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SHORT COMMUNICATION

**Kishor M. Wasan · Joyce C. Wong · Traci Corr
Sheila Pritchard**

Role of plasma lipids and lipoproteins in predicting amphotericin B-induced nephrotoxicity in pediatric oncology patients

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Abstract *Purpose*: The objective of this study was to determine if total plasma and lipoprotein cholesterol (C) and triglyceride (TG) concentrations could predict the degree of nephrotoxicity caused by the antifungal agent amphotericin B (AmpB); and to use the average amount of potassium supplementation received daily as a indicator of nephrotoxicity in pediatric oncology patients. *Patients and methods*: Plasma samples from 18 patients (ages < 17 years) who were receiving AmpB due to suspected or confirmed fungal infection at British Columbia Children's Hospital were analyzed for lipid concentrations. The high density lipoprotein (HDL) fractions were separated by precipitation; total (TOT) plasma and fraction C and TG concentrations were measured by enzymatic colorimetric assays; and low density lipoprotein (LDL) C levels were determined by Friedewald's formula. Changes in serum creatinine levels from baseline and amounts of potassium supplementation were used as indicators of nephrotoxicity; both were obtained from patients' medical charts. Pearson correlation coefficients (r) were determined and considered significant if $P < 0.05$. *Results*: The total cumulative AmpB dose, adjusted for weight, does not seem to predict AmpB-induced nephrotoxicity. Positive but relatively weak correlations were found between total potassium supplementation and LDL C ($r = 0.489$, $P < 0.02$); and TOT C ($r = 0.551$, $P < 0.01$). In addition, a positive but relatively weak correlation between the average amount of potassium supplementation per day above baseline and HDL C ($r = 0.407$; $P < 0.02$) was observed. *Conclusion*: Differences in total plasma and LDL cholesterol concentrations may be used as predictors of AmpB-induced nephrotoxicity in pediatric oncology patients.

Keywords Lipoprotein · Pediatric oncology · Amphotericin B nephrotoxicity

K. M. Wasan (✉) · J. C. Wong
Division of Pharmaceutics and Biopharmaceutics,
Faculty of Pharmaceutical Sciences,
The University of British Columbia,
2146 East Mall Avenue, Vancouver,
BC CanadaV6T 1Z3,
E-mail: kwasan@interchange.ubc.ca
Tel.: +1-604-8224889
Fax: +1-604-8223035

T. Corr · S. Pritchard
Department of Hematology/Oncology/BMT,
Children's and Women's Health Centre of British Columbia
Vancouver, British Columbia CanadaV6H 3V4

Introduction

Amphotericin B (AmpB) remains one of the most effective and widely used agents for the treatment of systemic antifungal infections (e.g. *Candida albicans*, *Histoplasma capsulatum*, and *Aspergillus niger*), which are specifically prevalent among patients with cancer [1, 2]. Although several of the new azole agents have demonstrated efficacy in the treatment of fungal infections in adults [1, 2], the lack of experience with these compounds in children makes AmpB the current therapy of choice in this population at most institutions.

However, the clinical use of AmpB has been limited by nephrotoxicity, which can result in a significant decrease in glomerular filtration rate (GFR) and renal plasma flow leading to renal potassium and magnesium wasting [1–4]. In most adult cases, the underlying electrolyte imbalances caused by AmpB therapy can be treated with electrolyte supplements [1–4]; however, the long-term consequences of AmpB-induced renal tissue damage remains unclear. In most children, a severe loss in renal potassium further limits AmpB use [5, 6].

Recommendations pertaining to the dosage and administration of AmpB (Fungizone Intravenous, Bristol-Myers Squibb Canada Inc., Montreal, Canada), in the treatment of candidemia and cryptococcal meningitis have been standardized and are well accepted. A 1-mg test dose of AmpB is given over 30 min and, if tolerated, is followed by an infusion of 0.2 mg/kg on the first day. The dosage in then increased by 0.1–0.2 mg/kg/day until a therapeutic of maximum-tolerated dosage is reached,

usually 0.5–1.0 mg/kg/day in adults and 1.0–1.5 mg/kg/day in children[3–8]. However, it should be noted that in many institutions the test dose is often not given, especially in children.

