

Short reports

Antimicrobial activity of cytotoxic drugs may influence isolation of bacteria and fungi from blood cultures

V Peiris, B A Oppenheim

Abstract

The potential antimicrobial activity of cyclophosphamide, vincristine, and adriamycin against Gram positive and negative bacteria and *Candida albicans* was examined. The time taken for different microbial inocula to turn a simulated blood culture positive in the presence of different concentrations of these drugs was measured. Doxorubicin retarded the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus sanguis* in a concentration dependent manner. Cyclophosphamide and vincristine showed minimal antimicrobial activity. *Escherichia coli* and *Pseudomonas aeruginosa* were unaffected by any of the drugs. An inoculum dependent effect was seen with some combinations of microbial inocula and cytotoxic drug concentrations.

(J Clin Pathol 1993;46:1124-1125)

Infections occur commonly in patients with cancer who have received chemotherapy, particularly if this has resulted in neutropenia. Many of these episodes, however, are fevers of unknown origin with negative blood cultures.¹ The reasons for this failure to isolate pathogens are often not known but may be due to the problem of recovering low numbers of organisms, fastidious organisms, which fail to grow using conventional culture media, or the presence of inhibitory substances in specimens.

Some cytotoxic agents have been shown to have antimicrobial activity^{2,3} although the clinical relevance of this has not been determined. Hopfer *et al*⁴ found that dacarbazine and 5-fluorouracil inhibited the growth of various organisms in Bactec 6B bottles.

Using a simulated blood culture system we investigated the action of three commonly used cytotoxic agents against the most frequently isolated organisms causing bacteraemia in neutropenic patients.⁵

Methods

Each drug was tested at three concentrations: one that reflected expected peak concentrations in adult patients; one 10-fold higher;

and one 10-fold lower. Cyclophosphamide was tested at 1, 10, and 100 µg/ml, based on human pharmacokinetic studies using a gas-liquid chromatography assay of its tri-fluoroacetyl derivative. Vincristine was tested at 1, 10, and 100 µg/ml, based on human pharmacokinetic studies using a radioimmunoassay. Doxorubicin was tested at 0.1, 1, and 10 µg/ml, based on data supplied by the manufacturers. All drugs were stored and prepared for use as recommended by the manufacturers. Dilutions used against any single organism were prepared in a single batch.

One isolate from each of the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus sanguis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* obtained from blood cultures of cancer patients were used in the study. At least three consecutive 10-fold dilutions of 20 hour broth cultures of each isolate were used to inoculate the bottles. The number of cells in each of the dilutions actually used was determined by a surface count technique on blood agar plates.

A glass universal container with 18 ml of nutrient broth was used as the blood culture bottle. Each bottle received 1ml of a dilution of cytotoxic drug and 1ml of an appropriate suspension of organism. Controls were prepared with: (1) saline replacing both drug and organism; (2) saline replacing drug; and (3) saline replacing organism. Bottles were then incubated at 37°C and observed for turbidity at 12 hour intervals for up to 84 hours. At the end of this time a selection of bottles, including controls, were subcultured to check that negative bottles were sterile and positive ones contained only the organism inoculated. Every combination of organism-inoculum and cytotoxic concentration was repeated three times to ensure reproducibility. The mean time to turn positive was recorded.

Results

The control bottles without cytotoxic drugs were positive within 24 hours except for low inocula of *S sanguis* which took up to 36 hours. *S aureus* and the Gram negative organisms generally gave earlier positive results than *S epidermidis* and *C albicans*.

Growth of *E coli* and *Ps aeruginosa* was not affected by any of the cytotoxic drugs.

Department of
Microbiology and
Public Health
Laboratory,
Withington Hospital,
Manchester M20 8LR
V Peiris
B A Oppenheim

Correspondence to:
Dr V Peiris

Accepted for publication
1 July 1993

Mean number of hours for simulated blood cultures to become positive for various cytotoxic drug concentrations and inocula of organisms

