Pharmacology Review: Chemotherapy in the Horse— The Penicillins

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The penicillin group of antibiotics was the first of the true antibiotics to be introduced into medicine, and they remain by far the most potent and effective of the antibiotic group of drugs. Over the years, their mechanism of action has been studied in some detail, and it turns out that their basic structure and mechanism of action determines many of their clinical properties. Figure 1 shows the basic chemical structure of penicillin G and points to the β-lactam bond," which is the "active" bond or active site of all members of the penicillin group and the closely related cephalosporins. This communication will review the pharmacology of the penicillins and show how the clinical characteristics of penicillins are determined and best understood in terms of their basic chemical structure.

All penicillins are pharmacologically active by virtue of their β-lactam bonds (Figure 1). It turns out that this bond is a structural analog of an alanine-alanine peptide bond which must be opened by a bacterial enzyme during the last step in bacterial cell wall synthesis (Figure 2). When this bacterial enzyme is presented with a penicillin molecule, it tries to open the β-lactam bond in the same way as it would normally open the alanine-alanine bond." It opens the β-lactam bond by forming a penicilloyl-enzyme complex, but unfortunately this complex is stable, and the enzyme cannot then get rid of the penicillin molecule (Figure 2). The final result of this interaction is that one molecule of penicillin chemically "sticks in the throat" of one molecule of the bacterial cross-linking enzyme and inactivates it. The bacterium is then unable to perform the final cross-linking step in synthesis of its cell wall and is left with a faulty cell wall. Then, if the medium in which the bacterium is growing is hypotonic relative to the protoplasm of the bacterium, its protoplasm swells and protrudes through the faulty cell wall, its plasma membrane ruptures, and the bacterium dies.

It is quite clear from this mechanism of action that penicillin does not directly kill bacteria, but just robs them of their protection against osmotic rupture. However, because most body fluids are hypotonic relative to bacterial protoplasm, loss of cell wall integrity is sufficient under most circumstances to cause bacterial death. Because penicillin binds irreversibly to the cross-linking enzyme, it never "washes" away from the enzyme, and is therefore a very potent drug. Because
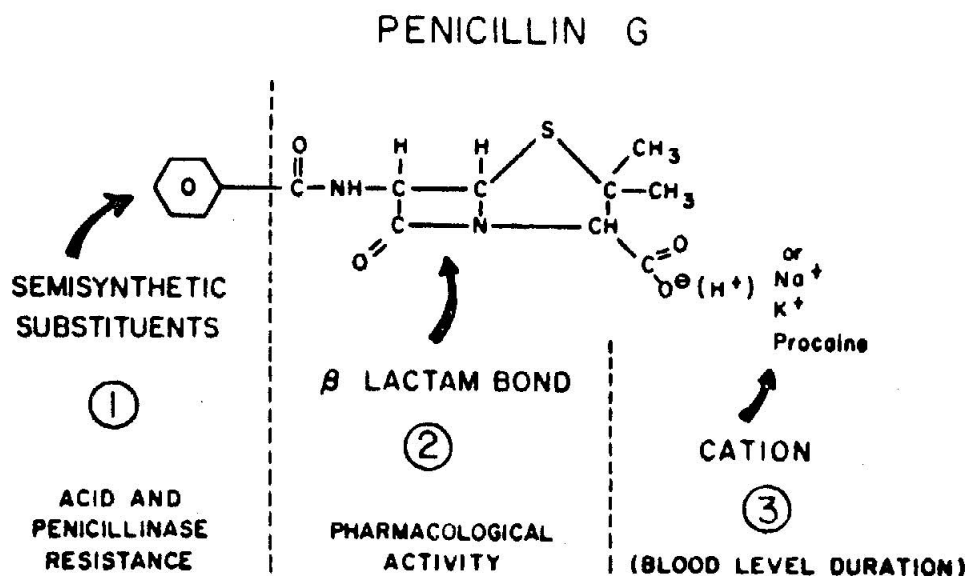
PENICILLIN G

