Biomed. Papers 148(2), 221–223 (2004)

INFLUENCE OF AMPHOTERICIN B DEOXYCHOLATE OR AMPHOTERICIN B COLLOIDAL DISPERSION ON RENAL TUBULE EPITHELIUM IN RAT

Lenka Krejčířová[a], Irena Lauschová[a], Drahomír Horký[a], Michal Doubek[b], Jiří Mayer[b], Jiří Doubek[c]

[1] *Department of Histology and Embryology, Medicine Faculty Masaryk University Brno*
[2] *Department of Internal Medicine – Haematooncology, Masaryk University Hospital Brno*
[3] *Department of Physiology and Pathophysiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic*
e-mail: lkrejcir@med.muni.cz

Received: September 20, 2004

Key words: Proximal tubule/Distal tubule/Amphotericin B desoxycholate/Amphotericin B colloidal dispersion/Rat

Amphotericin B deoxycholate (AmB) or Amphotericin B colloidal dispersion (ABCD) are used in clinics for the treatment of systemic fungal infections. The goal of our study was to compare the nephrotoxicity of these drugs in rat kidney. The effects of AmB and ABCD on the ultrastructure of the epithelium of renal tubules were studied and evaluated using morphometric and statistical methods. Two groups of 3 animals were established: group 1 was treated with AmB desoxycholate and group 2, to which ABCD was applied. AmB caused more than ABCD ultrastructural changes in the cytoplasm of the epithelial cells: damage to mitochondria, vacuolation of cytoplasm, and increased values of volume density of peroxisomes. However, we failed to observe significant differences in morphology and density of the other cell organelles. The proximal tubules seemed to be more sensitive to the nephrotoxic influence of both formulas than the distal tubules of rat kidney. Although, AmB causes more severe damage than ABCD, both drugs cause damage to renal tubuli.

Abbreviations used in text and tables: *AmB – amphotericin B deoxycholate, ABCD – amphotericin B colloidal dispersion, Dt – distal tubule, Pt – proximal tubule, VD (%) ± SD – volume density and standard deviation, * – statistical significance*

INTRODUCTION

Amphotericin B deoxycholate (AmB) is used as an efficacious medication for systemic fungal infections, in spite of the nephrotoxic effects mentioned by some authors[6, 3, 2]. Colloidal dispersions or lipid complexes of AmB seem to be more sparing on kidney parenchyma and its functions than AmB[10, 11]. Unfortunately, these new formulas of AmB are considerably more expensive. The majority of clinical references are concentrated on functional evaluation of the kidneys; morphological aspects are studied as a matter of peripheral importance. Light microscopy is normally used and as the result, necrosis of the epithelial cells is mentioned. The nephrotoxicity of these drugs at the ultrastructural level have been studied in previous research here[5]. This evaluation was objectively reviewed using stereological and statistical methods.

MATERIAL AND METHODS

Laboratory male rats (pathogen-free; source Anlab, Prague, Czech Republic), 265–380 g body weight, were used and individually housed in metabolic cages for urine collection during the study. The adaptation time to new conditions was 7 days for each experiment. The rats were maintained on standard rat chow ad libitum. Water was not restricted. The ethics committee of the Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, approved the studies. Two groups of 3 animals were established: group 1 – rats were treated with AmB deoxycholate (Amphotericin B Squibb®, Bristol-Myers Squibb; 4 mg/kg of body weight daily for a period of 14 days), group 2 – included animals, which received ABCD (Amphocil®, Torrex Pharma; 12 mg/kg of body weight daily for a period of 14 days). The rats received AmB or ABCD ip. Water for injections (Bieffe Medital) served as a vehicle for the dilution of drugs. Animals were killed 24 hours after application of the last dose of AmB or ABCD. Tissue samples from kidneys were taken lege artis and processed for transmission electron microscopic study.

Stereometric evaluation was done and values of volume density of selected organelles in epithelial cells of proximal and distal tubules were statistically analysed[12]. Volume densities of organelles (nuclei, mitochodria, Golgi

apparatus, granular endoplasmic reticulum, pinocytic vesicles, vacuoles, lipid droplets, peroxisomes and lysosomes) were counted. Statistical procedures included calculation of mean values of volume density (VD), standard deviation (SD), and standard error of the mean (SEM). A simple Student's test was used to determine the significance of differences ($p < 0.05$ and $p < 0.01$).

