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Antagonistic effect of amphotericin B on carboplatin antitumor activity in human osteosarcoma xenografts

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Amphotericin B (AmB), a polyene antifungal antibiotic, has been shown to potentiate the cytotoxic effect of different chemotherapeutic drugs *in vivo* and *in vitro*. The purpose of this study was to determine whether AmB could enhance the carboplatin antitumor activity in a human osteosarcoma xenograft model. Nude mice, bearing s.c. transplanted osteosarcoma xenografts, received i.p. an injection of AmB (5 mg/kg) 6 h prior to carboplatin (20 mg/kg) or each of the drugs separately. The effect of treatment was assessed by analyzing tumor growth delay and T/C ratio. Carboplatin clearly reduced tumor growth when administered alone. However, an unexpected interaction was seen where AmB significantly decreased the antitumor effect of carboplatin. The present results contradict some earlier *in vitro* studies and indicate the complexity of this interaction *in vivo*. Hence, it seems that interactive phenomena in one experimental model, and especially with regard to AmB, cannot be universally applied to all experimental situations.

Key words: Amphotericin B, carboplatin, interaction, nude mouse, osteosarcoma.

Introduction

Carboplatin (*cis*-diammine-1,1-cyclobutanedicarboxyplatinum) is developed as an analog of cisplatin with lower emetic potential and lower nephrotoxicity. It is used in the treatment of solid tumors and has been included in some chemotherapeutic protocols in the treatment of osteosarcoma.[1] Although the exact mechanism of action of carboplatin is not known, it is assumed that its antitumor activity is similar to cisplatin with binding to DNA and subsequent inhibition of DNA synthesis.[2]

Amphotericin B (AmB) is a macrolide polyene antifungal antibiotic that exerts its action by forming complexes with ergosterol molecules in the cell membrane. Insertion of AmB in the cell membrane leads to the formation of 4–10 Å pores, provokes a leakage of electrolytes and metabolites, and augments the cellular accumulation of chemotherapeutic drugs.[3] It has been shown that AmB can increase intracellular accumulation of carboplatin and augment the cytotoxic effect of this drug *in vitro*.[4,5]

The purpose of the present study was to investigate *in vivo* the interactive effects between AmB and carboplatin in human osteosarcoma xenografts.

Materials and methods

Mice and tumors

Female, *nu/nu* BALB/ca Bom, mice (Bomholtgård Breeding and Research Centre, Ry, Denmark), 6–12 weeks old, bearing s.c. transplanted xenografts from the eigth and ninth serial passages of a human osteoblastic (grade IV) osteosarcoma were used in the experiment. The mice were maintained under specific pathogen-free conditions at constant temperature (25 ± 0.5°C) and humidity (50%). Each experimental group consisted of eight to 11 mice. At the time of treatment the diameters of the tumors ranged from 5 to 7 mm. The study was approved by the Umeå University Ethical Committee for animal welfare.

Drugs

Carboplatin (Paraplatin®, Bristol-Myers Squibb, New York, NY) and AmB (Fungisone®, Bristol-Myers Squibb) were purchased as commercially available i.v. preparations. The mice were given i.p. injections of AmB 5 mg/kg 6 h prior to carboplatin 20 mg/kg or each of the drugs separately.

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Assessment of tumor growth

The tumors were measured at intervals during 33 days following treatment. The tumor volume was calculated for an ellipsoid with the formula $\pi \times a \times b \times c/6$, where a, b and c are the diameters in the three perpendicular planes.[6] The volume doubling time (VDT) was calculated by the formula $\log_2 \times (t_1 - t_0)/\log V_1 - \log V_0$. The volume V_0 was defined as the volume on day t_0, when the tumor was treated, and V_1 the volume on day t_1, which was chosen as the 33rd day after treatment. The growth delay (GD) was defined as the VDT of each treated tumor minus the mean VDT of the control group. Relative volume increase is presented as a percentage of pre-treatment volume, on day 33 after treatment. The T/C ratio was defined as the ratio of relative volume increase of each treated tumor to the mean relative volume increase of the control group.

