PAPERS AND ORIGINALS

New cause of penicillin treatment failure

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Summary

A large empyema infected with a penicillin-sensitive haemolytic group B streptococcus failed to respond to high doses of penicillin. After two weeks' treatment the pus aspirated was found not only to contain no penicillin, but also to inactivate penicillin added to it. We believe that the inactivating agent is an enzyme that may destroy various penicillins and cephalosporins but has no effect on other common antibiotics. When treatment was changed to doxycycline the patient made a rapid recovery.

Introduction

There are several possible explanations for the failure of penicillin treatment to overcome an apparently sensitive infection. The antibiotic may not be absorbed; the dose given may be inadequate; or it may not penetrate to the site of the infection; the organism may produce a β-lactamase (penicillinase), or a second, unrecognised pathogen may be present. None of these possibilities accounted for the failure of our patient (who had an empyema) to respond to large doses of intramuscular penicillin, and we thought that this might have been the result of inactivation of the penicillin at the site of the infection by leucocytes in the pus.

Case report

A 46-year-old alcoholic diabetic with a severe peripheral neuropathy was admitted to hospital with a deep ulcer of the left foot; a haemolytic streptococcus group B was isolated and the infection responded well to a three-week course of intramuscular penicillin, but he discharged himself before the ulcer was completely healed. Three months later he was readmitted with a three-week history of pleuritic pain, fever, and lassitude. He had a fever of 39°C, a large left pleural effusion, and the foot ulcer had broken down. Chest radiography showed a large, partially loculated pleural effusion and approximately a litre of pus was aspirated. There was no evidence of bronchopleural fistula.

Culture of the pus yielded a profuse growth of haemolytic group B streptococci; Gram-negative bacilli were also seen in the direct film but only a few *Bacteroides fragilis* were isolated with difficulty after prolonged incubation. A few *Corynebacterium xerosis* were also present. The same streptococcus was isolated from the foot ulcer and both appeared sensitive to penicilin by the disc test. Blood cultures were not done as the patient was taking co-trimoxazole when admitted, but his empyema had probably arisen from a septic pulmonary infarct.

Treatment was started with parenteral benzylpenicillin, 900 mg six-hourly, and frequent pleural aspirations were performed. Two weeks later film and culture again showed many streptococci present and a few different, penicillin-sensitive bacteroides were isolated; the original *B fragilis* had not at that time been reported. The penicillin was increased to 1200 mg six-hourly, and probenecid 500 mg six-hourly, and metronidazole, 400 mg eight-hourly by mouth, were added. The patient's clinical condition continued to show no improvement and nine days later (day 24) 600 mg penicillin was injected intrapleurally and further advice sought from the laboratory. A profuse growth of the streptococcus was again obtained from the pus but no other organism was either grown or seen in the direct film; the streptococcus was inhibited by 0·06 mg/l penicillin. Penicillin and metronidazole were assayed in both blood (collected three hours after a dose of penicillin) and pus. The serum contained 8 mg/l penicillin and 11·5 mg/l metronidazole; the pus contained 7·5 mg/l metronidazole but not only was no penicillin detected, but some evidence was obtained that the pus could inactivate it.

Three days later (day 27) the clinical and bacteriological picture was still unchanged and the pus aspirated was shown to contain 0·5 mg/l penicillin. A further 300 mg penicillin had been injected locally at aspiration, but as it had now been confirmed that the pus could inactivate penicillin, parenteral treatment with this was stopped and oral doxycycline, 200 mg daily, instituted; a tetracycline was chosen because the pus was acid (pH 5·2), which favours its action. Twenty-four hours later (day 28) the pus contained 9·4 mg/l penicillin and 1 mg/l doxycycline and the streptococci were less numerous; the serum contained 2 mg/l doxycycline. The fluid aspirated the next day contained clotted blood and little pus, and only scanty streptococci were grown; the doxycycline level was 2 mg/l. Thereafter the fluid was sterile and there was a corresponding clinical improvement with resolution of fever and no recurrence of the effusion.

