THE RATIONALE AND EXPERIENCE BEHIND THE USE OF OTHER ANTIBIOTICS IN COMBINATION WITH PENICILLIN FOR TREATMENT

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TABLE OF CONTENTS

HISTORICAL (1, 2, 3, 4)

The intense activity that is taking place in the field of antibiotic research and development tends to obscure an interesting, though neglected, past. Inhibition of the growth of one organism by another had been observed long before Fleming noticed the clearing of a culture plate by a fungus contaminant. Thousands of years before the advent of bacteriology crude mold preparations were being used in the treatment of wounds and infections. At present similar preparations still enjoy popularity among the practitioners of folk medicine in many parts of the world. The effectiveness of these remedies is to be doubted, but it is interesting to speculate about them in the light of our present knowledge.

The medical literature of centuries past is sprinkled with references to the use of fungal products. We find that John Parkinson (1640), Apothecary of London and King's Herbarist, particularly recommended "the Mosse upon dead men's Sculle's" as being most efficacious in the treatment of wounds. A copy of Lancet from the year 1852 contains a report of the successful treatment of furunculosis with common yeast.

In 1876 Tyndall described the clearing of broth infusion cultures which followed the overgrowth by a species of Penicillium. Similarly the great Pasteur noted the antagonistic effect of common aerobes when grown together in culture with anthrax bacilli. In the closing years of

the last century bacterial antagonism was described over and over again by many workers. Methods for demonstrating this phenomena on solid media which differs little from present day techniques were evolved by Babes (1885) and Garre (1887). Garre inoculated gelatin plates with alternate rows of B. flourescens and Staphylococcus pyogenes and found the growth of the staphylococcus to be inhibited by the presence of the other organism. He surmised that the inhibition was caused by a toxic product elaborated by the B. flourescens which could be of therapeutic value.

It had been observed for many years that persons with chronic infectious diseases often showed much improvement following an attack of erysipelas. Experimenters following this lead were able to show that inoculations of streptococci, pneumococci or B. pyocyaneus protected a significant percentage of rabbits from experimental anthrax. A great deal of effort was expended in research of a similar nature, but the results were not consistent and nothing of proven clinical value was produced.

In 1898 two Russians, Houri and Bukovsky, reported excellent results in the treatment of chronic skin ulcers with the applications of a B. pyocyaneus preparation. The following year an extract of pyocyancus culture was introduced into clinical medicine by Emmerich and Lowe under the name "pyocyanase". The substance was quite definitely bacteriocidal and was able to produce rapid lysis of many

pathogenic organisms. A flood of clinical and laboratory research was devoted to this material but was, for the most part, confused and unproductive. Pyocyanase received a wide clinical trial over a period of several years and was found to be an effective topical agent, but interest in it gradually waned.

A bacteriocidal substance produced by B. subtilis and B. mesentericus was found to have marked in vitro activity against the tubercle bacillus. Following this observation Rappin (1912) reported that injections of filtrate from cultures of B. mesentericus protected guinea pigs from what would have otherwise been fatal inoculations of tubercle bacilli. Observations of this type were fairly common but were often misinterpreted by workers preoccupied with the concept of immunity.

At this time much interest was being shown in treating infectious diseases by replacing the pathogen with other organisms. Good results were claimed for the treatment of diphtheria carriers by spraying their throats with suspensions of staphylococci. Harmless organisms such as B. acidophilus were employed in enteric and pharyngeal infections in the hope that their growth would "crowd out" the infecting organism. It is interesting to note that similar therapy is being used at the present time in the treatment of conditions resulting from the disturbance of normal gut flora by oral antibiotics.

Throughout the nineteen-twenties many new organisms with antibiotic properties were discovered, especially among the soil bacteria. In 1929 Fleming observed the marked antibiotic effect of a colony of Penicillium notatum that had accidentally contaminated a culture of Staph. aureus. The crude liquor from cultures of this mold possessed remarkable antibiotic properties and was not toxic to animals. No attempt was made to evaluate this substance in experimental infections and its clinical possibilities were not explored.