Serum creatinine (SCr) has previously been used as an indicator for nephrotoxicity; however, it is a marker subject to misinterpretation [9]. Reductions in GFR of 20–40% may occur before significant increases in SCr can be detected [5]. Since children are still undergoing the process of organ development, SCr may not be the most accurate indicator of nephrotoxicity. However, children are extremely sensitive to renal potassium wasting, which results in abrupt changes in plasma potassium concentrations [5, 6]. Physicians frequently monitor these concentrations carefully during AmpB therapy to ensure they do not fall out of normal ranges (typically 3.5–5 mmol/l in children) and elicit severe consequences (e.g. heart and nerve malfunction) [5, 6]. To treat subnormal potassium concentrations, physicians administer potassium through various formulations. We could not use absolute potassium concentrations, as indicators of nephrotoxicity as this would require the complete abstinence of potassium treatment. Thus, we have chosen potassium supplementation as an additional indicator of nephrotoxicity.

We have previously shown that serum and plasma lipoproteins alter the pharmacokinetics and tissue distribution of a number of lipophilic compounds 16 including AmpB [10, 11]. We have observed that AmpB, which distributes into both serum high-density (HDL) and low-density (LDL) lipoproteins when incubated in human plasma for 1 h at 37°C [12, 13], is less toxic to pig renal endothelial cells (located in the proximal tubular region of the kidney) when associated with HDL, but equally toxic when associated with LDL as free AmpB [14]. However, when the cells were pretreated with trypsin, which decreased the number of high-affinity surface LDL receptors (LDLr), LDL-associated AmpB was less toxic to these cells than free AmpB. Since these cells exhibited both high- and low-affinity LDLr, but only low-affinity HDL receptors, we concluded that the nephrotoxicity associated with AmpB may be a result of its interaction with renal cells through the uptake of LDL-associated AmpB by the LDL receptors.

While these lipid/protein complexes are best known for their role in regulating plasma cholesterol metabolism [15], we have observed significant differences in the AmpB pharmacokinetics, serum lipoprotein and tissue distribution following its administration to hypercholesterolemic rabbits compared to normolipidemic controls [10, 16]. In addition, hypercholesterolemic rabbits exhibited enhanced AmpB-induced nephrotoxicity compared to controls [10, 16]. Thus, we hypothesized that the plasma level of LDL-cholesterol might modulate the nephrotoxicity of AmpB. In order to test this hypothesis, we conducted a preliminary study in adult cancer patients receiving AmpB and compared the amount of AmpB associated with plasma LDL-cholesterol to the relative kidney toxicity observed. We found enhanced AmpB kidney toxicity in these patients with elevated levels of LDL-cholesterol [11].

The objective of the present study is to determine the relationship between total plasma and lipoprotein cholesterol and triglyceride concentrations and the degree of AmpB-induced nephrotoxicity in children receiving AmpB, using both changes in SCr and the average amount of potassium supplementation administered throughout the duration of AmpB therapy. Our hypothesis was that patients with higher plasma LDL cholesterol (LDL C) levels were more suspectible to AmpB-induced nephrotoxicity than patients with lower plasma LDL C levels.