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Bacteriological investigations

MATERIALS AND METHODS

All assays were done by the plate diffusion method; penicillin was assayed against *Bacillus subtilis* (NCTC 8236), doxycycline against the Oxford *Staphylococcus aureus* (NCTC 6051), and metronidazole against *Bacteroides fragilis* (NCTC 9343). For the last of these 5% horse blood was added to the medium and the plates were incubated in Gaspak jars.

Inactivation experiments were done by adding 0·1 ml of the appropriate concentration of the antibiotic to 0·9 ml pus and to 0·9 ml pooled human serum as controls. These mixtures were placed in wells cut in thin agar plates, pre-seeded with the Oxford staphylococcus, immediately on mixing, and after three and 24 hours' incubation at 37°C. The plates were inoculated with *B subtilis* instead of the staphylococcus for tests with specimens containing doxycycline, and with *Escherichia coli* (NCTC 10418) for tests with chloramphenicol. Oxoid Isosensitest agar was used except for tests with *B subtilis* as the assay organism, when it was replaced by Oxoid diagnostic sensitivity test agar.

RESULTS

The pus aspirated on day 24 contained no penicillin. Furthermore, the zone made by a penicillin disc on agar seeded with the Oxford staphylococcus was distorted by the pus placed in a well cut at the side of the disc, suggesting that the pus had destroyed the penicillin. There appeared to be three possible explanations: that the infecting organism produced a β-lactamase; that the penicillin was destroyed by the acidity of the pus, which had a pH of 5·2; or that it was destroyed by the metronidazole known to be present. When 10 or 1 mg/l penicillin was added to overnight broth or glucose broth cultures of the infecting streptococcus (final pH of the latter 4·2), to buffer solution at pH 5·0, or to metronidazole 10 mg/l in water and incubated overnight, there was no major loss of activity in any of them. On the other hand, when these concentrations of penicillin were added to the pus, none was detected after three hours' incubation, and when the concentration was increased to 100 mg/l it was substantially reduced in three hours and none remained after overnight incubation.

The pus aspirated on day 27 contained 0·5 mg/l penicillin when it was first assayed but none was detectable after overnight storage at 4°C, and when penicillin was added to this it was inactivated at the same rate as in the first specimen. The specimen aspirated 24 hours after the intrapleural injection of 300 mg penicillin (day 28) contained 9·4 mg/l when first assayed. This concentration fell to nil during three days' storage at 4°C, its ability to inactivate further penicillin being somewhat reduced—both 1 and 10 mg/l being only partially inactivated in three hours, though both had disappeared after overnight incubation. The specimen aspirated the following day (day 29) was heavily blood-stained and contained little pus and only scanty streptococci; its pH was 7·4 and there was little if any inactivation of penicillin added to it.

NATURE OF THE INACTIVATING AGENT

The capacity of the pus to inactivate four other penicillins and seven cephalosporins was tested in the same way (table I). Its low activity against ampicillin and its rapid destruction of cephaloridine and cephalothin suggest that the inactivating agent was not a β-lactamase. Various attempts were made to prove that it was an enzyme. The capacity to inactivate penicillin was lost when the pus was heated to 56°C for 30 minutes and was considerably reduced when potassium fluoride was added to the pus at a concentration of 350 mmol/l and incubated overnight before adding the penicillin.

We studied the rate of inactivation of penicillin by adding 100 mg/l to the pus, holding this at 37°C, and assaying the penicillin at hourly intervals. The results showed that the reaction rate is proportional to substrate concentration up to a concentration of at least 100 mg/l. The same concentrations of each of the 12 antibiotics were added to buffer solution at pH 5·0 and incubated overnight. Only methicillin showed any significant loss of activity and this was appreciably less than that produced by the pus. However, when the pH of the pus was raised to 7·2, not only was the rate of inactivation of penicillin reduced, but that of ampicillin was considerably increased. Indeed, the inactivation of the two antibiotics was then very similar, in contrast to their behaviour at pH 5·2.