Figure 1. Structure and activity of penicillin G. The antibacterial activity of penicillin resides in the C-N β-lactam bond (2) which is attacked by, but blocks, the bacterial "cross-linking" enzyme. This lactam bond is sensitive to acidic or penicillinase hydrolysis. Alterations at (1) on the penicillin molecule give rise to the semisynthetic penicillins which can be acid stable (oxacillin), penicillinase-resistant (methicillin), or broad spectrum (ampicillin). In crystalline penicillins, the H at (3) is substituted for by Na or K, which gives rise to highly water soluble compounds suitable for intravenous administration. If procaine is substituted for these ions, a slowly dissolving crystal is formed, which is a useful intramuscular depot preparation of penicillin.
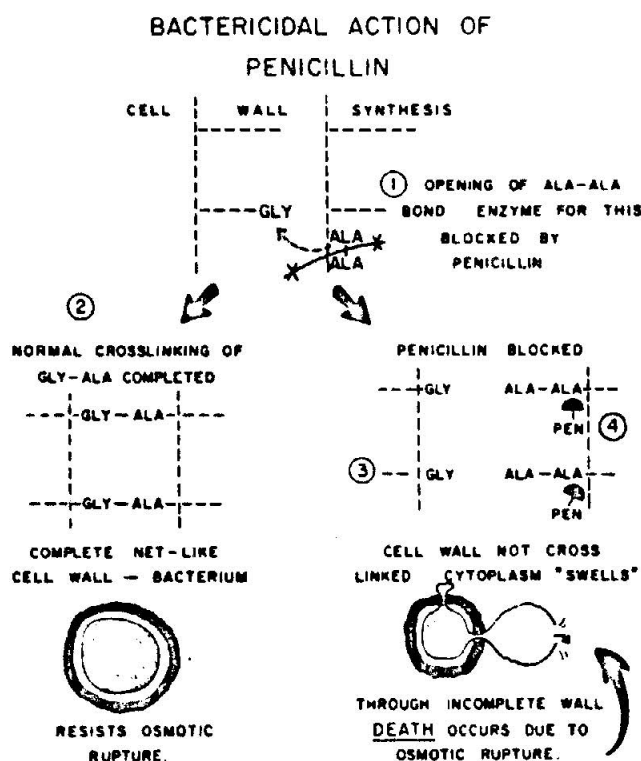
BACTERICIDAL ACTION OF PENICILLIN

Figure 2. Bactericidal action of penicillin. The last step in bacterial cell wall synthesis is the opening of an alanine-alanine bond at (1) and the linking of the remaining alanine to a glycine on the next strand. This cross-linking (2) completes the cell wall which protects the bacterium from osmotic rupture. In the presence of penicillin, the cross-linking enzyme attacks and attempts to open the β-lactam bond of the penicillin molecule. Penicillin binds covalently to the active site on the enzyme (Pen ◆), blocking its action with the result that the cell wall is not cross-linked and the bacterium is not protected against osmotic shock. Bacterial death, when it occurs, is due to osmotic rupture and not to any direct action of penicillin.

mammals do not have rigid cell walls and cross-linking enzymes, penicillin has virtually no direct toxicity in mammalian systems. This highly specific and stable linking of penicillin to a specific bacterial enzyme is therefore the basis of the very potent and specific antibacterial action of penicillin.

