

Amphotericin B binding of Magnesium: Contribution to its toxicity, and therapeutic implications

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Zusammenfassung

Trotz ernster Nebenwirkungen ist Amphotericin B (AmB) weiterhin das Mittel der Wahl bei schweren Pilzinfektionen. Allein eingesetzt wird AmB oft nicht lang genug in ausreichend hohen Dosen vertragen, um die Infektion zu beherrschen. Es ist wiederholt mit wechselndem Erfolg versucht worden, die Toxizität zu senken. Aufgrund der Ähnlichkeit der klinischen, funktionellen und strukturellen Störungen nach parenteraler Gabe von AmB mit denen nach Magnesium-(Mg)-Verarmung im Tierversuch wird eine neue Möglichkeit vorgeschlagen. Es ist möglich, daß die Symptome eines Mg-Mangels bei mit AmB behandelten Patienten durch eine Inaktivierung von Mg erfolgt, das durch das Antibiotikum an das Cholesterin der Zellmembran gebunden wird. Bei entsprechend mit AmB behandelten Patienten könnten Mg-Gaben die Nebenwirkungen verhindern. Da jedoch die antifungale AmB-Wirkung auf einer Schädigung der Zellmembran beruht, die durch die Bindung mit Cholesterin und divalenten Kationen erfolgt, muß für die Verabreichung von Mg ein Weg gefunden werden, der nicht mit den therapeutischen AmB-Effekten interferiert. Da AmB auch bei Gabe in 2-tägigem Abstand wirksam ist, scheint der Versuch lohnend, an den dazwischenliegenden Tagen Mg zu verabreichen.

Summary

AmB is still the mainstay of treatment of serious fungal infections, despite its serious side effects. When used alone, it is often not tolerated in sufficiently high doses for long enough to eradicate the infection. There have been several approaches to reduction of its toxicity, with varying degrees of efficacy. A new possibility is suggested by the similarity of the clinical, functional, and structural abnormalities, caused by parenteral administration of AmB, to those produced by Magnesium depletion in experimental animals. It is possible that the manifestations of Magnesium depletion, in patients being treated with AmB, are caused by inactivation of Magnesium when it is bound by the polyene antibiotic to the cell membrane cholesterol. Administration of Magnesium to AmB treated patients might protect them from some of the adverse effects of the antifungal therapy. However, because the antifungal activity is mediated by the cell wall damage that occurs with binding of the polyene with ergosterol and divalent cations, a means of administration must be developed that will not negate the therapeutic effect. Since AmB retains its efficacy when given on alternate days, worth study is Magnesium administration on the days on which no AmB is given.

Résumé

L'Am. B est encore l'élément essentiel du traitement des infections fongiques graves, malgré ses effets secondaires sérieux. Quand elle est utilisée seule, elle n'est souvent pas tolérée à des doses suffisamment élevées pour entraîner une éradication de l'infection. Il y a eu plusieurs approches pour une réduction de sa toxicité, avec un degré varié d'efficacité. Une possibilité nouvelle est suggérée par la similitude des anomalies cliniques fonctionnelles et structurelles, provoquées par l'administration parentérale de l'Am. B, avec celles produites par une déplétion en Magnésium chez les animaux d'expérience. Il est possible que les manifestations de la déplétion

magnésique chez les patients qui sont traités par l'Am. B, soient provoquées par l'inactivation du Magnésium, quand celui-ci est lié par l'antibiotique polyénique au cholestérol membranaires cellulaires. L'administration de Magnésium aux patients traités par l'Am. B pourrait les protéger de certains des effets secondaires de la thérapeutique antifongique. Cependant, étant donné que l'activité antifongique admet comme médiateur une lésion de la paroi cellulaire qui se produit avec la fixation du polyène à l'ergostérol et aux cations bivalents on doit élaborer des procédés d'administration qui n'annulent pas l'effet thérapeutique. Du fait que l'Am. B conserve son activité lorsqu'elle est administrée à des jours alternés, il est valable d'étudier l'administration de Magnésium les jours où l'Am. B n'est pas administrée.

