SYNERGYLABS

CLINICAL AND SCIENTIFIC DOCUMENTATION SUBSTANTIATING THE SAFETY AND EFFICACY OF EAR THERAPY

Section I - A CLINICAL STUDY OF OTITIS EXTERNA IN THE DOG

INTRODUCTION

MANY PAPERS have been written concerning the effectiveness of or preparations for the treatment of otitis externa (O.E.) (8, 11, 12, 13, 14). A recent comprehensive study of the etiology of O.E. and the medical treatment of the condition has been made by Pugh et al (12). Causes such as trauma, foreign bodies, and hirsute ear canals are obvious but microorganisms isolated from an established otitis may actually be a secondary infection and not the primary cause of the condition. As suggested by Pugh et al (12) the etiology may be difficult to determine as often the primary cause may no longer be present at the time of examination. Multiplication of organisms in the environment provided by the initial inflammatory reaction may contribute to clinical otitis. The exudate in the external auditory canal may serve as a guide to the nature of the infection and therefore to the likely prognosis. It is reported that a dark brown or black discharge is associated with Pityrosporum canis or Pityrosporum in association with staphylococcal infection. A dark vellow/light brown discharge may represent a Staphylococcus or Streptococcus infection and a pale/light yellow discharge is often associated with Pseudomnonas and Proteus infections (3,4). However, with mixed infections the discharges may vary in colour when yeasts are involved, either singularly or with bacteria, then the discharges are usually brown, dark brown or black (12). The purpose of this study is to report the findings and results of clinical tests and to evaluate various treatments used for O.E. in dogs.

MATERIALS AND METHODS

Veterinarians from seven veterinary clinics (four in Vancouver area and three on Vancouver Island) voluntarily participated in the project. Each clinician was asked to record his etiological diagnosis of O.E. in dogs, treatment, preparations used for the treatment and the relative response to the treatment. The color of the exudate was recorded in this study but not correlated with the species of microorganism. In one clinic, culture and sensitivity tests of infected ears were routinely performed and the results for the year 1974 have been included. A proportion of the clients failed to return their animals following initial therapy. These cases have been included in the results on the assumption that many of these treatments may have been successful.

RESULTS AND DISCUSSION

A total of 126 treatments of O.E. were performed at the seven clincs. A further 192 cases that had plate cultures and sensitivity tests performed have been recorded, primarily as an indication of the sensitive and resistant strains of microorganisms. In this study 60.3% of all the dogs treated were poodles, spaniels and terriers. Dark brown/black discharge were reported in 36.5% of the dogs. Fifty-three percent had pale/ yellow discharges while the remainder were not reported. On the basis of previous studies (4, 12) this finding would suggest that at least 53% of the dogs were infected with Proteus or Pseudomnonas species. However, Fraser et al (4) reported Proteus and Pseudomonas species in only 13 and 16% respectively of infected ears. This discrepancy may be due to inaccurate colour classification of the discharge by the clinicians. The clinicians listed bacterial infections as the cause of 61.1% of cases and yeast/fungi as causing 23.8% of the otitis. Bacterial or yeast/fungi infections were generally assumed to be the cause of the otitis when other causes were not apparent and before bacteriological test results were available. Matted hair (12.6%) in the ear canal was the next most frequently listed cause. Factors such as concurrent dermatitis, trauma, mites and foreign bodies were less frequently noted. The role of mites as a causative agent of O.E. in dogs have been reported (2, 5, 12). Many workers (3, 4, 6, 10, 11, 12) have discussed the significance of microorganisms as causative agents of O.E. The Gram negative organisms, Pseudomonas spp., Proteus mirabilis and coliforms were found to be responsible for many of the chronic cases of otitis. These microorganisms generally occur in association with other microorganisms and it has been suggested they originate from the intestinal flora (4). However, they may only invade tissue following trauma, pH change or changes in the normal ear flora. Pityrosporum canis is commonly found in

normal ears. However, it is present more frequently in infected ears and has been suggested as a contributor to O.E. (7). Many microorganisms, staphylococci, streptococci and Pityrosporumn spp., are present in normal ears indicating that they are nonpathogenic. Alteration of the internal environment may well cause an increase in the size of their population. The Gram negative organisms, Proteus mizirabilis, Pseudomonas spp. and E. coli which are seldom associated with the normal flora may be pathogens. The results of the 192 culture and sensitivity tests are listed in Table I. The isolations are indicative of the major types of microorganisms and their relative sensitivity to commonly used antibiotics. Twenty-four of the 192 cultures showed "no growth", 31 of the 105 yeasts isolated were single infections and the remaining yeasts were in conjunction with bacteria. Many of the bacteria especially the Pseudomonas and Proteus spp. were commensurate with other bacteria which accounts for the 188 isolations from the 137 plates with bacterial growth (192 total - 24 no growth - 31 yeasts only).