	Log ₁₀ dilution of 20 hour broth culture used for inoculum	Range of cfu in inoculum used	Cyclophosphamide			Vincristine			Doxorubicin			Growth control
			0.1 × P	P*	10 × P	0.1 × P	P*	10 × P	0.1 × P	P*	10 × P	
<i>S aureus</i>	-6	10-75	12	12	12	12	12	12	12	12	36	12
	-7	2-30	12	12	12	12	12	12	12	12	36	12
	-8	1-10	12	16	12	20	16	12	12	24	NP	12
<i>S epidermidis</i>	-6	50-80	16	16	20	12	20	20	24	24	NP	24
	-7	5-10	24	24	24	24	24	24	24	20	NP	24
	-8	0-2	24	24	30	24	NP	24	24	NP	36	24
<i>E coli</i>	-6	200-400	12	12	12	12	12	12	12	12	12	12
	-7	25-55	12	12	12	12	12	12	12	12	12	12
	-8	1-10	12	12	12	12	12	12	12	12	12	12
<i>Ps aeruginosa</i>	-6	290-450	12	12	12	12	12	12	12	12	12	12
	-7	50-85	24	24	24	24	24	24	24	18	24	24
	-8	0-4	24	24	24	24	24	24	24	24	24	24
<i>S sanguis</i>	-4	NE	24	20	24	20	24	24	20	24	12	20
	-5	204-222	20	20	20	24	20	20	16	24	NP	24
	-6	19-35	32	24	24	24	28	24	24	32	NP	32
<i>C albicans</i>	-7	1-4	36	24	24	32	24	28	40	24	NP	36
	-4	106-137	12	20	20	12	12	20	24	20	20	16
	-5	14-18	20	20	20	20	20	20	24	24	24	16
	-6	3-4	12	12	30	12	18	20	24	12	18	24
	-7	0-5	24	NP	12	24	24	NP	24	24	24	24

*P = notional peak serum concentration

NP = not positive after 84 hours

NE = not examined

Doxorubicin showed the greatest inhibitory effects, delaying growth of *S aureus*, *S epidermidis*, *S sanguis* and *C albicans*. Vincristine and cyclophosphamide showed minimal increases in the time to turn positive (table). Considerable delays were mainly shown at high concentrations of drug and using low inocula of organism.

Discussion

Our results confirm the findings that certain cytotoxic agents may delay or prevent in vitro growth of micro-organisms, and show that this is particularly important for Gram positive bacteria, such as coagulase negative staphylococci and viridans streptococci, organisms which are becoming increasingly prominent as causes of infection in neutropenic patients.⁶

We should be aware that other cytotoxic agents, many of which were discovered as potential antibiotics, may display some of the activities we have demonstrated. In addition, synergistic or antagonistic interactions might occur when multiple cytotoxic drug or cytotoxic-antibiotic drug combinations are used in a similar manner to that seen with some antimicrobial drugs.

As courses of chemotherapy usually precede periods of high risk of infection, any antimicrobial activity that a cytotoxic agent possesses may be less relevant as treatment continues. Dilution of samples of blood by their collection into larger volumes of broth in blood culture bottles may be sufficient to mitigate any antimicrobial activity that may be present, as well as any other inhibitory factors that may be present in whole sampled blood. The possibility that blood products may interact with the drug or organisms used would need to be explored in further studies.

- 1 Rogers T R. Investigation of infection in immunocompromised patients. *Br J Haematol* 1985;61:195-201.
- 2 Bodet C A, Jorgensen J H, Drutz D J. Antibacterial activities of antineoplastic agents. *Antimicrob Agents Chemother* 1985;28:437-9.
- 3 Hamilton-Miller J M T. Antimicrobial activity of 21 antineoplastic agents. *Br J Cancer* 1984;49:367-9.
- 4 Hopfer R, Moore D G, Fainstein V, Watkins S, Wenglar M. Routine use of BACTEC 16B bottles to remove antibacterial and anti-tumor agents from blood cultures of cancer patients. *J Clin Microbiol* 1979;18:759-64.
- 5 Chan C C, Oppenheim B A, Anderson H, Scarffe J H. Randomised trial comparing ciprofloxacin plus netilmicin versus piperacillin plus netilmicin in empiric treatment of fever in neutropenic patients. *Antimicrob Agents Chemother* 1989;33:87-91.
- 6 Burden A D, Oppenheim B A, Crowther D, et al. Viridans streptococcal bacteraemia in patients with haematological and solid malignancies. *Eur J Cancer* 1991;27:409-11.