Over a period of 80 years many observations of antibiotic phenomena were made by a host of investigators but still no antibiotic of proven and accepted value had been produced. Although much of the work that had been done was excellent, the substances producing the observed effect were never properly isolated and identified except in a few instances. The excellent work done by Dubos in isolating and characterizing an antibiotic obtained from one of the soil bacteria put a solid foundation under this field of research and helped recreate interest in these substances. When, in 1940, reports on penicillin began to come out of Oxford, a floodtide of enthusiastic research was initiated which shows no sign of abating even now, 14 years later. In 1944 Waksman and his group contributed streptomycin and this was followed in rapid succession by scores of other antibiotics many of which are of great importance.

If nothing else is accomplished by this brief and incomplete history it should be pointed out that antibiotics as we know them today did not result from any one man's inspiration, but were arrived at after repeated observations over a long period of time culminated by the intensive, coordinated efforts of well trained groups of experimenters.

INTRODUCTION

At present we have at our disposal some 300 different antibiotics of which perhaps a dozen can be used clinically. The rate at which the search for new antibiotics is being carried on is the best indication that we still do not have the therapeutic answer to every infection. Some of the newer antimicrobials are effective against a wide variety of organisms but there is no single agent that is satisfactory in all cases. Many pathogens remain for which no effective, clinically acceptable drug has been found. The viruses, and fungi are still, for the most part, inaccessible to specific therapy. Many bacteria that once fell easy prey to antibiotics have become resistant to these substances with discouraging rapidity (5). A large number of infections are caused by a mixture of organisms all of which may not be included in the spectrum of any one antibiotic. With these circumstances in mind it is easy to understand why combinations of two or more antimicrobials

would come to be tried in an effort to achieve greater effectiveness. Perhaps, by the simultaneous administration of several of these substances, organisms already susceptible will be eliminated more rapidly and the resistant ones made susceptible to therapy. I'm sure that the philosophy which states that if one drug is good, two are twice as good is often the motivating factor behind combined therapy.

Penicillin is still the most widely used antibiotic because it is effective, inexpensive, and has relatively few side effects. It is quite often the first treatment prescribed for an infection with the subsequent addition of another antibiotic if it proves ineffective. It has become common for it to be given in conjunction with another antibacterial with little regard given to the result the second drug may have on its effectiveness.

When one antibiotic is administered simultaneously with another any one of four things can happen. (1) The action of the pair can be greater than would be expected from a corresponding size dose of the original agent. This is referred to as synergism. (2) The antibacterial effect of the combination may merely be equal to the sum of the two components acting individually. This is an additive action. (3) There may be no effect whatever and the action of the pair will equal that of the original drug alone. (4) The effectiveness of the original anti-

biotic may be reduced by the addition of the second. This is an antagonistic response.

This paper will concern itself with the result of combining penicillin with other antibiotics.

PENICILLIN AND BACITRACIN

Bacitracin is a substance having marked antibacterial effect against gram-positive organisms with a spectrum of activity similar to that of penicillin. Unfortunately, it has been found to be quite toxic when administered parenterally. It causes renal tubular damage regularly in mice and in man urinary changes occur after only a few days of therapy with 100,000 units daily (6). For this reason it has received only very limited parenteral use but it is widely employed topically.

In combination with penicillin it has been shown by Eagle to have a marked synergistic action in the treatment of syphilis in rabbits. (7) Only a small fraction of the CD 50 of each drug was required to produce rapid cures in the infected animals. Bacitracin-penicillin combinations have been tested against several strains of Alpha and Gamma hemolytic streptococci by Bachman who found the combination to act synergistically in all cases (8). On the strength of this report, Volini and Kadesan treated 3 cases of subacute bacterial endocarditis with penicillin and bacitracin. Two of these cases had not responded to treatment with

penicillin alone prior to the start of combination therapy. The addition of 6,000 units of bacitracin daily to high doses of penicillin gave prompt remission of symptoms and persistently negative blood cultures without any sign of nephrotoxicity (9).