Flushing of the ear canal is considered a necessary prelude to the successful treatment of O.E. Foreign material left in the canal blocks therapeutic agents from complete contact with affected areas, thus impairing healing. Various cleaning agents were used by the clinicians, the most common being Surfak' (38%), Cerex2 (33%) and V. Tergent3 (11%). Four dogs' ears were not cleaned. The pH of the ear has been suggested as an indicator of infection. If the pH is greater than six then the infection may be severe (1). Hughes (9) suggested that a weak acid (5% acetic acid), may be both bacteriostatic and bactericidal. The weak acid returns the ear to its normal pH thus inhibiting growth of most pathogenic bacteria. In chronic conditions, with thick waxy exudates, ether and ethyl alcohol can be applied to facilitate defatting and drying the canal, therefore eliminating the compatible medium for bacterial growth. Care should be taken when using alcohols as they can be irritating and painful especially in chronic conditions. Cerumenolytic agents, light mineral oils and propylene glycol, are suitable for softening compact cerumen crusts, dried exudates and other compacted debris. Following installation of these agents, digital massage of the canal will assist in loosening and breakup of the entrapped and adherent material. A pulsating jet of warm water, can be an effective method of irrigating and cleaning the ear of debris that may adhere to the tympanic membrane, especially if a cerumenolytic agent such as sodium sulfosuccinate is added. The various preparations used in this study are listed in Table II. Treatments number six and ten were both prepared by the veterinarian. The antibiotic based treatments are separated from those containing no antibiotics. Treatment numbers one to five contain neomycin, treatment six contains chloramphenicol, seven contains gentamycin and number eight has tetracycline as the antibiotic. The response to treatments wvas based on a return visit within approximately a three week period. Many of the owners returned their animals not only once but twice in situations where the initial response was not satisfactory. It is possible that some of the satisfactory responses may have relapsed at a later time but this case study did not lend itself to such evaluation. Comparison of satisfactory to nonsatisfactory responses to treatment have been considered in Table IV. In this table the satisfactory responises include the parameters "not retumed", "very good" and "good" as well as a separate analysis to include only the "very good" and "good" responses as satisfactory. This separation was made because the parameter "not returned" may or may not be considered as a satisfactory (including the "not returned" as satisfactory) response of 84.45% or a 72.16% satisfactory response - not including those not returned. The means for treatments containing no antibiotics have a satisfactory response of 71.4% (including those not returned) and 62.0% satisfactory if the "not returned" parameter was not included as being satisfactory. The suppression of clinical O.E. symptoms are not always sufficient to warrant an assumption of recovery. Animals that do not show evidence of persisting discomfort may still have an integument far from normal and in these cases it may be still difficult to persuade an owner that further examination and treatment is required. The comparisons made in Table V show that the calculated values which are less than the chi square value (X2 .05 (1) = 3.841) are indicative that the responses for those treatments are independent of the type of treatment; and those greater than the chi square value (3.841) are independent on the treatment type. The treatments containing neomycin and the total of all antibiotic based treatments did not show a significantly greater response at the first return than all the treatments containing no antibiotics. The treatments containing chloramphenicol did however show a significantly better

response than treatments with neomycin and those containing no antibiotics. No analysis was performed with the tetracycline and gentamycin based treatments due to the small population size. In interpreting these results from this case study, one must not make unqualified assumptions. The species of bacteria found in the ears of all treatments were not cultured, and although treatments without antibiotics did show satisfactory responses, highly pathogenic bacteria such as Pseudomonas and Proteus may not be effectively controlled by such a treatment and may require the usage of sufficiently potent broad spectrum antibiotics. As suggested by Fraser et al. (4) these pathogenic bacteria can be assumed present with reasonable accuracy when the ear contains pale/ yellow exudate, then it is possible that only in these circumstances antibiotics would be required, and not when the ear contains the normal commensal microorganisms. However, as shown in Table I, the microorganisms, Pseudomonas and Proteus are resistant to many of the antibiotics. Therefore, if it were only the antibiotics within the treatments that were responsible for a positive recovery, then many of the cases containing these microorganisms would not be successfully treated. The high incidences of resistant bacteria are definitely a concern of most practitioners. Houdeshell and Hennessey (8) and Webster et al. (14) have reported the success of gentamycin for the treatment of O.E. In this study only six cases of gentamycin usage were reported for the initial therapy. It appears that many clinicians consider such a drug to be the "standby" or "backup" in cases where all else fails. It is suggested that clinical examination is of paramount importance to the successful treatment of O.E. Elimination of the cause will, with suitable treatment eliminate the infection. It must be kept in mind however, that bacterial infections are of secondary importance as causative agents - therefore primary antibacterial treatment may not be the most preferential order of attack.