Patients and methods

Patient selection

The target population was all patients less than 17 years of age with an anticipated or confirmed diagnosis of a systemic fungal infection and a physician's order of AmpB therapy. At the time of study enrollment, all patients were from the Pediatric Oncology Unit at British Columbia Children's Hospital (Vancouver, BC, Canada). Parents and/or legal guardians of the children signed an informed consent form before participating in the study. The mean patient age was 8.8 years (range 1.2–15.8); 10 patients were males and 8 females. None of these patients were receiving cyclosporine A, cisplatin, gentamicin, vancomycin (known nephrotoxins) or liposomal AmpB therapy at the time of conventional AmpB therapy. Furthermore, none of the patients were previously administered conventional AmpB therapy. All patients had acceptable lipid levels as defined by the National Cholesterol Education Program for children and adolescents 2–19 years old. The SCr and potassium supplementation received were measured immediately prior to AmpB administration and daily until each patient received a cumulative AmpB dose of 6 mg/kg. This dose was chosen to represent an average duration of therapy for most patients. The duration of therapy for all patients was between 4 days and 12 days (Table 1). In addition, potassium supplementation was administered such that serum potassium levels were maintained between 3.5 mmol/l and 5.0 mmol/l on a daily basis. This was done to rule out the possibility that cumulative potassium supplementation reflected individual physician therapeutic choice and not renal tubular injury.

Sample collection

Prior to and each day of AmpB therapy, a 2-ml blood sample was obtained in a heparinized collection tube, centrifuged at 12,000 *g* for 5 min and stored at 4°C. Plasma was harvested from each blood sample and stored at −20°C until lipid analysis could be performed.

Table 1 Individual patient cumulative amphotericin B (AmpB) dose, changes in serum creatinine (SCr) and potassium supplementation and plasma cholesterol and triglyceride concentrations

Age (years)/sex	Cumulative AmpB dose[a] (mg/kg)	Serum Cr[b] (%)	Total K + [c] supplementation (mEq)	Average K + [d] supplementation (mEq/day)	Serum chol			Serum TG total (mg/dl)
					HDL (mg/dl)	LDL (mg/dl)	Total (mg/dl)	
7.8/F	6.0	19	−129	−2.4	47	32	94	71
3.7/F	5.8	25	158	8.5	19	9	58	148
5.3/M	5.9	−18	435	13.5	36	24	70	48
8.9/F	6.0	46	10	3.6	56	19	105	149
15.8/M	6.1	50	1,188	10.4	28	106	146	63
8.3/F	6.0	5	191	5.6	15	90	134	143
11.8/M	5.5	118	1,036	6.9	12	86	138	202
14.4/F	6.3	52	298	11.6	18	56	86	59
15.8/M	4.4	2	143	19.4	30	85	124	124
12.8/F	5.9	8	1,078	28.6	47	37	94	50
3.5/M	4.3	21	−430	−21.5	16	15	57	133
2.8/F	5.3	20	291	10.1	25	38	90	133
10.1/M	6.5	5	−9	−1.7	24	57	96	79
11/M	6.0	69	697	21.0	69	35	129	122
1.6/F	6.0	41	471	6.2	20	46	80	71
1.2/M	5.5	−43	9	0.8	5	44	78	149
15.8/M	6.2	22	41	0.2	28	44	91	95
8.4/M	4.3	8	595	5.9	42	98	164	116

AmpB amphotericin B, *Chol* cholesterol, *TG* triglycerides, *HDL* high-density lipoproteins, *LDL* low-density lipoproteins, K + , potassium; Cr, creatinine

[a]Cumulative AmpB dose to the day closest to 6 mg/kg

[b]Percent change in SCr concentration from the day prior to AmpB therapy to the day of 6 mg/kg cumulative dose

[c]Difference between cumulative amount of K + supplementation to the day of 6 mg/kg cumulative dose and the baseline amount on the day prior to AmpB therapy

[d]Difference between the average amount of K + supplementation per day to the day of 6 mg/kg cumulative dose and the baseline amount on the day prior to AmpB therapy

Lipoprotein separation

An aliquot of plasma was separated into its HDL (α)fraction by precipitation as previously described.[11]. Twenty microliters of precipitating reagent was added to 200 µl of plasma samples in eppendorfs, incubated at room temperature for 10 min, and microcentrifuged at 43,000 rpm for 30 min at 4°C.

