Subinhibitory Action of Antibiotics

Predicting Antibiotic Effectiveness

Antibiotics are utilized to prevent, control and treat disease in livestock. An animal's response to an antibiotic depends on numerous factors, perhaps the most important of which are the animal's overall nutrition and immune status, and individual animal pharmacokinetics. Because antibiotics do not kill all of the target bacteria, but rather suppress them by reducing their multiplication (bacteriostatic action) or by killing most of them (bactericidal action), the animal's immune system will ultimately be required to fight off infection. Those animals experiencing compromised nutritional status or immune systems should not be expected to respond optimally to antibiotic therapy.

In an attempt to predict which antibiotics are most effective against a particular pathogen, minimum inhibitory concentrations (MIC) for that pathogen are determined. Minimum inhibitory concentration is defined as the smallest amount of antibiotic required to inhibit bacteria. MIC values are determined in the laboratory (in vitro) through Kirby-Bauer (disc diffusion) or microwell dilution (breakpoint) applications from tissue, fluid or exudate obtained from live or dead animals. Based on the results obtained from these laboratory tests, an attempt is made to predict animal response (in vivo) to administration of a specific antibiotic.

Prediction of in vivo response based on in vitro sensitivity patterns can be difficult. Some MIC determinations are based on breakpoints specifically derived for veterinary medicine (tilmicosin, ceftiofur), while others (tetracycline, beta-lactams) utilize human breakpoints. Other factors potentially affecting interpretation of MIC data can include extent of disease progression in the sample submitted, source of samples (live or dead), previous antibiotic exposure, and timing of sample acquisition relative to disease progression in the animal. None of the MIC determination methods can account for the pharmacokinetics of the antibiotic within the individual animal. These and other factors make transposition of information from the laboratory to the animal tenuous.

While not fully understood or completely reliable, the supposition of treatment success is generally based on antibiotic concentrations at MIC levels. The effect of antibiotics when administered at subinhibitory (sub-MIC) levels is even less understood. Experiences of health professionals indicate that sub-MIC antibiotics can have positive effects on health, generally expressed by reduction in morbidity and mortality.

Mechanisms of Sub-MIC Action During Antibiotic Administration

In vitro sub-MIC levels of antibiotics may affect bacteria by (a) altering ultra-structure and antigenicity, (b) altering adherence to epithelial cells, (c) affecting growth rate, (d) changing the synthesis/excretion of pathogenic enzymes,[6] and (e) increasing the efficiency of phagocytosis.[18] Bacteria residing in the host animal may be similarly affected, and any structural impairment or disruption of normal bacterial processes can inhibit their ability to grow and produce pathophysiological changes in the animal's body.

Low concentrations of antibiotics, between 1/3-1/8 MIC and perhaps as low as 1/20 MIC may cause the modification of bacterial structure.[6] Sub-MIC concentrations of antibiotics can cause gram-negative rods to become filamentous and Staphylococci to become clusters. These changes are caused by antibiotic activity on specific enzymes responsible for septation, as in the case of gram-negative organisms, or by lysis of the cross walls, such as in gram-positive organisms.[6] Similarly, *Pasteurella multocida* exposed to subinhibitory levels of tetracycline has been reported to show filamentation, but no changes in capsule production were observed.[4]

Antibiotics may help decrease adhesion of bacteria to epithelial surfaces, making colonization more difficult. Oxytetracycline has been shown to decrease the adhesion of wild-type, induced-resistant, and genetically resistant K88+ enterogenic *Escherichia coli* to porcine small intestinal cells at antibiotic concentration several orders of magnitude below the MIC.[1] Small doses (1/200 of the usual dose) of antibiotic plus large volumes of fluids cured humans of urinary tract infections, while fluids alone resulted in no cure or improvement. Authors attributed the cure to decreased adherence of the bacteria to the bladder wall in those patients exposed to low concentrations of the antibiotic.[6] Tets et al. (1991) found that sub-MIC levels of tetracycline interacted with epithelial cells of *Shigella* spp and various enteroinvasive bacilli. Events such as adhesion, penetration, intracellular multiplication and transfers between contacting cells were affected. The cumulative net effect of these actions was the decrease in virulence of the bacteria.[9]

Subinhibitory concentrations of antibiotics may also affect the production of virulence factors by various bacteria.[2] This mechanism of action may in part account for the effectiveness of low doses of Aureomycin® (chlortetracycline) against bovine respiratory disease (BRD). Bovine lungs are commonly infected with *Mannheimia haemolytica* (formerly *Pasteurella haemolytica*). One of the key virulence determinants used by this bacterium is the secretion of an exotoxin specific for ruminant leukocytes (leukotoxin). At low concentrations, the leukotoxin can activate ruminant leukocytes, whereas at higher concentrations, it inhibits leukocyte functions and is cytolytic, presumably as a result of pore formation and a subsequent increase in membrane permeability. These observations may represent an important mechanism by which *M. haemolytica* overwhelms host defenses, contributing to the fibrinous pleuropneumonia characteristic of bovine pasteurellosis.[10]