TABLE I—*Rate of inactivation of various β-lactam antibiotics by pus*

Antibiotic	Concentration (mg/l)	Rate of inactivation	
		3 h	24 h
Penicillin ..	10	Complete	
	100	Partial	Complete
Ampicillin ..	1	Partial	Almost complete
	10	NR	Partial
Cloxacillin ..	20	NR	Partial
Carbenicillin ..	20	Partial	Complete
	100	NR	Partial
Methicillin ..	20	Partial	Complete
Cephaloridine ..	10	Complete	
	100	Partial	Complete
Cephalothin ..	10	Complete	
	100	Partial	Complete
Cephalexin ..	20	None detected	None detected
Cephradine ..	20	None detected	None detected
Cefuroxime ..	10	Partial	Complete
Cephazolin ..	10	Partial	Complete
Cefatrizine ..	10	NR	Complete

NR = No reduction in zone size observed at three hours; the method was not sufficiently accurate, however, to assume that there had been no loss of activity.

SITE OF THE ENZYME

Four ml pus was ultracentrifuged and the small amount of supernatant removed. One ml water was added to the cells; these were then disrupted with an ultrasonic probe and ultracentrifuged again. The supernatant was removed and passed through a 0·2-μ membrane filter and the cell debris was washed twice and resuspended in 1 ml water. Ten and 1 mg/l penicillin was added to each of these components and tested for inactivation in the usual way (table II).

TABLE II—*Inactivation of penicillin by different components of pus*

Component	Penicillin (mg/l)	Time of assay		
		Immediate	3-h contact	24-h contact
Pus supernatant ..	1	Partial	Complete	
	10		Partial	Complete
Cell contents ..	1		Complete	
	10		Partial	Complete
Cell walls ..	1	Complete		
	10	Partial	Complete	

Each was also placed in wells cut in agar plates inoculated with the Oxford staphylococcus but containing an inhibitory concentration of penicillin (0·03 mg/l). Both the cell walls and the unfiltered supernatant from the pus enabled the staphylococcus to grow in a narrow band round the wells containing them. This suggests that, although the enzyme clearly arises from the cell walls, a little can diffuse from them into the surrounding medium.

ACTION ON OTHER ANTIBIOTICS

Various other antibiotics were tested in the same way and no important loss of activity was seen with sodium fusidate 5 mg/l, clindamycin 5 mg/l, chloramphenicol 20 or 50 mg/l, tetracycline 10 mg/l, doxycycline 1 or 10 mg/l, or gentamicin 10 mg/l. Erythromycin 5 mg/l was inactivated in 24 hours, but when the pH of the pus was raised to 7·2 this did not happen, and this concentration was also destroyed in serum when the pH was lowered to 5·2. We therefore presumed this inactivation to be the effect of acidity.

Discussion

It has long been accepted that treatment of a fully sensitive infection with penicillin may fail if a β-lactamase-producing organism is also present, since it may inactivate the penicillin before it takes effect. No such organism was present in any of our specimens of pus shown to inactivate penicillin. No one has suggested that treatment may fail because of the production of a smilar type of enzyme by the host. This appears to be the only explanation for the failure of the vigorous penicillin treatment

given to our patient to influence the course of his infection with a highly sensitive organism. Although no penicillin was found in the pus aspirated before he received intrapleural injections, the high serum level (8 mg/l) together with the presence of a substantial amount of metronidazole in this specimen, and of doxycycline in subsequent ones, and his prompt response to treatment with this drug, make it highly improbable that the failure of parenteral penicillin could have been due to its failure to reach the site of the infection.

The evidence suggests that the inactivating agent was an enzyme arising from the cell walls of the leucocytes. At first sight its pattern of inactivation of β-lactam antibiotics suggest that it was not a β-lactamase, but the effect of pH on its activity against penicillin and ampicillin raises the question of whether other β-lactam antibiotics are similarly affected.