Because bacteria which are not multiplying do not make much new cell wall, the penicillins are not very active against bacteria which are not actively dividing. On the other hand, if bacteria are rapidly dividing, the presence of penicillin prevents the formation of new cell wall, and the bacteria will undergo osmotic rupture and die. This is the classical bactericidal' action of penicillin, and under these conditions, penicillin is a very effective drug. However, if the bacterial population is relatively stable and not actively dividing, the production of new cell wall and thus the effectiveness of

penicillin is greatly reduced, and the action of penicillin is primarily bacteriostatic.[b] Similarly, if growth of a bacterial population is slowed by use of a bacteriostatic drug, penicillin will be less effective, and this effect accounts for the antagonism between penicillin and the bacteriostatic antibiotics. On the other hand, if the osmotic pressure in the vicinity of the bacteria is high, like that which may occur in the renal medulla or in abscesses, osmotic rupture and bacterial death may not occur. In this case, the bacteria may survive as "cell wall deficient" (CWD) species or spheroplasts.[6] Then, when penicillin is withdrawn, the bacteria are once again able to form their protective cell walls, and the infection can spread from the area of high osmolarity which protected it from the bactericidal action of penicillin, and reinfect the patient.

Because penicillin binds irreversibly to the bacterial enzyme, the antibacterial action of penicillin persists for a period after plasma levels of the drug decline. For this reason, penicillin is one of the very few antibiotics for which one can let blood levels drop below a "minimum inhibitory concentration" (MIC) for a period during therapy and not get into trouble. Though this practice is not recommended, it is helpful to know that one has this extra margin of safety with penicillin.

Because the pharmacological activity of penicillin resides in the β-lactam bond, any factors which alter this bond will block the action of penicillin. Thus, highly acidic (i.e. stomach pH) or basic conditions, or exposure to alcohol, all of which open the β-lactam bond, can rapidly inactivate penicillin. The clinically most important factor which affects the β-lactam bond, however, is bacterial penicillinase, which is nearly always a β-lactamase. In fact, these β-lactamases are thought to have developed from cross-linking enzymes which modified sufficiently to complete the splitting of the β-lactam bond, and thus remain free to complete the synthesis of the bacterial cell wall.[6] So it appears that the clinically very important property of penicillinase production again relates back to the β-lactam bond and the basic mechanism of action of the penicillin molecule.

Finally, the principal problem associated with the clinical use of penicillin is the relatively high incidence of hypersensitivity reactions following its use. This incidence runs about 5% in humans and is probably less in horses. These allergic reactions are thought to involve linkage of the penicillin molecule via an opened

* Bactericidal—kills bacteria

* Bacteriostatic—prevents bacterial growth

β-lactam bond to tissue proteins. These new penicilloylproteins then constitute foreign antigens in the body and give rise to the various hypersensitivities that may occur in response to penicillin therapy. Again, the β-lactam bond is central to the clinical characteristics of this drug.

Once in the blood, penicillin is rapidly excreted by the kidney, pumped out through the renal tubules by the organic acid transport system.[5] This system apparently operates very efficiently in the horse, penicillin being excreted by the kidney almost five times as fast as the kidney excretes inulin.[5] The upshot of this is that penicillin has a half-life in equine blood of only about 30 to 40 minutes, which is a very short half-life indeed.[1,11] On the other hand, urinary concentrations of the drug are correspondingly high, and very effective concentrations of penicillin are readily obtained in the urinary tract.

Because penicillin is so rapidly pumped out of the bloodstream, it can be difficult to maintain effective plasma levels of the drug. There are two principal approaches to this problem. The simplest is to put an animal on intravenous (IV) infusion and maintain it on penicillin as long as is required. This approach is suitable for very ill animals in intensive care. It allows very high plasma levels of penicillin to be attained rapidly and the drug levels to be altered readily if the condition of the animal warrants a change.