PENICILLIN AND STREPTOMYCIN

Of all of the various antibiotic combinations, penicillin and streptomycin is probably the most widely used and accepted. Like penicillin, streptomycin is bacteriocidal when employed in suitable concentration against susceptible organisms. In vitro, these two drugs have shown very definite synergism in their bacteriocidal action when tested against staph. aureus and the hemolytic streptococcus (10). The addition of streptomycin, even though it in itself is ineffective in high concentrations, greatly increases the bacteriocidal rate of optimal levels of penicillin on enterococci in culture (11). Not only are the enterococci killed at a greater rate, but the killing proceeds until the cultures are sterilized which cannot be done with penicillin alone except in very high doses. This, indeed, must represent synergism for streptomycin alone has no effect. In this experiment the viable bacteria remaining after exposure to optimal (most rapidly bacteriocidal) levels of penicillin showed no increase in resistance when recultured. This indicates that in this

instance the synergistic effect of streptomycin is not due to its effect on a few resistant cells.

In recent years streptomycin has found frequent use in combination with penicillin for treating subacute bacterial endocarditis. It is generally agreed that penicillin in massive doses is the treatment of choice in SBE. However, in a certain percentage of cases, especially those involving an enterococcus, treatment with penicillin alone is attended by a very high relapse rate. Even where the causitive organism is quite sensitive to penicillin very large doses must be used continuously for periods of six to eight weeks in order to obtain permanent cure. Because of the nature of the lesion Hunter refers to SBE as a special therapeutic problem (12). The infected valve leaflets are poorly vascularized and thick layers of fibrin and even calcium deposits enclose the heavy concentrations of bacteria. Under such circumstances it is difficult to maintain adequate antibiotic concentrations in the tissues and the natural defenses of the host cannot be brought to bear because of the poor vascularity. Owing to the poor host reaction, bacteriocidal activity is required of an antibiotic for it to be effective. The organisms deep in the lesions are deprived of adequate metabolites and, therefore, are at their maximum population density and growing quite slowly. As Eagle has demonstrated, slowly growing cultures are quite resistant to penicillin which

requires rapidly dividing organisms for it to act maximally (14). Constant levels of penicillin will temporarily reduce the population of slowly growing cultures but after a short period of time growth recurs. Hunter has shown that in this same situation a combination of penicillin and streptomycin have a steady, progressive killing effect ultimately rendering cultures sterile.

In order to more closely parallel the situation in SBE Hunter has investigated the ability of antibiotics to sterilize blood clots in vitro (13). He has found that here, too, penicillin-streptomycin mixtures were often more effective than penicillin alone. Very high antibiotic concentrations in the media surrounding the infected clot are much more effective than are the lower concentrations that are optimal for organisms in a liquid menstruum.

In clinical trial, combined penicillin and streptomycin therapy has proven to be quite effective in cases of enterococcal and endocarditis that have failed to respond to massive penicillin therapy alone. Robbins and Tompsett have reported very good results in a series of eight cases of enterococcal endocarditis treated with 10,000,000 units of penicillin and 2 grams of streptomycin daily in divided doses for a period of 28 to 42 days (15). In most cases the fever fell rapidly, the patients felt better, and the blood cultures promptly became negative and remained so.

The organisms isolated from these patients were relatively more sensitive to streptomycin than to penicillin, but streptomycin therapy was ineffective. Further in vitro studies on these organisms showed that penicillin and streptomycin acted in a true synergistic manner. Cates successfully treated a series of cases of SBE using penicillin and streptomycin after penicillin alone, streptomycin alone, aureomycin, chloramphenicol and aureomycin plus penicillin had failed (16). He also showed the bacteriocidal effects of penicillin to be increased by the addition of streptomycin.