Since we began this work, specimens of pus received from two other patients have been tested for their ability to inactivate penicillin. Neither had this property, but de Louvois and Hurley,[1] who have recently been examining specimens of pus with this object, report that four out of 22 were able to inactivate penicillin to a variable degree. Thus the phenomenon may be relatively common, though it may only be of clinical significance where there is a collection of pus, as in an empyema or a joint, and seemingly it will be accentuated where the pus is of low pH. It may account, however, for some unexplained failures of penicillin treatment. Nevertheless, the failure of our patient to respond to the high concentration of penicillin that must have been achieved by the intrapleural injection of 600 mg—which resulted in an adequate concentration still being present three days later—remains unexplained.

We thank Dr Lyall Watson for his permission to report on a patient under his care, and Dr D Gardner for his helpful advice on the properties of enzymes.

Reference

[1] De Louvois, J, and Hurley, R, *British Medical Journal*, 1977, **1**, 998.

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High TSH concentrations in "euthyroidism": explanation based on control-loop theory

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Summary

High concentrations of thyroid-stimulating hormone (TSH) in the serum have often been reported in apparently euthyroid patients with damaged thyroids. We have confirmed this finding in 14 patients 18 months after subtotal thyroidectomy for Graves's disease (group 1) and in 14 patients with manic-depressive psychosis (group 2) receiving lithium carbonate, which reduces thyroid reserve. One factor common to groups 1 and 2 but not to the controls was reduced thyroid reserve or functioning capacity, and, using established physical principles of servo-control, we have tried to define the mechanism. A series of curves were projected to indicate how TSH might be expected to vary with functioning thyroid capacity.

Introduction

Raised thyroid-stimulating hormone (TSH) concentrations have been reported in apparently euthyroid patients with symptomless thyroiditis[1 2] after radioiodine treatment for thyrotoxicosis,[3 4] and subtotal thyroidectomy.[5 6] Evered *et al*[7] claimed that a raised serum TSH concentration is the most sensitive index of thyroid failure and placed patients with normal circulating thyroid hormone but raised TSH concentrations into a group designated "subclinical hypothyroidism." Himsworth and Fraser[8] found this terminology confusing and argued that to divide euthyroid patients into two arbitrary groups according to their serum TSH concentrations may conceal other determinants of TSH secretion. Functioning thyroid mass or capacity (FTC) has been tentatively suggested as one such determinant,[3 4 8 9] but to date no attempt has been made to evaluate its importance. (FTC is defined in terms of the thyroid response to a standard concentration of TSH.)

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Present series

Study A was carried out on three groups of 14 subjects with near-identical (within 1 nmol (0·8 μg)/l) free thyroxine (FT4) indices. Group 1 comprised 14 women who were clinically (Billiewicz's diagnostic index[10]) and biochemically (FT4 index and serum triiodothyronine (T3) concentration) euthyroid 18 months after subtotal thyroidectomy for Graves's disease; group 2, 12 women and two men, all biochemically (FT4 index and serum T3) euthyroid and all under treatment for manic-depressive psychosis with enough lithium carbonate to obtain therapeutic plasma levels of 0·8-1·1 mmol (5·6-7·6 mg)/l (lithium at these concentrations reduces the thyroid response to TSH[11]); and group 3, 14 healthy women cleaners all biochemically (FT4 index and serum T3) euthyroid, who acted as controls (see table). No subject in group 1 or 3 was receiving any form of medication. There was no significant difference in mean serum T3 concentrations between the groups.

Study B was carried out on a 56-year-old woman with severe hypothyroidism due to Hashimoto's thyroiditis during incremental replacement with thyroxine. TSH and the FT4 index were measured before treatment and again at monthly intervals before each increment (25 μg) in the dose of thyroxine.

Serum TSH,[12] T4,[13] and T3[13] were measured by radioimmunoassay. T3 uptake was measured by a method similar to Thyopac-3 (Radiochemical Centre, Amersham), and the FT4 index derived from the ratio of T4:T3 uptake expressed as a percentage.