** Naveed Ahmed Khan, Department of Biology, Chemistry and Environmental Sciences, College of Arts and Sciences, American University of Sharjah, University City, Sharjah, United Arab Emirates, PO Box 26666, Tel: +971-6515-4752; E-mail: naveed5438@gmail.com

Received: 08-Jun-2020, Manuscript No. jbtbm-20-13027; **Editor assigned:** 10- Jun-2020, Pre QC No. jbtbm-20-13027 (PQ); **Reviewed:** 17-Mar-2022, QC No. jnp-55809; **Revised:** 21-Mar-2022, Manuscript No. jbtbm-20-13027 (R); **Published:** 28-Mar-2022, DOI: 10.4172/2155-952X.1000265

Citation: Khan NA, Siddiqui R, Ong TYY (2022) The Use of Intranasal Versus Intravenous Route to Limit Adverse Effects of Amphotericin B Administration. J Biotechnol Biomater, 12: 266.

of neutrophilic infiltrations.

Summary and Conclusion

These findings undoubtedly revealed that utilising the intranasal route, revealed limited adverse side effects of Amphotericin B, *in vivo,* in contrast to the intravenous route. As *N. fowleri* exhibit unequivocal affinity to the olfactory bulb and frontal lobe in the CNS and the fact that parasite portal of entry is via nose, it makes sense that intranasal administration can directly reach the amoebae bypassing the blood-brain barrier selectivity and achieve the MIC at the target site, and with limited side effects to the host's tissues. Imminent studies are needed to further scrutinise and optimise this route of administration in combination with the intravenous route or as a mode of treatment in its own right [3]. Moreover this route needs to be evaluated with other potential novel drugs against *N. fowleri.* This work is ground breaking and could lead to the utilization of a much looked for new approach for therapy for this infection.

Materials and Methods

C57BL/6 male adult mice between 22 – 26 weeks old, weighing 20-38g (n=50) were kept under specific pathogen free (SPF) conditions. Based on the principle of the three Rs i.e. (Replacement, Reduction and Refinement),animals were divided into three groups and treated as follows: (i) animals injected with saline alone, (ii) animals infected with *N. fowleri* followed by IV administration of Amphotericin B to achieve an effective concentration of 4mg/kg, and (iii) animals infected with *N. fowleri* followed by IN administration of Amphotericin B (2mg/kg) plus IV administration of Amphotericin B (2mg/kg) to achieve an effective concentration of 4mg/kg based on earlier studies and as our objective was determine the effects of Amphotericin B toxicity in both routes of administration [4]. For simplicity, group 2 is referred to as IV group, while group 3 is referred to as IN group. *N. fowler* were cultured using HeLa cells as feeder cells and used for *in vivo* studies as previously described. Next, animals were anaesthesized by intraperitoneal injection of 0.9 ml/kg (4.5 mg/kg) of ketamine-xylazine-zoletil and then intranasally infected with 10^5 amoebae per nare. At 72 h post-infection, Amphotericin B was administered IV for Group 2 animals via tail vein (4 mg/kg) daily. For Group 3 animals, mice were given Amphotericin B IN (2 mg/kg) and IV (2 mg/kg). The mice were observed on a daily basis for activity, alertness, body condition and weight, breathing, coat, dehydration, drinking and eating, movement/gait, eyes, faeces, nose, urine, vocalisation, motor incoordination/ataxia, lethargy, pain signs. On day 14, the mice were sacrificed and brains, livers and kidneys were collected in 4% PFA and transferred to 30% sucrose in 0.1 M PBS and finally stored at -80°C. Sections of 5-15µm in sagittal planes were rehydrated, followed by hematoxylinand eosin staining and images captured using inverted microscope [5]. The foci of examination were primarily aimed at the olfactory bulb, anterior olfactory nucleus, cerebral cortex, hippocampus, thalamus, midbrain, cerebellum, pons and medulla while liver were examined for distorted architecture and kidney for medulla tubular necrosis and glomerular ischemia.

Ethics

All procedures involving animals in this study were approved by the Sunway University Research Ethics Committee (SUREC2017-029). As the animals were housed at Monash Animal Research facility, the procedures were also approved by the Animal Ethics Committee Monash University (MUM2017-06).

Acknowledgement

This work was funded by the Sunway University, Malaysia and American University of Sharjah, UAE.

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