

Inhibitory effect of miconazole on mitogen-induced lymphocyte proliferative responses

Miconazole is a synthetic imidazole with broad-spectrum antimicrobial activity, which has potential value for treating systemic mycotic infections.^{1,2} Recent reports indicate that some antimicrobial agents, such as rifampicin³ and co-trimoxazole,⁴ may have immunosuppressive properties. Patients with deep mycoses are usually having immunosuppressive treatment, and further immunosuppression produced by antimycotic agents may be detrimental to recovery from infection. We have therefore studied the effects of miconazole on mitogen-induced human lymphocyte proliferative responses, the in-vitro correlates of immunocompetence.

Materials, methods, and results

A stock solution of miconazole was prepared by dissolving 5 mg of the drug in 1 ml propylene glycol and stored at 4°C. For these experiments further dilutions were made with RPMI 1640 tissue culture medium, and the pH was maintained close to 7.4. Preliminary studies showed that lymphocyte viability, as assessed by trypan blue dye exclusion, was not affected by miconazole in the concentrations used in these experiments for as long as four days in culture. Moreover, the diluent propylene glycol at the concentration of 0.2% did not inhibit lymphocyte transformation.

Lymphocytes were purified from heparinised blood of healthy adult donors by Hypaque-Ficoll centrifugation. They were washed three times and resuspended in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum.

Lymphocyte transformation studies were performed by a microtechnique.⁵ All cell cultures were performed in sterile microtitre plates. Each round-bottom well received 2×10^5 lymphocytes in 0.2 ml of medium and either phytohaemagglutinin (PHA) or pokeweed mitogen (PWM) in the final concentrations of 1 µg/ml and 50 µg/ml, respectively. These concentrations of mitogens had been determined to produce optimal stimulation. To the test wells was added miconazole nitrate dissolved in 0.02 ml of medium to reach final concentrations of 1 µg, 5 µg, and 10 µg/ml; control wells received 0.02 ml medium containing appropriate concentrations of the diluent only. All experiments were performed in triplicate.

The cultures were incubated for 72 hours at 37°C in a 5% CO₂ atmosphere and high humidity. Six hours before harvesting, 1 µCi of ³H-thymidine was added to each well. Harvesting was performed with the aid of a Skatron multiple sample harvester.

Additional experiments were performed in which lymphocytes were incubated in 10 µg/ml of miconazole nitrate for one hour and then washed three times before culture with PHA. A separate set of experiments was also performed to determine the effects of the drug when added to cultures at four hours, 24 hours, 48 hours, or immediately after the addition of mitogens.

Pronounced dose-dependent suppression of lymphocyte transformation was observed (see table). At miconazole concentrations of 1 µg/ml, 5 µg/ml, and 10 µg/ml, percentage inhibition of ³H-thymidine uptake was 12.6, 67.0, and 99.1 for PHA-stimulated cultures and 26.0, 76.2, and 97.8 for PWM-stimulated cultures.

Effect of varying concentrations of miconazole on mitogen-induced lymphocyte proliferative responses

Miconazole (µg/ml)	PHA ³ H-thymidine uptake (cpm)	PWM ³ H-thymidine uptake (cpm)
0	43 388 ± 15 395	14 965 ± 6 902
1	36 668 ± 9 449*	9 808 ± 4 472†
5	15 959 ± 6 749†	3 268 ± 1 146†
10	387 ± 272†	236 ± 71†

The results represent mean ± SD of seven experiments using seven donors. *P<0.05. †P<0.001. ‡P>0.05.

The inhibitory effect of miconazole was not reversed by washing; percentage inhibition of washed cells was 99.2 and of unwashed cells 99.6. Miconazole may therefore bind irreversibly to receptors in lymphocytes. The inhibitory effect was quite substantial even when the drug was added four hours (91.8%) or 24 hours (57.5%) after the start of the experiment, but not 48 hours (10.5%).

Discussion

These results indicate that miconazole has a potent inhibitory effect on mitogen-induced lymphocyte proliferative responses, at concen-

trations (1-10 µg/ml) reached during systemic treatment for deep mycotic infections.²

Since recovery from severe mycotic infections may need at least minimal immune responses, this immunosuppressive property of miconazole may be undesirable. The problem is compounded because deep mycotic infections usually occur in patients with cancer or other chronic diseases who are already receiving immunosuppressive drugs. Further studies are needed to examine the effect of miconazole on the immune system and find out how it works.