Statistics

The data are presented as the mean ± SD. Statistical significance was tested using Wilcoxon rank sum W test. p values of less than 0.05 were considered to be significant.

Results

The mean relative volume increase of the control tumors was 12.1 ± 1.4 -fold, 33 days after treatment and VDT was 9.2 ± 0.4 days. Carboplatin reduced the mean relative volume increase to 7.9 ± 3.1 -fold ($p = 0.0119$ compared with controls) and prolonged the mean VDT to 12.4 ± 3.1 days ($p = 0.0028$ compared with controls) (Table 1).

In mice treated with AmB 6 h prior to carboplatin, the effect of carboplatin was significantly reduced resulting in a mean relative volume increase of 14.6 ± 6.7-fold ($p = 0.0134$ compared with carboplatin alone) and VDT of 9.1 ± 1.6 days ($p = 0.0043$ compared with carboplatin alone) (Table 1).

Tumor growth delay was significantly decreased ($p = 0.0043$) when AmB was combined with carboplatin (GD = −0.1 ± 1.6) as compared with carboplatin alone (GD = 3.2 ± 3.1) (Figure 1A).

The 33-day T/C ratio values were as follows: carboplatin alone, 0.67 ± 0.26; AmB alone, 1.01 ± 0.53; AmB + carboplatin, 1.21 ± 0.53 ($p = 0.0133$ compared with carboplatin alone) (Figure 1B).

No post-treatment morbidity was observed that could be related to the toxicity of the drugs.

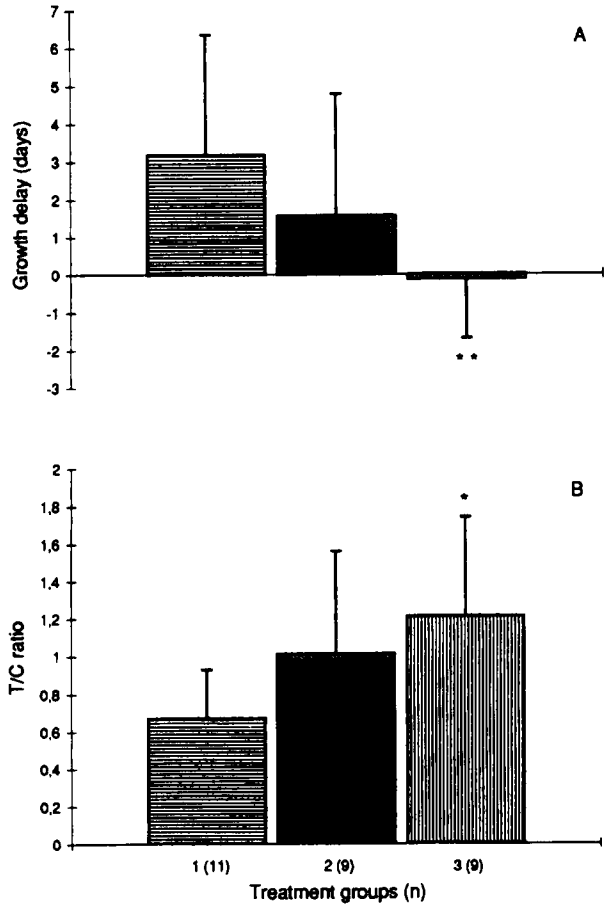

Figure 1. Effect of the combination of AmB and carboplatin on growth of osteosarcoma xenografts expressed as growth delay (A) and T/C ratio (B). Treatment groups: (1) carboplatin (20 mg/kg); (2) AmB (5 mg/kg); (3) AmB (5 mg/kg) + carboplatin (20 mg/kg). The data represent the mean ± SD. *$p < 0.05$ and **$p < 0.005$ compared with carboplatin alone. n = number of tumors.