The treatment of SBE requires a long and expensive treatment even when it is successful. In an attempt to produce a more rapid cure of this disease, King maintained very high penicillin levels in his patients for ten days by giving 10,000,000 units of intravenous penicillin daily along with oral caronamide (17). The results were very disappointing. Out of eight cases only one permanent cure was obtained by this regimen. However, four of the remaining seven patients were ultimately rid of their infection by prolonged penicillin therapy. Geraci has been able to successfully treat patients with Strep. viridans endocarditis in only 14 days by combining penicillin and streptomycin (18). Out of 23 patients, 18 were given permanent cures in this length of time by treatment with moderate doses of penicillin (1 - 2 million units daily), plus 1.5

- 2.5 grams of dihydrostreptomycin per day. Blood cultures reverted to negative and remained that way for an average follow-up period of one year.

All available information indicates that the combination of streptomycin with penicillin can be very valuable in treating conditions refractory to penicillin alone. This combination holds special promise in the treatment of penicillin resistant SBE. It may be possible to greatly shorten the treatment period for penicillin sensitive cases by the concurrent administration of streptomycin and penicillin, but this requires more work before it can be put into routine use.

PENICILLIN IN COMBINATION WITH THE BROAD SPECTRUM ANTIBIOTICS

When penicillin is combined with bacitracin or streptomycin the antibiotic activity of the combination is frequently greater than the sum of the actions of the individual drugs (i.e. synergism). Rarely, if ever, are these drugs antagonistic in combination. However, the results of combining a broad spectrum antibiotic (aureomycin, terramycin or chloramphenicol) with penicillin have not been so uniformly favorable. In vitro studies by Gunnison have shown that the addition of bacteriostatic doses of aureomycin or terramycin to bacteriocidal doses of penicillin causes a lower death rate in cultures of Strep. pyogenes than does penicillin alone (19). When relatively

ineffective doses of either of these two drugs were added to optimal levels of penicillin they had no effect at all. When the aureomycin or terramycin were increased to mildly bacteriocidal levels they no longer gave interference. Approximately similar results were obtained when the combinations were tested against K. pneumoniae. In another study by the same author aureomycin and chloramphenicol were found to be bacteriostatic for 17 strains of enterococci which were rapidly killed by penicillin. Both of these agents slowed the bacteriocidal rate of penicillin when added to it (20). In a series by Jawetz, also employing the enterococcus, streptomycin alone in high doses (50 mcg/ml) was completely without effect, and chloramphenicol was bacteriostatic. When combined with optimal levels of penicillin, streptomycin displayed marked synergism, whereas chloramphenicol slowed the rate of killing (11).

The antagonistic effect of the broad spectrum antibiotics on penicillin as demonstrated by Gunnison and Jawetz has not been reproduced with the same consistency by other workers. Romansky's data not only fails to substantiate their results, but indicates that chloramphenicol and aureomycin frequently act synergistically with penicillin (5). This does not invalidate their findings, but should serve as a reminder of the great number of variables operating in experiments of this type.

After a culture has been exposed to penicillin for 24 hours the remaining organisms begin to multiply and the population rises toward its original level. When a similar culture is exposed to a broad spectrum antimicrobial in addition to the penicillin, the bacteriolysis is greatly slowed, but the killing is progressive and the cultures ultimately become sterile. When certain strains of streptococci and staphylococci are subjected to concentrations of penicillin that are above the optimum, the bacteriocidal rate is similarly slowed (21). Here too, however, the killing is relentless and all of the cells are ultimately killed.