Miconazole was kindly provided by Dr Leon E Harris of Ethnor Pty Ltd, New South Wales, Australia.

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Department of Paediatrics, University of Adelaide, Adelaide Children's Hospital, North Adelaide 5006, South Australia

Y H THONG, MB, FAAP, senior lecturer

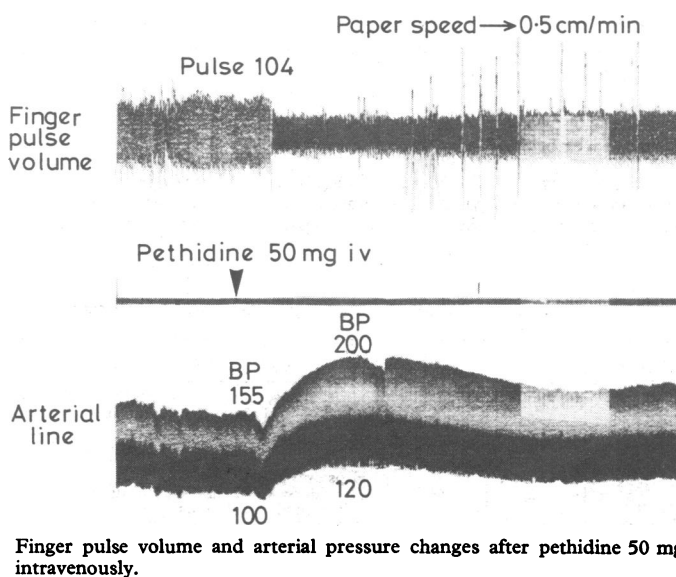
BRENTON ROWAN-KELLY, BSC, technician

Pethidine-induced hypertension in phaeochromocytoma

Continuous postoperative blood-pressure monitoring in a case of malignant phaeochromocytoma showed that pethidine induced paroxysmal hypertension. Although the histamine-liberating action of pethidine, opiates, and other drugs is known¹ and has been implicated in the diagnosis of phaeochromocytoma,² the risk of provoking hypertension with these drugs appears to be often overlooked,^{3,4} as in our case.

Case report

A 55-year-old normotensive and mildly diabetic housewife with clinical and radiological evidence of a large right-sided adrenal mass and raised blood catecholamines (noradrenaline 3.5 µg/l, normal 0.79 µg/l; adrenaline 2.1 µg/l, normal 0.28 µg/l) was found at laparotomy to have an inoperable



tumour with metastatic deposits in the liver and mesenteric lymph nodes. Preoperative alpha- and beta-adrenoreceptor blockade was not used, and biopsy of both primary and secondary tumour tissue produced short bursts of hypertension and extrasystoles. On her return to the intensive care unit her blood pressure rose to 240/140 mm Hg. Labetalol (50 mg), a drug with both alpha- and beta-blocking activity, was given intravenously, and continuous monitoring showed a fall in blood pressure to 75/50 mm Hg over three to four minutes. There was an increase in finger pulse volume (as detected by a Philips infrared sensor) but no change in heart rate. Pethidine 50 mg, given intravenously at this time and during two other short periods of labetalol infusion in the first 24 hours after operation, caused no change in blood pressure. It was noticed, however, on subsequent examination of the trace, that in the absence of labetalol, pethidine had produced a rise in systolic pressure of 30-80 mm Hg and in diastolic pressure of 10-30 mm Hg (see figure). The maximum pressures developed over four minutes and lasted about 10 minutes. Five episodes were recorded and in three a slight drop in blood pressure lasting 10 seconds preceded the rise. The hypertension was accompanied by a simultaneous decrease in finger pulse volume.

Comment

Pethidine and other histamine-liberating drugs should be used with caution in patients with pheochromocytoma, as episodes of hypertension may occur. Alpha-adrenergic blockade abolished the hypertension in this patient.

I am grateful to Dr F D Thompson and Dr A M Joekes for permission to report this case and to the department of medical illustration for the figure.