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Abstract

AmB is still the mainstay of treatment of serious fungal infections, despite its serious side effects. When used alone, it is often not tolerated in sufficiently high doses for long enough to eradicate the infection. There have been several approaches to reduction of its toxicity, with varying degrees of efficacy. A new possibility is suggested by the similarity of the clinical, functional, and structural abnormalities, caused by parenteral administration of AmB, to those produced by magnesium depletion in experimental animals. It is possible that the manifestations of magnesium depletion, in patients being treated with AmB, are caused by inactivation of magnesium when it is bound by the polyene antibiotic to the cell membrane cholesterol. Administration of magnesium to AmB treated patients might protect them from some of the adverse effects of the antifungal therapy. However, because the antifungal activity is mediated by the cell wall damage that occurs with binding of the polyene with ergosterol and divalent cations, a means of administration must be developed that will not negate the therapeutic effect. Since AmB retains its efficacy when given on alternate days, worth study is magnesium administration on the days on which no AmB is given.

Introduction

Many patients with serious fungal infections in inaccessible sites, such as the central nervous sys-

tem or in endocardial vegetations, are unable to tolerate requisite long-term toxic antifungal therapy. The polyene antibiotics, such as amphotericin B (AmB), are almost as toxic to the host as to the fungi. There have been many procedures employed to decrease the incidence and severity of adverse effects of parenterally administered AmB, long the therapeutic standby for serious fungal infections. Alternate day therapy, combination drug therapy, and use of agents shown to have some ameliorating effects on the manifestations of AmB toxicity have all been attempted, with varying degrees of success. The most promising approach, thus far, has been the combination of AmB with drugs with which it has synergistic activity [1—8]. The fact that side effects of AmB resemble those of serious magnesium deficiency, and that AmB inactivates magnesium by binding it to cell membranes, thereby removing it from the available body pool (*infra vide*), suggests that replacing the magnesium might protect against AmB toxicity. The major drawback to this approach is that AmB's antifungal activity may be dependent, in part, on cell damage caused by magnesium (ergosterol) polyene binding at the cell wall [9—13]. Since AmB is an effective antifungal agent when given on alternate days [14], it might be possible to develop a regimen whereby the toxicity of the drug to the host could be lessened without blocking its antifungal activity, by giving magnesium on the days free of AmB treatment. Before undertaking such therapeutic modification in the clinic, it should be investigated in infected experimental animals.

Mechanisms of amphotericin B toxicity

Functional and Structural Damage

The toxicity of AmB at the high doses needed for its use, alone, in serious fungal infections is an extension of the mechanism of polyene-antifungal activity to the cells of the host. Polyenes damage cell walls of fungi and cell membranes of the host (e.g. erythrocytes and renal tubular cells) by binding to the sterols: ergosterol of the fungi and cholesterol of mammalian cells, with resultant leakage of cellular constituents [9—13], alteration of membrane fluidity [15, 16], and ultimately cell death. AmB-induced renal malfunction and structural damage are well documented. Among functional changes are decreases in glomerular filtration rate [17—20], in renal blood flow [21, 22], and in urinary acidification [20] with renal tubu-

lar cellular acidosis [23—25]. Also seen are hypokalemia [20, 26, 27], and hypomagnesemia [20, 28, 29]. Hypomagnesemia occurs more often than hypokalemia but without increased Mg urinary excretion [30]. Renal lesions include tubular atrophy, intraluminal calcification, interstitial edema, fibrosis, and finally calcification around the tubules, and thickening of glomerular capillary and tubular basement membranes [17, 18, 21, 31—35].

Anemia, with marked decrease of hematocrit, is also frequently seen in patients receiving long-term and repeated courses of AmB [21, 28, 36]. This effect has been correlated with erythrocyte lysis produced *in vitro* by low concentrations of polyene antibiotics [12, 37], and with bone marrow suppression [21]. Cardiac arrhythmia has also been produced by AmB infusions [28]. High intravenous doses of AmB (2—5 mg/kg), given to dogs, have caused transient bradycardia, then tachycardia, elevation of T-waves, prolongation of PR, and shortening of QT intervals, and terminally aberrant ventricular complexes and fibrillation or arrest. Electrocardiographic studies in patients receiving long-term AmB showed development of prominent U waves.