RESULTS

Vacuolation of cytoplasm and some mitochondria, the presence of damaged mitochondria, increased number of peroxisomes (especially after AmB), dilated extracellular space between epithelium and basal lamina in basal labyrinth, reduction of microvilli in brush border were observed and described on the ultrastructural level in this study. This was found in both experimental groups.

Apart from the mitochondria, vacuoles and peroxisomes, volume density of the other followed organelles was not significantly different after administration of AmB or ABCD (Table 1).

Table 1. Volume densities of cell organelles

		VD (%) ± SD	
		proximal tubules	**distal tubules**
Nuclei	*AmB*	12.58 ± 3.84	13.54 ± 2.60
	ABCD	8.32 ± 2.67	13.83 ± 1.89
Mitochondria	*AmB*	13.42 ± 0.95	14.70 ± 4.82
	ABCD	16.86 ± 2.09	16.28 ± 0.67
Golgi apparatus	*AmB*	0.71 ± 0.27	0.55 ± 0.06
	ABCD	0.57 ± 0.18	0.68 ± 0.41
Granular endoplasmic reticulum	*AmB*	2.08 ± 0.42	2.55 ± 0.79
	ABCD	1.70 ± 0.05	1.92 ± 0.19
Pinocytic vesicles	*AmB*	3.25 ± 0.39	2.48 ± 0.29
	ABCD	3.33 ± 0.35	2.33 ± 0.06
Lipid droplets	*AmB*	0.67 ± 0.35	0.51 ± 0.12
	ABCD	0.78 ± 0.52	0.04 ± 0.04
Vacuoles	*AmB*	4.77 ± 1.83	2.48 ± 0.29
	ABCD	4.97 ± 3.94	0.36 ± 0.19 *
Peroxisomes	*AmB*	1.05 ± 0.45 *	1.33 ± 0.81 *
	ABCD	0.36 ± 0.01	0.32 ± 0.15
Lysosomes	*AmB*	0.81 ± 0.47	0.93 ± 0.48
	ABCD	0.61 ± 0.39	0.34 ± 0.17

The mitochondria did not show statistically significant differences in volume density, but morphological signs of their damage, more after AmB than after ABCD, were observerd. These signs were represented by the occurrence of enlarged mitochondria (somtimes to about 2.5 μm in diameter), some showed an indistinct inner structure or they lost their cristae and were vacuolated. The percentage of damaged mitochondria was established from total number of mitochondria found: in proximal tubules 46% (± 3.12) after AmB and 21% (± 1.08) after ABCD; in distal tubules 35% (± 2.40) after AmB and 17% (± 1.56) after ABCD. These differences were significant at $\alpha = 0.01$.

Statistically significant difference at the $p < 0.05$ level was found in density of vacuoles. The value was significantly lower in epithelial cells of distal tubules in animals after ABCD in comparison with AmB and values observed in proximal tubules after administration of both formulas. Significantly higher values of peroxisome density at $p < 0.05$ level were registered in the AmB group of animals. These findings were true for both types of tubules.

DISCUSSION

It is known, that AmB is a nephrotoxic drug and its influence on renal functions has been described by many authors[1, 4, 13]. According to[6, 14] AmB acts by binding to sterols, ergosterol in fungal membranes or cholesterol in biological membranes of mammalian cells. Some authors believe that AmB and other polyene antibiotic-induced renal toxicity is mediated by the drug anchoring to cholesterol within the mammalian cell membrane, resulting in pore formation, abnormal electrolyte flux, decrease in adenosine triphosphate, and eventually a loss of cell viability[8].