Antagonism can be demonstrated with these combinations in vivo as well as in vitro. A single dose of 120 mcg of penicillin will cure 95% of a group of mice inoculated with 60 - 100 LD_{50} of Beta hemolytic streptococci. The concomitant administration of 10 mcg of aureomycin or terramycin decreases the number of cures to 40% (22). However, if the interval between the administration of the two drugs exceeds 30 minutes no antagonism is observed. Similar results are obtained when K. pneumoniae is the infecting organism. In an experiment reported by Jawetz a single dose of either penicillin (30 mcg) or chloramphenicol (800 mcg) protected 80% of a group of mice against 100 LD_{50} of Beta hemolytic streptococci. When similarly infected animals were treated with 30 mcg of penicillin,

plus 800 mcg of chloramphenicol only 40% of the animals survived (23). With the dosage of penicillin kept constant an increase in mortality was produced by increasing the quantity of chloramphenicol. However, with any therapeutic concentration of chloramphenicol the mortality was lowered by increasing the dose of penicillin. This rather clearly indicates that it is the penicillin whose action is being interfered with. Ahern has repeated these experiments, but instead of using a single dose of the antibiotics, the animals were placed on a multiple dose schedule for several days so as to maintain a fairly constant blood level. When the drugs were administered in this way 40% of all of the treated animals survived whether they had received chloramphenicol, penicillin, or a combination of the two (24). From this he concludes that although the broad spectrum antibiotics interfere with the early killing action of penicillin this is probably of little importance clinically when both drugs are administered in full therapeutic doses. Dowling and Lepper found no consistent antibiotic antagonism in treating pneumococcus infected mice with multiple doses of aureomycin and penicillin. In 112 experiments in which the two drugs were given in varying ratios, aureomycin caused interference in 21%, additive effects in 21%, and no effect in 58%. Antagonism occurred most frequently (33%) with high penicillin-aureomycin ratios, and least frequently in low ratios (25).

Clinical data on penicillin-broad spectrum antibiotic combinations is quite scanty and largely limited to two series of cases. Lepper and Dowling have reported upon a series of cases of penumococcic meningitis in which alternate patients were treated with penicillin alone and with penicillin plus aureomycin (26). In those patients treated with 1,000,000 units of penicillin daily there was a 30% mortality. The addition of from 2 - 4 grams of aureomycin daily increased the fatalities to 79%. However, this combination was not found to be antagonistic when administered to patients ill with pneumococcal pneumonia. In a study of 50 cases Ahern was not able to find any difference in patients treated with penicillin alone and those receiving penicillin plus aureomycin (27). Each treatment group had the same number of deaths and the clinical responses and length of hospitalization were nearly identical. Why this combination should show antagonism in one instance and not in another is probably explained by the differences in the disease processes. In meningitis, where the defensive response of the host is poor, any interference with the bacteriocidal action of penicillin will reduce its effectiveness. The pneumonias differ in that phagocytosis is quite effective in the lungs and bacteriostasis is all that is required for the successful treatment of infections of this organ.

ORGANISM RESISTANCE AND COMBINATIONS OF ANTIBIOTICS WITH PENICILLIN

If the development of antibiotic resistant organisms could be prevented or suppressed by combination therapy a valid argument for their use would be presented. For some time PAS and streptomycin have been used together in the treatment of tuberculosis for this very reason. Recently Finland and his group have published several reports on this aspect of antibiotic therapy.

They have demonstrated that organisms repeatedly subcultured in media containing both erythromycin and penicillin develop less resistance to this combination than organisms show to each of the components after being subcultured in them individually (28). Organisms cultured in this combination become less refractory to each of the components than do organisms cultured in the single members of the combination. These results have been reproduced with certain strains of staphylococci and streptococci using several different antibiotic combinations (20). When the development of resistance to the individual components of an antibiotic combination varies greatly the resistance of the pair most closely follows that of the component which shows the least change. In order for resistance to one member of an antibiotic pair to be suppressed both of the antibiotics must have inhibitory action (30).