Possible Mediating Role of Magnesium-Inactivation in AmB Toxicity

The divalent cations, Mg and calcium, participate in the polyene-sterol binding process [10] that damages the cell membranes of fungi and host. Possibly the binding of Mg, a vital cellular constituent, makes it unavailable for enzymatic activities, leading to a functional deficiency [38]. Support for this premise is the similarity of signs of Mg-depletion to the toxicities of AmB. Magnesium deficiency has interfered with urinary acidification [39], been implicated in clinical renal tubular acidosis [40], decreased urinary citrate excretion [41], and decreased renal blood flow [42, 43]. It has also long been known to cause refractory cellular and plasma potassium (K) depletion [38, 44—50]. Thus the hypokalemia associated with AmB toxicity might well be a consequence of its production of functional Mg deficiency. The renal lesions resemble those of Mg-depletion [46, 50—53], more than they do those of K-depletion [54, 55].

It is possible that Mg inactivation contributes also to the less frequently observed toxicities of AmB: hemolysis and arrhythmia. Erythrocyte membrane instability, as demonstrated by shortened survival time and tendency towards hemo-

lysis is seen in Mg deficiency [56]. Cardiac arrhythmias have been seen in Mg deficient animals and patients and reversed by Mg administration [57].

Protection against AmB Toxicity

Lowering AmB daily dosage [2] or giving it on alternate days [14] have been found to reduce its toxicity without notably impairing its curative effects in most instances. This approach has been made more applicable to very serious infections now that other antifungal agents are available, that gain access to the interior of the fungi that are damaged by low doses of AmB [1, 5].

Measures to decrease AmB-induced renal damage include correcting impaired renal hydrogen ion secretion, and counter-acting AmB reduced renal blood flow [1]. Because urinary citrate excretion is also decreased by AmB, urine alkalization with K-citrate has been suggested [20] to protect against tubular cellular acidosis, the tendency towards renal calcification, and hypokalemia. Bicarbonate has been utilized [24] to protect against renal tubular injury of acidosis [2]. Since I.V. mannitol improves renal blood flow in ischemic kidneys, it was given to dogs simultaneously with AmB (1 mg/Kg/L of dextrose and water over four to six hours) [58]. The dogs so treated had no demonstrable diminution in renal function, as measured by blood urea nitrogen and creatinine, and only minimal histological renal tubular changes, as compared with the changes in dogs given the AmB alone. Neither tolazoline nor hydralazine (each of which increases renal blood flow) ameliorated AmB nephrotoxicity. A patient given 50 mg AmB with 25 g mannitol I.V. on alternate days for five weeks had no diminution of renal clearance [58]. A modification of this regimen: 12.5 g of mannitol before and after I.V. AmB in doses up to 50 g, and totalling 775 to 3 000 mg [59] did not influence the blood urea nitrogen or creatinine, but produced some decrease in urinary acidification. A lower dosage AmB (17.5 mg/ml), given with mannitol (25 g) in a liter of 5% dextrose and water, was given thrice weekly to a patient with mucocutaneous candidiasis and renal impairment from calcemic therapy of infantile hypocalcemia with favorable results [60, 61]. However, since mannitol causes magnesiumuria [62, 63], its use with AmB might increase the risk of Mg depletion.

Possible Protection by Magnesium

Like mannitol, I.V. MgSO₄ causes renal vasodilation [64, 65]. Magnesium citrate might better protect against nephrotoxicity than the acid sulfate salt, in view of AmB impairment of citrate excretion [20]. However, before undertaking a clinical trial, the effect of Mg on AmB's antifungal activity must be ascertained. Magnesium [9, 10], participates in polyene-binding of fungal cell wall ergosterol and has interfered with the anticandidal effects of polyenes [16, 66, 67]. Conceivably, its administration at a time separate from that of AmB might protect the host without blocking antifungal activity. A regimen worth study is mannitol I.V. with AmB, Mg and K salts of citrate to be infused on alternate days — optimal amounts to be determined. In view of the K-sparing effects of Mg, it is possible that, with its use, less supplemental K might be necessary than the 100 mEq described as necessary by [20]. The amount of Mg, and its mode of administration for this use, will have to be determined.

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