Lipid measurement

Total cholesterol (TOT C), total triglyceride (TOT TG), and HDL cholesterol (HDL C) concentrations were determined using enzymatic cholesterol and triglyceride assay kits (Sigma Chemical, St. Louis, MO, USA) [10] . One milliliter of C or TG reagent was added to 10 µl of plasma sample, incubated at 37°C for 5 or 10 min, respectively, and scanned at 505 nm or 500 nm, respectively using an ultraviolet spectrophotometer. LDL C concentrations were obtained using Friedewald's Equation: LDL C = TOT C − (HDL C + TOT TG/5) mg/dl [11].

Renal function assessment

Renal function was determined by SCr levels and amount of potassium supplementation as obtained from patient medical charts. "Serum creatinine" in this paper refers to the percent change in SCr concentration from the day prior to AmpB therapy to the day of 6 mg/kg cumulative dose. "Potassium supplementation" was calculated by two methods (a) the total amount of potassium supplemented to the day of 6 mg/kg cumulative dose and (b) the difference between average amount of potassium supplementation per day to the day of 6 mg/kg cumulative dose and baseline amount on the day prior to AmpB therapy (Tables 1, 2). The latter method of calculating potassium supplementation was done to rule out that a change in supplementation was not a function of length of therapy but of total cumulative AmpB dose. Sources of potassium included total parenteral nutrition (TPN); IV infusion and oral potassium supplements. The percentage of each potassium supplement varied from patient to patient. For this study renal toxicity was defined as an increase of at least 50% in SCr compared to baseline (prior to AmpB therapy being initiated).

Statistical analysis

Correlations were made between two parameters in a nonnormal population. Thus, Pearson correlation coefficients (r) were calculated and considered significant if $P < 0.05$ (Table 2).

Table 2 Correlation coefficients (r) of total plasma and lipoprotein cholesterol and triglyceride concentrations versus SCr and potassium supplementation

Total or lipoprotein lipid component	Pearson correlation coefficient (r)		
	Serum Cr[a]	Total K + Suppl.[b]	Total K + Suppl.[c]
HDL cholesterol	0.144	0.200	0.407*
LDL cholesterol	0.173	0.489*	0.205
Total cholesterol	0.343	0.551**	0.314
Total cholesterol/HDL cholesterol	−0.098	0.038	−0.181
HDL cholesterol/total cholesterol	−0.009	−0.020	0.269
LDL cholesterol/total cholesterol	0.013	0.333	0.149
LDL cholesterol/HDL cholesterol	−0.043	0.142	−0.095
Total triglycerides	0.285	−0.150	−0.260

r Pearson correlation coefficient, *HDL* high-density lipoproteins, *LDL* low-density lipoproteins, Cr, creatinine; K + suppl., potassium supplementation

[a]Percent change in SCr concentration from the day prior to AmpB therapy to the day of 6 mg/kg cumulative dose

[b]Difference between cumulative amount of K + supplementation to the day of 6 mg/kg cumulative dose and the baseline amount on the day prior to AmpB therapy

[c]Difference between the average amount of K + supplementation per day to the day of 6 mg/kg cumulative dose and the baseline amount on the day prior to AmpB therapy

*$P < 0.01$; **$P < 0.02$

Results

The total cumulative AmpB dose using changes in SCr does not predict AmpB-induced nephrotoxicity (Table 1). A positive but relatively weak correlation was found between total potassium supplementation and LDL C ($r = 0.489$, $P < 0.02$); however, this correlation was not observed when SCr was used as an indicator of nephrotoxicity (Table 2). A positive but relatively weak correlation was found between total potassium supplementation and TOT C ($r = 0.551$, $P < 0.01$). This correlation was not observed when SCr was used (Table 2). Only a positive but relatively weak correlation was found between the average amount of potassium supplementation per day and HDL C (Table 2).

Discussion

In this study, we investigated whether total plasma and lipoprotein cholesterol and triglyceride concentrations could predict the severity of amphoterin B-induced nephrotoxicity. We discovered that the cumulative dose (up to 6 mg/kg) in relation to SCr and plasma lipoproteins does not predict amphotericin-B induced nephrotoxicity; however, the LDL C and TOT C concentrations may possibly predict nephrotoxicity when using potassium supplementation. In addition, we observed a positive correlation between HDL-cholesterol levels and the mean daily potasssium supplementation.