In equine medicine, the more usual method of obtaining prolonged plasma levels of penicillin is to administer intramuscular depot preparations such as procaine or benzathine penicillin. The penicillin in these preparations is not readily available for absorption, and is only slowly released into the bloodstream. In this way, useful plasma concentrations of penicillin can be maintained for up to 12 hours with procaine penicillin.[2] In studies with combination preparations of penicillin in the horse, Rollins et al. (1972) showed that about 2,000 units/lb gave serum levels of between 0.6 and 1.3 IU/ml, which fell to about 0.1 units/ml at 24 hours (Figure 3). These doses, at about 1 million units to a 1,000 lb horse, must be considered relatively small doses. Currently, doses of procaine penicillin administered intramuscularly to humans range up to about 5 million units,[3] and 5 million units would presumably be a conservative dose for a horse. With regard to penicillin dosage, it is important to remember that because penicillin has little or no inherent toxicity for the adult horse and is relatively inexpensive, doses are limited only by what the practitioner thinks is required. It should also be remembered that high plasma levels help the diffusion of penicillin into areas of difficult access by simple mass action, and that high doses tend to delay the appearance of resistant forms of bacteria. The watchword with penicillin therapy is therefore, in general, the higher the plasma levels, the better.
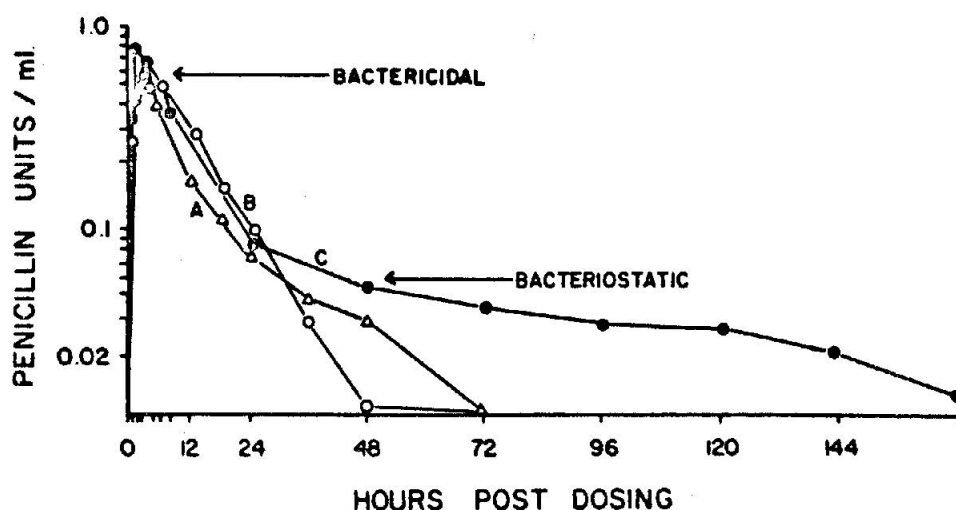

Figure 3. Mean concentrations of penicillin in serum of horses after intramuscular administration of antibiotic preparations. Preparation A: procaine penicillin G and dihydrostreptomycin (DSM); penicillin dosage, 2,000 units/lb (0.45 kg) of body weight. Preparation B: procaine penicillin G, DSM, dexamethasone and chlorpheniramine; penicillin dosage, 2,000 units/lb. Preparation C: benzathine penicillin G (50%), procaine penicillin G (50%), and DSM; penicillin dosage, 4,000 units/lb. The arrows represent approximate bacteriostatic and bactericidal concentrations. It should be kept in mind that the concentrations required to produce these effects depend on the organism in question, whether or not it produces penicillinase, and the accessibility of the site of infection to penicillin. (Modified from Rollins et al. 1972).

Another reason to administer high doses of penicillin is that penicillin generally crosses cell membranes poorly, and may be difficult to get to the sites where its action is required. Thus, there can be problems in attaining useful levels of penicillin in joint cavities, pleural and peritoneal cavities, and in cerebrospinal fluid. It may be helpful, under some circumstances, to administer penicillin directly into the affected area. Administration into the cerebrospinal fluid, however, should be performed carefully, because about the only direct toxicity associated with penicillin is central nervous system excitation and convulsions induced by direct application of penicillin to nervous tissue. Penicillin administered intrathecally should have a clear-cut indication, be isotonic (20 to 50,000 units/ml), and be administered very carefully.

