THE EFFECTS OF THE POLYENE ANTIBIOTIC MEPARTRICIN ON POLYMORPHONUCLEAR LEUCOCYTE FUNCTION: AN IN-VITRO STUDY

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The normal defence mechanisms against infection with Candida albicans include opsonisation, phagocytosis, and subsequent killing by an oxidative mechanism involving hydrogen peroxide and myeloperoxidase. Genetic defects in either peroxide production (Oh et al., 1969) or myeloperoxidase activity (Lehrer and Cline, 1969a) result in defective killing by polymorphonuclear leucocytes (PMN). The functional integrity of the PMN is thus a requirement for elimination of Candida, and drugs to be used in topical or systemic therapy should not inhibit these phagocytic cells. With this in mind we have studied the possible effects of the polyene antibiotic mepartricin (Bruzzese, Cambieri and Recusani, 1975), which is active against Trichomonas and Candida, on PMN functions in vitro. Like other polyene antibiotics, mepartricin interacts with the sterols present in cell membranes (Pellegrini, Ruozzi and De Bernardi, 1974) causing a rapid drop in oxygen consumption by fungal cells (Ritzerfeld, 1972) and changes in the external layers of the cell wall (Ruozzi, Zara and Pellegrini, 1974).

MATERIALS AND METHODS

Mepartricin (MP) was supplied by Società Produzione Antibiotici (SPA), Milan. Normal human serum (NHS) was from a pool of sera from 10 donors divided into small samples and kept in liquid nitrogen until use. Human leucocytes were obtained from heparinised venous blood by erythroprecipitation (1% w/v dextran, at 37°C for 40 min.) and centrifugation (1000 g for 10 min.) in phosphate buffered saline (PBS, Oxoid) at room temperature, and the cells were resuspended in Hanks’s medium at a concentration of 10⁷ PMN/ml. C. albicans, a strain from a hospital source, was maintained on Sabouraud’s agar slants, and was harvested from Sabouraud’s broth while still showing active growth and resuspended in PBS (10⁸ cells/ml). Baker’s yeast was prepared by the method of Lachman, Hobart and Aston (1973) and stored in PBS at 4°C at a concentration of 10⁹ cells/ml.

Opsonisation of Candida or baker’s-yeast cells by normal serum was carried out in plastic tubes; 10⁷ cells were incubated for 30 min. at 37°C with serum 10% v/v in PBS. The cells were then washed twice in PBS and stored at 4°C until use. The opsonisation index was the average number of intracellular yeasts per PMN. Phagocytosis and nitro-blue tetrazolium (NBT, British Drug Houses) reduction were determined by the method of Preisig and Hitzig (1971) modified for use with PMN attached to polystyrene rather than as cell suspension; 10⁵ PMN in 2 ml Hanks’s solution were incubated in small Petri dishes (Nunc, Tissue Culture Dishes, 26 mm) at 37°C for 30 min. The dishes were rinsed with PBS at 37°C, and 2 ml of PBS (adjusted to pH 6.8 with HCl) containing 10⁷ opsonised fungal cells and 1 mM NBT were added. After 20 min. at 37°C the dishes were rinsed with PBS, air dried, fixed with methanol for 30 s, stained with safranin, rinsed with water, and air dried. About 150 PMN

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were then inspected \((\times 1000,\) oil immersion) in each dish. Three indices were then calculated: the proportion of the PMN containing yeasts (PhF), the average number of intracellular particles (PhI), and the proportion of the PMN with evidence of phagocytosis that also showed reduction of NBT (NRF).

Intracellular killing of \textit{Candida} was assayed by the method of Lehrer and Cline (1969\textit{b}); this is based on the recognition of dead \textit{Candida} cells by their uptake of methylene blue and counts were made at 60 and 120 min.

Increased metabolic activity associated with phagocytosis was measured as the cyanide-resistant production of \(^{14}\text{CO}_2\) from \(^{14}\text{C}-1\)-glucose (Amersham, UK; specific activity 0·1 \(\mu\text{Ci}/\mu\text{mol}; 0·07 \mu\text{Ci}/\text{assay}; 5 \times 10^5\) PMN/assay) in the presence and in the absence of latex (Keusch and Douglas, 1973).

The minimal inhibitory concentration of mepartricin on \textit{C. albicans} in Sabouraud's medium is less than 0·1 mg/litre as measured on 18-h cultures from a small inoculum (10^3 \textit{Candida} cells/ml at 30°C, with agitation).

The minimal dose of mepartricin which causes some killing (\(\approx 10\%\)) in the conditions of the intracellular killing test at 60 min. and in the absence of PMN is 3 mg/litre.

\section*{RESULTS}

The effects of mepartricin on serum opsonisation of fungal cells were investigated; table I shows the data from a typical experiment in which fungal cells were treated with serum, washed and then added to human PMN adhering to polystyrene. The serum opsonic activity, measured by the opsonisation index, was destroyed by heating the serum at 56°C for 30 min. or by the addition of 2 mM ethylenediamino-tetra-acetic acid sodium salt (EDTA) or ethyleneglycol (2-amino ethyl) tetra-acetic acid sodium salt (EGTA); the addition of 4 mM Mg_2SO_4 to EGTA-treated serum (but not to EDTA-treated serum) restored the opsonic activity, suggesting the involvement of the alternative pathway of complement (Fine et al., 1972). The addition of mepartricin up to concentrations of 100 mg/litre was without effect on the opsonic activity of normal human serum and of EGTA-Mg\textsuperscript{++}-serum.