Table 1. Volume doubling time (VDT) and relative volume increase (RVI) of the xenografts treated with AmB alone (5 mg/kg), carboplatin alone (20 mg/kg) or the combination of the drugs

Treatment groups	VDT (days)[a]	RVI (fold)[a]
Controls ($n = 8$)	9.2 ± 0.4	12.1 ± 1.4
Carboplatin ($n = 11$)	12.4 ± 3.1[b]	7.9 ± 3.1[c]
AmB ($n = 9$)	10.8 ± 3.2	12.3 ± 6.4
AmB + carboplatin ($n = 9$)	9.1 ± 1.6[d]	14.6 ± 6.7[e]

[a]Mean ± SD.
[b]$p < 0.005$ and [c]$p < 0.05$ compared with controls.
[d]Significantly ($p < 0.005$) shorter than carboplatin alone.
[e]Significantly ($p < 0.05$) higher than carboplatin alone.

Discussion

The main finding of the present study on human osteosarcoma xenografts is that AmB showed not only lack of potentiation, but also significantly reduced the antitumor effect of carboplatin. This is contradictory to the results of some previous studies.[4,5,7–10]

It is known from earlier *in vitro* studies that AmB can enhance the effect of several chemotherapeutic agents such as doxorubicin,[7] actinomycin D,[8] nitrogen mustard[9] and cisplatin.[10] The augmentation of the carboplatin cytotoxicity by AmB has been demonstrated in ovarian[4] and lung cancer[5] cell lines. It has been assumed that this property of AmB is connected to its effects on the cell membrane integrity and subsequent alteration in permeability followed by increased intracellular drug accumulation.[3] However, in the present study AmB significantly hampered carboplatin cytotoxicity in human osteosarcoma xenografts. The results of our study are in concordance with the observation of Sharp *et al.*[11] who reported the lack of potentiation of carboplatin cytotoxicity with AmB in ovarian carcinoma xenografts.

AmB is known to bind to serum protein, mainly lipoproteins.[3] Assem *et al.*[12] demonstrated on human colon carcinoma cell lines that the cell incubation in pure human serum instead of serum-free medium significantly inhibited the potentiating effect of AmB on cisplatin accumulation and cytotoxicity. This inactivation of the membrane effects of AmB in the presence of serum proteins could thus explain the different results from *in vitro* and *in vivo* studies, and consequently could explain disappointing effects of some clinical trials where AmB was administered with cyclophosphamid, lomustine and doxorubicine.[13,14] However, other experimental *in vivo* studies have shown that potentiation of some anticancer drugs (i.e. doxorubicin, melphalan) can be achieved with AmB.[15,16]

In our experimental osteosarcoma model, carboplatin showed significant reduction of tumor growth when administered alone. This sensitivity of the tumor to carboplatin could also be a possible explanation to the lack of sensitization with AmB, as proposed by Morikage *et al.*[5] who demonstrated *in vitro* that the synergistic effect of AmB with cisplatin and carboplatin could be achieved only in resistant but not in sensitive cell lines. However, the fact that AmB significantly reduced the effect of carboplatin in our tumor model indicates the complexity of this interaction *in vivo*. In contrast to cisplatin, carboplatin is primarily excreted via the kidneys.[17] It can be speculated that AmB may affect the kidney, resulting in increased excretion of carboplatin and decreased drug content in plasma as well as in tumor tissue.

Differences in scheduling of platinum drugs *in vivo* have made it difficult to compare the results of different experimental studies. Gately *et al.* reported that a cisplatin dose of 30 mg/kg in the nude mouse could be expected to generate the same degree of toxicity as a dose of 92 mg/m^2 in a human.[18] Although carboplatin has comparable antineoplastic activity but less toxicity than cisplatin we used a dose of 20 mg/kg which is lower than the equivalent doses in clinical trials. Due to the expected overlapping nephrotoxicity between carboplatin and AmB, we chose a lower dosage. No post-treatment morbidity was observed that could be related to the toxicity of the drugs indicating that the maximal tolerated dose of AmB and carboplatin had not been achieved in the nude mouse.

In summary, the present *in vivo* study on human osteosarcoma xenografts imply that interactive effects in general and especially with regard to AmB cannot be universally applied to all experimental situations, a fact that has also been emphasized by our earlier *in vitro* study[10] where AmB decreased the cytotoxic effect of epirubicin, bleomycin and estramustine while the effect of cisplatin was markedly increased.