Inhibiting *Mannheimia* growth in vivo may decrease leukotoxin production sufficiently to allow animal's immune system to overwhelm the pathogen.[14] Subinhibitory concentrations of tetracycline resulted in a decrease in virulent factors of *Shigella* spp.[9] The effect of antibiotics on the production of representative extracellular enzymes and toxins produced by *Staphylococcus aureus* has been investigated. Protein synthesis inhibitors reduced the production of coagulase and protein A, and almost completely inhibited the production of the haemolysins.[2]

Any effect an antibiotic has on nutrients needed for bacterial growth, such as iron, can have an effect on animal health. Iron is an essential nutrient for the growth of most pathogenic microorganisms; however, in vivo iron is complexed with host proteins such as transferrin in the blood and lactoferrin in secretions so that it is not available as a free ionic iron.[11] Therefore, microbes have developed various strategies for acquiring iron while simultaneously protecting themselves from iron's potential toxic effects. The major strategies used by bacteria to acquire iron include production and utilization of siderophores (ferric specific chelators); utilization of host iron compounds such as heme, transferrin, and lactoferrin; and reduction of Fe (III) to Fe (II) with subsequent transport of Fe (II).[12] When iron is freely available, the phagocytic system may be overwhelmed by rapidly growing organisms.[13] The outcome of every infection is therefore dependent on both the level of free iron present in the host and the efficiency of siderophore-mediated iron uptake system of the pathogen.[11]

The interaction of tetracycline and endogenous levels of iron is important. It has been suggested that a moderate excess of readily available iron, endogenous or exogenous, was not likely to diminish the action of tetracycline. Conversely, research suggests that it was much more likely for tetracycline to decrease the infection enhancing effect of iron.[7]

The effects of subinhibitory concentrations of tetracycline on *Pseudomonas aeruginosa* ferripyochelin (a siderophore) binding protein (FBP) and *P. aeruginosa* virulence in pulmonary infection of rats has also been examined. No FBP was detectable on the surface of bacteria isolated from the lungs of animals treated with tetracycline, and the degree of pathology was significantly less in those animals treated with tetracycline. These studies suggest that exposure to subinhibitory concentrations of tetracycline can repress FBP surface expression as well as proteolytic activity in *P. aeruginosa* leading to a significant decrease in lung injury during infections.[5] Sub-MICs of tetracycline and other antibiotics reduced ferripyochelin, presumed to be a precursor of FBP, uptake by whole cells. This suggests that exposure of the organism to sublethal doses of tetracycline can alter the ability of the organism to acquire iron.[17]

Mechanisms of Sub-MIC Action After Antibiotic Withdrawal

Several mechanisms of action may exist that allow antibiotics to affect bacteria post-drug administration have been suggested. The post-antimicrobial effect (PAE) occurs in vivo when a drug falls below MIC and is slowly removed from the body. For a period of time, bacteria remain disabled by the remnants of the antibiotic. A true PAE may well be the result of previous exposure of bacteria to high concentrations of the antibiotic, rather than the low concentrations still present. The PAE is most common among gram-positive organisms, less common in gram-negative organisms and may last for up to 6 hours.[15]

Of more questionable significance in vivo is the post antibiotic leukocyte effect (PALE). PALE refers to a period of prolonged bacterial growth suppression induced by as little as 10% of the MIC. The mechanisms for both PALE and PAE are complex and perhaps correlated.[15]

Effectiveness of Aureomycin at Sub-MIC Levels

Aureomycin has been used for over 50 years to prevent and treat infectious disease. Diagnostic sensitivities are routinely obtained for tetracycline, and not for other tetracyclines within the class, such as chlortetracycline and oxytetracycline. As is the case with laboratory sensitivity testing, many literature references dealing with MIC and sub-MIC action are commonly made to tetracyclines as a class of antibiotics, and not individual drugs.

Studies indicate that bacteria can be positively affected by sub-MIC concentrations of Aureomycin. Oral doses of Aureomycin at sub-MIC levels have reduced lung lesions due to *Haemophilus somnus*.[16] The positive effects of low doses of Aureomycin in controlling *Anaplasma marginale* infection in beef cattle have also been demonstrated.[3,8]

Conclusions

Intrinsic factors contribute greatly to the ability of animals to ward off disease. Antibiotics only assist the animal's natural defense mechanisms to prevent or cure disease. Predicting treatment success based on MIC values alone is difficult. Clinical studies and field experience suggest there are defined mechanisms by which sub-MIC levels of antibiotics can positively affect the health of animals. Additional work is needed to better understand how various concentrations of antibiotics in blood and tissue affect the health and performance of animals.

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