Colloid dispersion or lipid complex of AmB are considered to be more sparing on the kidney parenchyma and its functions[10, 11]. In regard to the volume density measurement, values of mitochondria in animals treated with AmB or ABCD did not show any statistically significant differences. However it is important to mention the morphological appearance of these organelles. Generally, more or less mitochondria were damaged after an administration of not only AmB, but also ABCD. Insignificantly higher values of pinocytic vesicles and lysosomes density in epithelial cells of renal tubules may reveal the fact that the resorptive function of tubules need not be irreversibly affected after either AmB and or ABCD administration. We observed, that nephrotoxicity of conventional AmB can be reduced after some nephroprotective measures, such as vigorous hydratation and ion supplementation proportionate to their loss via the kidney[6, 7]. The difference in values of volume density of vacuoles observed in proximal tubules was insignificantly higher in both drugs application, while in distal tubules there was significant difference between values after AmB and ABCD. It can be assumed, that the different values in distal tubules are not the result of the infuence of these formulas or that

the epithelium of proximal tubules is more sensitive to both formulas than the cells in distal tubules. Significantly higher values of peroxisomes density after AmB administration can be explained as a result of increased detoxicating activity in the cells.

ACKNOWLEDGEMENT

The study was supported by grant No. NL 6514-3/2001 of the Internal Grant Agency, Ministry of Health, Czech Republic.

REFERENCES

1. Cheng JT, Witty RT, Robinson RR, Yarger WE (1982) Amphotericin B nephrotoxicity: increased renal resistance and tubule permeability. Kidney Int *22*, 626–633.
2. Costa S, Nucci M (2001) Can we decrease amphotericin nephrotoxicity? Current Opin Crit Care *7*, 379–383.
3. Fisher MA, Talbot GH, Maislin G, Mc Keon BP, Tynan KP, Strom BL (1989) Risk factors for amphotericin B-associated nephrotoxicity. Am J Med *87*, 547–552.
4. Kavlock RJ, Rehnberg BF, Rogers EH (1985) Amphotericin B – and folic acid-nephropaties in developing rats. Toxicol Appl Pharmacol *81*, 407–415.
5. Krejčířová L, Lauschová I, Doubek M, Horký D, Mayer J, Doubek J (2003) Changes of rat kidneys after administration of Amphotericin B colloidal disperion and conventional Amphotericin B deoxycholate. Morphological observation. Scripta medica (Brno) *76*, 257.
6. Mayer J, Doubek M, Vorlíček J (1999) Must we really fear toxicity of conventional amphotericin B in oncological patients? Support Care Cancer *7*, 51–56.
7. Mayer J, Doubek M, Doubek J, Horký D, Scheer P, Štěpánek M (2002) Reduced nephrotoxicity of conventional amphotericin B therapy after minimal nephroprotective measures: animal experiments and clinical study. J Inf.ect Dis *186*, 379–388.
8. Ng AW, Wasan KM, et al. (2003) Development of liposomal polyene antibiotics: an historical perspective. J Pharm Pharm Sci *6*, 67–83
9. Terrel CL, Hughes CE (1992) Antifungal agents used for deep-seated mycotic infections. Mayo Clin. Proc. *67*, 69–91.
10. Walsh TJ, Hiemenz JW, Seibel NL, Perfect JR, Horwith G, Lee L, Silber JL, Dinubile MJ, Reboli A, Bow E, Lister J, Anaissie EJ (1998) Amphotericin B lipid complex for invasive fungal infections: analysis of safety and efficacy in 556 cases. Clin Infect Dis *26*, 1383–1396.
11. Walsh TJ, Finberg RW, Arndt C, Hiemenz J, Schwartz C, Bodensteiner D, Pappas P, Seibel R, Greenberg N, Dummer S, Schuster M, Holcenberg JS (1999) Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. N Engl J Med *340*, 764–771.
12. Weibel ER (1969) Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol *26*, 235–302.
13. Zager RA, Bredl CR, Schimpf BA (1991) Direct amphotericin B – mediated tubular toxicity: assesment of selected cytoprotective agents. Kidney Inter *41*, 1588–1594.
14. Zhang L, Zhang Y, Zhou Y, An S, Zhou Y, Cheng J (2002) Response of gene expression in Saccaromyces cerevisiae to amphotericin B and nistatin by microarrays. J. Antimicrob. Chemother. *49*, 905–915.