References

1. Ettinger LJ, Gaynon PS, Krailo MD, *et al.* A phase II study of carboplatin in children with recurrent or progressive solid tumors. A report from the Childrens Cancer Group. *Cancer* 1994; **73**: 1297–301.
2. Van Echo DA, Egorin MJ, Aisner J. The pharmacology of carboplatin. *Semin Oncol* 1989; **16**: 1–6.
3. Brajtburg J, Powderly WG, Kobayashi GS, Medoff G. Amphotericin B: current understanding of mechanisms of action. *Antimicrob Agents Chemother* 1990; **34**: 183–8.
4. Kikkawa F, Kojima M, Oguchi H, *et al.* Potentiating effect of amphotericin B on five platinum anticancer drugs in human *cis*-diamminedichloroplatinum (II) sensitive and resistant ovarian carcinoma cells. *Anticancer Res* 1993; **13**: 891–6.
5. Morikage T, Ohmori T, Nishio K, Fujiwara Y, Takeda Y, Saijo N. Modulation of cisplatin sensitivity and accumulation by amphotericin B in cisplatin-resistant human lung cancer cell lines. *Cancer Res* 1993; **53**: 3302–7.
6. Tomayko MM, Reynolds CP. Determination of subcutaneous tumour size in athymic (nude) mice. *Cancer Chemother Pharmacol* 1989; **24**: 148–54.
7. Krishan A, Sauerteig A, Gordon K. Effect of amphotericin B on adriamycin transport in P338 cells. *Cancer Res* 1985; **45**: 4097–102.
8. Vertut-Croquin A, Brajtburg J, Medoff G. Two mechan-

isms of synergism when amphotericin B is used in combination with actinomycin D or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea against the human promyelocytic leukemia cell line HL-60. *Cancer Res* 1986; **46**: 6054–8.

9. Presant CA, Carr D. Amphotericin B (Fungizone) enhancement of nitrogen mustard uptake by human tumor cells. *Biochem Biophys Res Commun* 1980; **93**: 1067–73.
10. Bergström P, Grankvist K, Holm S, Henriksson R. Effects of antimicrobial drugs on the cytotoxicity of epirubicin, bleomycin, estramustine and cisplatin. *Anticancer Res* 1991; **11**: 1039–44.
11. Sharp SY, Mistry P, Valenti MR, Bryant AP, Kelland LR. Selective potentiation of platinum drug cytotoxicity in cisplatin-sensitive and -resistant human ovarian carcinoma cell lines by amphotericin B. *Cancer Chemother Pharmacol* 1994; **35**: 137–43.
12. Assem M, Bonvalot S, Beltramo JL, *et al.* Deleterious effect of serum proteins on the amphotericin B-induced potentiation of cisplatin in human colon cancer cells. *Br J Cancer* 1994; **70**: 631–5.
13. Chabot G, Pazdur R, Valeriote FA, Baker LA. Pharmacokinetics and toxicity of continuous infusion Amphotericin B in cancer patients. *J Pharm Sci* 1989; **78**: 307–10.
14. Presant GA, Multhauf P, Metter G. Reversal of cancer chemotherapeutic resistance by amphotericin B. A broad phase I–II pilot study. *Eur J Cancer Clin Oncol* 1987; **23**: 683–7.
15. Medoff G, Valeriote F, Dieckman J. Potentiation of anticancer agents by amphotericin B. *J Natl Cancer Inst* 1981; **67**: 131–5.
16. Valeriote F, Dickman J, Chabot G. Schedule-dependent potentiation of lomustine cytotoxicity by amphotericin B in mice. *J Natl Cancer Inst* 1986; **76**: 521–5.
17. Chatelut E, Canal P, Brunner V, *et al.* Prediction of carboplatin clearance from standard morphological and biological patient characteristics. *J Natl Cancer Inst* 1995; **87**: 573–80.
18. Gately DP, Jones JA, Christen R, Barton RM, Los G, Howell SB. Induction of the growth arrest and DNA damage-inducible gene GADD153 by cisplatin *in vitro* and *in vivo*. *Br J Cancer* 1994; **70**: 1102–6.

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