The penicillins are primarily active against gram-positive organisms, and with the exception of the broad spectrum, penicillins have limited activity against gram-negatives. They are almost always effective against Streptococci, and are usually effective against Staphylococci, Corynebacteria, Erysipelothrix spp. and Clostridia spp. The minimum effective concentrations against these species vary, however, from 0.0033 units/ml to 0.33 units/ml against Streptococcus, to 0.1 unit/ml for Corynebacteria, and 2.5 units/ml for Staphylococci.[1] It should be kept in mind that the plasma concentrations required to cure experimental infections in laboratory animals are from two to five times higher than those required to kill the organisms in vitro. This discrepancy is presumably due to the fact that penicillin is at least partially protein bound in vivo, and that diffusion of penicillin into the infected area may also be a problem.

Among the many penicillins, penicillin G, or benzylpenicillin, remains the most potent antibacterial agent, and is the penicillin of choice unless a penicillinase-resistant or broad-spectrum agent is required. However, the appearance of penicillinase-producing bacteria encouraged scientists to study the effect of modifications to the penicillin molecule, and a number of penicillinase-resistant penicillins have been developed. The principal member of this group used in equine practice is oxacillin, which is useful in treating infections due to penicillinase-resistant organisms. It is important to remember, however, that though resistance to penicillinase has been gained, some antibacterial activity has been lost, so full dose schedules of all the more expensive semisynthetic antibiotics must be maintained.

Because penicillin G and its congeners had long had little activity against gram-negative organisms, the development of the broad spectrum penicillins represented a considerable advance. Thus, ampicillin possesses substantial activity against gram-negative bacteria, including Escherichia coli, Shigella, Salmonella, and Proteus. Ampicillin is particularly useful in equines in the treatment of enteritis and septicemia in the newborn and young foal, metritis, including apparently contagious equine metritis, and bacterial infections secondary to upper-respiratory infections, especially those associated with equine influenza.

Carbenicillin is another broad-spectrum penicillin which is often useful against Pseudomonas and Proteus and certain gram-negative organisms, and has been found useful against strains of these organisms resistant to gentamycin.

As mentioned previously, the toxicity associated with the penicillins is minimal. Encephalopathy has been seen in a human patient on 20 million units daily, but this was reversible following discontinuation of the drug. In experimental studies in cats, 1.3 million units/kg has produced encephalopathy which was reversed by intravenous penicillinase.[7] The likelihood of producing encephalopathy with therapeutic doses of penicillin in the horse appears small.

Indirect toxicities associated with the use of the penicillins include problems associated with the cations with which penicillin is complexed. When giving large doses of crystalline potassium penicillin intravenously, care should be taken not to cause hyperkalemic depression of the heart. With procaine penicillin, a possible cause of CNS disturbance in the horse following procaine penicillin could be CNS effects due to procaine. Occasional bizarre behavioral changes have been seen in humans following procaine penicillin and these have been attributed to high plasma levels of procaine.[3] Because the horse is about 20 times more sensitive than the human to plasma levels of procaine, the likelihood of responses of this type must be greater in the equine. However, judging from data developed in this laboratory, at least 30 million units, and more likely, about 60 million units of penicillin, would have to be given to allow procaine toxicity to develop after procaine penicillin. Particular care should also be taken when procaine penicillin is administered to racing animals, because procaine may show up in equine urine for very long periods (up to two weeks) after administration of procaine penicillin and give rise to embarrassing procaine "positives."[8]

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Recessive Lethal White in Two Foals

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The lethal white foal is a genetic abnormality that occurs from crossing two overo parents. It is characterized by the absence of pigment from the skin and iris and by possible congenital intestinal tract defects.

Introduction

Two types of lethal white have been recognized in the foal.[1,3] One is a dominant gene which causes early embryonic death in the homozygous condition.[3] The other results from breeding an overo (color pattern where white is continuous over the body) paint to an overo paint.[3] The pleiotropic effect of the gene affects the intestinal system. This paper describes two white foals affected with intestinal congenital defects.