PMN on polystyrene surfaces ingest opsonised particles and reduce NBT. Both these processes were unaffected by mepartricin concentrations up to 100 mg/litre (table II). Phagocytosis-dependent enhancement of glucose oxidation by the hexose monophosphate shunt also proved insensitive to high concentrations of mepartricin (table II).

\begin{table}[h]
\centering
\caption{Lack of effect of mepartricin upon serum opsonic activity on fungal cells}
\begin{tabular}{|l|c|c|}
\hline
Treatment of fungal cells & \textit{Candida} & \textit{baker's yeast} \\
\hline
None & 0·30 & 0·25 \\
10\% NHS\textdagger & 4·75 & 3·95 \\
10\% NHS & 4·55 & 3·85 \\
\text{+ MP}\text{ 10 mg/litre} & 4·75 & 4·00 \\
10\% NHS & 4·80 & 3·80 \\
\text{+ MP}\text{ 100 mg/litre} & 4·65 & 3·90 \\
10\% NHS & 4·65 & 3·90 \\
\text{+ EGTA}\text{+ Mg\textsuperscript{++}} & 4·65 & 3·90 \\
10\% NHS & 4·65 & 3·90 \\
\text{+ EGTA}\text{+ Mg\textsuperscript{++}} & 4·65 & 3·90 \\
\hline
\end{tabular}
\end{table}

\begin{flushleft}
* Opsonisation index = average number of intracellular yeasts per PMN.  \\
\textdagger NHS = normal human serum; MP = mepartricin.
\end{flushleft}
TABLE II
Effect of mepartricin upon phagocytosis by PMN leucocytes, NBT reduction, and phagocytosis-dependent enhancement of glucose oxidation by the hexose-monophosphate shunt

<table>
<thead>
<tr>
<th>Mepartricin (mg/litre)</th>
<th>Phagocytosis PhF*</th>
<th>PhI*</th>
<th>NBT reduction NRF*</th>
<th>Glucose oxidation P/R†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.92</td>
<td>4.78</td>
<td>0.91</td>
<td>6.2</td>
</tr>
<tr>
<td>10</td>
<td>0.93</td>
<td>5.00</td>
<td>0.89</td>
<td>5.9</td>
</tr>
<tr>
<td>30</td>
<td>0.90</td>
<td>4.72</td>
<td>0.95</td>
<td>6.1</td>
</tr>
<tr>
<td>100</td>
<td>0.93</td>
<td>4.90</td>
<td>0.94</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* PhF = proportion of PMN containing yeasts; PhI = average number of intracellular particles; NRF = proportion of the PMN with evidence of phagocytosis that also showed reduction of NBT. † P/R = ratio of the ¹⁴CO₂ (c.p.m.) released by phagocytosing (P) and by resting (R) leucocytes.

Pretreatment of Candida cells for 60 min. at 37°C with low concentrations of mepartricin (up to 3 mg/litre) enhanced to some extent their susceptibility to PMN killing (figure, a). Conversely, addition of high doses of mepartricin (up to 50 mg/litre) to the system in which PMN have already phagocytosed Candida cells did not substantially alter the rate of killing (figure, b). There was no additive effect of mepartricin with human PMN; presumably the drug did not enter the human cells. Experiments with PMN from a patient with chronic granulomatous disease (CGD) have confirmed this hypothesis; with CGD leucocytes (figure, b) the intracellular killing of Candida was markedly reduced even in the presence of large doses of mepartricin.

FIGURE—Effects of mepartricin on candida killing by PMN leucocytes. CK is the proportion of candida cells stained with methylene blue.
(a) Serum-treated candida cells were incubated with the indicated doses of mepartricin for 60 min. at 37°C. Human PMN (PMN: Candida = 1 : 1) were then added and CK was determined at 60 min. (○) and at 120 min. (△). A control without PMN (120 min. ●) was used.
(b) Human PMN were incubated with candida cells (PMN: Candida = 1 : 1) for 10 min. at 37°C; the indicated doses of mepartricin were then added and CK was determined at 120 min. PMN from a healthy control (△), from a CGD patient (□); control without PMN (●).
DISCUSSION

Mepartricin was found to be without toxic effects for human polymorphonuclear leucocytes at levels up to 100 times greater than its minimal inhibitory concentration for C. albicans; phagocytosis and intracellular killing by PMN were unaffected by the drug. Some synergism between the antifungal activities of mepartricin and human PMN was shown when cells of Candida were pre-incubated with sub-lethal concentrations of the drug. Mepartricin seems to be unable to enter the human cells because after cells of Candida were phagocytosed there was no enhancement of the killing even at high doses of the drug. Experiments with PMN from a patient with chronic granulomatous disease have confirmed this finding. Another polyene antibiotic, amphotericin B, has however been shown to exert a "non-specific potentiation" of host resistance (Thomas, Medoff and Kobayashi, 1973) and stimulates in vitro the bactericidal activity of peritoneal macrophages from untreated mice (Lin, Medoff and Kobayashi, 1977). The explanation for the different behaviour of the two drugs is unknown.

SUMMARY

Mepartricin, a polyene antibiotic with candidacidal and trichomomicidal activity, was found to be without toxic effects for human polymorphonuclear leucocytes; the drug seems to be unable to enter the human cells. Some synergism between the antifungal activities of mepartricin and of human leucocytes is seen if Candida cells are pre-incubated with sub-lethal concentrations of the drug.

REFERENCES