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Institute of Urology, St Peter's Hospitals, London WC2

C A LAWRENCE, MB, CHB, research fellow, intensive care

Mixed crystal deposition disease and osteoarthritis

Deposition of calcium pyrophosphate dihydrate in articular cartilage (chondrocalcinosis) is associated with several different types of joint disease, including a chronic polyarthritis clinically indistinguishable from generalised osteoarthritis.¹ Recently hydroxyapatite crystals have also been found in the synovial fluid of some patients with typical osteoarthritis.² We describe six cases in which evidence of deposition of both hydroxyapatite and pyrophosphate crystals in the same joint has been found.

Patients, methods, and results

Samples of synovial fluid, synovial membrane, and cartilage were obtained at operation from six patients undergoing biopsy or prosthetic orthopaedic surgery for arthritis. Samples were prepared for routine histology and

electron microscopy. Analysis of mineral deposits was carried out by infrared spectroscopy and analytical electron microscopy.³

All the patients presented with a chronic polyarthritis diagnosed clinically as osteoarthritis (see table). In cases 1, 2, and 3 the joint was particularly severely affected, with x-ray evidence of pronounced destructive changes similar to those described by Richards and Hamilton⁴ in chondrocalcinosis. Four patients had spotty calcification in and around the joint, and three had chondrocalcinosis.

The histological appearances of the cartilage were compatible with osteoarthritis in all cases, with varying degrees of destructive change and clumping of chondrocytes. The synovium showed evidence of a mild inflammatory cell infiltrate in cases 1, 2, 3, and 4. Polarised light microscopy of the synovial fluid disclosed pyrophosphate crystals in all cases and other minute birefringent particles in two cases. Mineral deposits were identified in the cartilage in three cases and in the synovium and capsule in three cases. In each case analytical electron microscopy disclosed deposits with calcium:phosphorus ratios characteristic of hydroxyapatite in addition to the pyrophosphate crystals. Infrared spectroscopy showed patterns similar to those obtained with artificial mixtures of the two salts. The deposits seen were deep in the tissues, often close to cell clusters, and their morphology was quite unlike that of bone fragments.

Discussion

Clinically, these six patients were indistinguishable from others with osteoarthritis. The joint damage was severe in three cases, but these patients were clinically similar to others with advanced osteoarthritis undergoing surgery.

It is perhaps surprising that both crystals were being actively deposited in the same tissue, as conditions favouring hydroxyapatite formation inhibit pyrophosphate deposition.⁵ There were possibly regional changes in the concentrations of alkaline phosphatase and inorganic pyrophosphate in our cases.

There are, however, several similarities between patients with pyrophosphate and with hydroxyapatite deposition. Osteoarthritis occurs in many people with chondrocalcinosis, and hydroxyapatite deposition often occurs in patients with osteoarthritis. The biochemical changes in articular cartilage are similar in both pyrophosphate arthropathy and osteoarthritis,⁵ and the two crystals have similar inflammatory properties.

There are therefore no obvious clinical differences in patients with this type of chronic polyarthritis, whether there is evidence of pyrophosphate deposition, hydroxyapatite deposition, both together, or neither. Calcification appears to be intrinsic to the evolution of many cases of osteoarthritis.

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Department of Rheumatology and Experimental Pathology, St Bartholomew's Hospital, London EC1A 7BE

P A DIEPPE, MRCP, senior registrar

D V DOYLE, MRCP, research registrar

E C HUSKISSON, MD, MRCP, consultant physician

D A WILLOUGHBY, DSC, FRCPATH, professor of experimental pathology

P R CROCKER, senior chief technician

Details of six patients undergoing biopsy or prosthetic orthopaedic surgery for arthritis

Case No	Age	Sex	Affected joints	Operated joints	X-ray findings
1	80	F	Hips, knees, shoulders, digital interphalangeal joints, and carpometacarpal joints of thumb	Right shoulder	Advanced osteoarthritis and spotty calcification
2	69	F	Shoulders and knees	Left shoulder	Advanced osteoarthritis and spotty calcification
3	62	M	Ankles, knees, wrists, and distal interphalangeal joints	Right ankle	Advanced osteoarthritis and chondrocalcinosis
4	64	M	Hips and knees	Left hip	Osteoarthritis and chondrocalcinosis
5	72	M	Shoulders, knees, ankles, and distal interphalangeal joints	Left knee	Osteoarthritis, spotty calcification, and chondrocalcinosis
6	62	F	Knees	Right knee	Osteoarthritis, spotty calcification, and chondrocalcinosis