It should be emphasized that although bacteria may develop less resistance to combinations than they do to individual antibiotics the emergance of resistance is merely delayed or suppressed and not prevented. In clinical use the advantages of suppressing refractory organisms must be weighed against the possibility of making the patient sensitive to larger numbers of antibiotics.

THE MECHANISM OF ANTIBIOTIC SYNERGISM AND ANTAGONISM

At present there is little that can be said on this subject that is not purely speculative. However, on the basis of experimental findings some generalizations can be made.

It has been the experience of Jawetz that antagonism occurs only when an effective concentration of one antibiotic is combined with a relatively ineffective concentration of another (31). Antagonism is not an expression of pharmacologic incompatibility, but is probably related to changes in the organism caused by the interfering drug. Penicillin is known to act maximally upon organisms that are rapidly dividing and has relatively little effect upon dormant cultures (14). From this it is logical to assume that primarily bacteriostatic drugs (e.g. the broad spectrum antibiotics) would reduce the susceptibility of organisms to penicillin by slowing their rate of growth.

As Spicer has shown, all antibiotics leave some residuum of viable cells. The number of organisms that persist varies with different bacteria and the antibiotic used (32). The synergistic behavior of certain antibiotic pairs could be the result of one member of the combination acting upon the few remaining organisms not killed by the other. In work done by Klein it was found that synergism occurred regularly if the bacteria promptly became resistant to both members of the antibiotic pair when grown separately in each (33). Many workers deny this thesis since they have not found the persisting organisms to be more resistant to the antibacterial when recultured. Cells persisting after exposure to penicillin do not show any increase in resistance upon retesting. However, these organisms are very resistant to the killing action of this drug and do not undergo lysis as do the cells of the original culture. The cells remaining after exposure to penicillin have been found to be quite sensitive to streptomycin where the organisms of the original culture were quite resistant (34). This seems to be a very able explanation of the synergistic behavior frequently displayed by penicillin and streptomycin.

WHEN SHOULD PENICILLIN BE COMBINED WITH OTHER ANTIBIOTICS?

There are very few occasions in which the combination of penicillin with other antibiotics is indicated or justifiable. In infections caused by single organisms susceptible to penicillin the treatment of choice is penicillin alone in adequate doses. If the offending organism does not respond, a suitable antimicrobial should be selected by sensitivity testing if necessary. Mixed infections should be treated with a single antibiotic whose range of activity includes all or most of the offending organisms. Although antibiotic antagonism is probably of little clinical importance, it does occur, and is a factor to be considered. Mutiple drug therapy often has little advantage over the single agent and increases the possibility of inducing multiple drug sensitivities in the patient.

Treatment with antibiotic mixtures is valid therapy in certain conditions where the effectiveness of combinations has been well established, such as penicillin resistant enterococcal endocarditis. If it is impossible to successfully treat an infection with a single antibiotic, due either to the nature of the organism, or to drug sensitivities in the patient, one may have to resort to combination therapy. In this situation it would be advisable to select the proper combination by in vitro study if this is possible. As facilities for this type of study are rarely

available one will do best to follow a scheme presented by Jawetz(31). He divides the antibiotics into two general classes, bacteriocidal and the bacteriostatic. The bacteriocidal group consists of penicillin, streptomycin, bacitracin and neomycin. In the bacteriostatic class are found aureomycin, terramycin, and chloramphenicol. According to his scheme members of the bacteriocidal group can be combined with impunity the possible effects being additive or synergistic but never antagonistic. When bacteriostatic drugs are combined, simple additive effects are found. However, when drugs from different groups are mixed one runs the danger of antagonism. This danger is small and if full doses of each drug are used there is little chance of interference.

A practice that is to be deplored is the use of "shotgun" mixtures of antibiotics as a substitute for diagnostic marksmanship. If one makes an accurate precise diagnosis and treats accordingly with the simplest effective regimen, he will be sure of deriving the maximum benefit from a wonderful family of drugs.

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