Three fairly recent studies of heterogeneous patient populations suggested that acute AmpB-induced nephrotoxicity is a function of total dose [17–19]. By contrast, Miller and Bates discovered no relationship between total dose and acute or permanent AmpB-induced nephrotoxicity; they also observed that this nephrotoxicity varied considerably in extent [20]. Our findings are similar to this observation, that is, the total cumulative AmpB dose using changes in SCr cannot predict the severity of AmpB-induced nephrotoxicity.

Previous studies have used SCr as an indicator of AmpB-induced nephrotoxicity; 12–15, however, we also used potassium supplementation as an indicator of nephrotoxicity. It must be emphasized that SCr is a relatively insensitive marker of renal function as GFR values can decline by up to 40% before changes in SCr are noticed. Thus, we have turned to potassium measurements as a more accurate indicator of renal function as these are extremely sensitive to changes in renal function, particularly in children. Since serum potassium values cannot be used as an indicator for reasons stated above, we have chosen potassium supplementation as an indicator of nephrotoxicity, given that physicians administer supplements immediately upon monitoring serum potassium values.

In this study, the positive correlation that was observed between total potassium supplementation (as an indirect measure of renal toxicity) and LDL C ($r = 0.489$, $P < 0.02$); and TOT C ($r = 0.551$, $P < 0.01$) (Table 1) supports our original hypothesis and confirms our previous work completed in animals [10, 16] and adult cancer patents [11]. However, the positive correlation between the average amount of potassium supplementation per day above baseline and HDL C ($r = 0.407$; $P < 0.02$) was unexpected and may be a function of the limited patient number. Taken together this data seems to suggest that changes in TOT C may be the most important determinant in predicting AmpB-induced renal toxicity independent of which pool (HDL and/or LDL) the cholesterol is associated with. Further studies would be required to confirm this hypothesis.

The negative values in the total and average potassium supplementation reported for several patients (Table 1) indicates that these patients received on average less potassium during AmpB therapy than they did

prior to AmpB therapy. Taken together with the lack of changes in SCr from baseline, this data suggests that AmpB treatment did not cause any renal toxicity.

One limitation of our study is a small sample size ($n = 18$). Given the homogenous population and the nature of the study, it was difficult to gather a large number of patient samples. In addition, 6 mg/kg cumulative dose is quite small and thus only a small number of patients had significant changes in SCr ($> 50\%$ increase in SCr from baseline; indicative of renal toxicity). In this small pilot study only 4/18 patients had SCr levels $> 50\%$ from baseline. However, the purpose of this study was to determine if total plasma and lipoprotein lipid concentrations could predict the degree of AmpB-induced nephrotoxicity. In order to test this hypothesis, we were able to enroll patients that exhibited a wide range of changes in SCr following AmpB treatment (Table 1). Future studies are currently underway to increase the sample size.

Another limitation of this study are the many variables, other than AmpB related renal dysfunction that can cause loss of potassium including the type of cancer, chemotherapy agents (i.e. carboplatin and methotrexate), fluid balance, acid-base balance and nutritional status. We were able to account for nutritional status and fluid balance in these studies by monitoring food and fluid intake. It should be noted that the patients served as their own control and we found that their food and fluid intake did not change before and during AmpB therapy. However, we could not account for the other potential variables. In addition, our model does not account for children that actually develop renal failure. However, we do feel that our model would be useful in limiting the number of children that develop AmpB-induced renal toxicity.

In conclusion, the relationship between total plasma and lipoprotein cholesterol concentrations and AmpB-induced nephrotoxicity in children could further aid in the development of a prognostic tool that would limit the excessive use of AmpB and prevent unwarranted AmpB-induced nephrotoxicity in children.

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