SUMMARY

A case study of otitis externa in the dog was performed in the Vancouver region and on Vancouver Island. The treatment types and relative responses to the treatments were recorded. The cases treated with antibiotic based preparations did show a slightly better response than the preparations containing no antibiotics, however the differences were not statistically significant.

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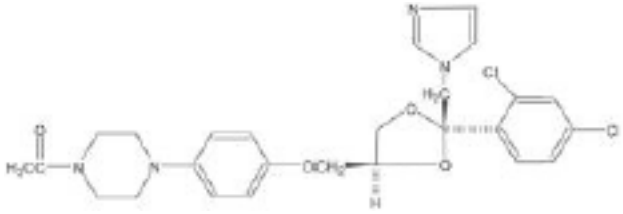
Section II - KETOCONAZOLE

Ketoconazole is an imidazole antimycotic. Its fungistatic effect is based primarily on enzyme inhibition due to a bond with the cytochrome P450. It thus inhibits, amongst others, biosynthesis of ergosterol and thereby alters the permeability of the fungal cell membranes.
Ketoconazole is cis - 1 - acetyl - 4 - [4 - [[2 - (2,4 - dichlorophenyl) - 2 - (1H - imidazol - 1 - ylmethyl) - 1,3 - dioxolan - 4 - yl]methoxy]phenyl]piperazine and has the following structural formula:



The azoles are classified as imidazoles (miconazole,econazole, clotrimazole and ketoconazole) or triazoles(fluconazole, itraconazole and voriconazole) according to whether they contain, respectively, two or three nitrogen atoms in the five-member azole ring. The azole class has become the initial treatment of choice for all but the most rapidly progressing and most severe systemic fungal infections.

FUNGICIDAL ACTIVITY

Ketoconazole is indicated for topical use or orally, with broad therapeutic potential for the treatment of superficial fungal infections and systemic. The distribution of ketoconazole is limited and their penetration of the liquid cerebrospinal is minimal.

Imidazoles act by interfering with cell wall formation in fungal and yeast organisms, which increases cellular permeability, thus suppressing metabolic function and inhibiting growth. There has also been evidence that ketoconazole exerts an inhibitory effect on keratinocytes in culture.
The antimycotic activity of ketoconazole against fungi Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum and Blastomyces dermatitidis is reached at concentrations of 0.125 mg / m up to 0.5 mg / m. The corresponding values for Sporothrix schenckii, Candida sp and Aspergillus sp range from 6mg / m to concentrations equal to or greater than 100 mg / m. (Sand & Mandell, 1987; LACAZ & BLACK, 1991, Richardson & Warnock, 1993).

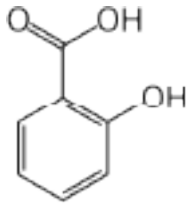
In vitro studies on the fungicidal activity of ketoconazole against samples of filamentous fungi and yeast isolated from animals have been varied. Concentrations from 10mg / m have fungicide effect on Trichophyton verrucossum and Malassezia pachydermatis, while the yeast phase of Sporothrix schenckii in Cryptococcus neoformans, Histoplasma capsulatum in and Blastomyces dermatitidis are needed to Coccidioides immitis 20mg/me 50mg /. The concentration of ketoconazole 50mg / m on Trichophyton mentagrophytes and Microsporum canis was moderately fungicidal. Regardless of the tested concentrations of ketoconazole (10 to 1,000 mg / m), the best effects strongly fungistatic and fungicidal were obtained respectively equinum against the Trichophyton and Microsporum nanum, whereas for Aspergillus fumigatus, Candida albicans, Candida tropicalis and Candida albicans activity of the drug was low (Gabal, 1986). The minimum inhibitory concentration (MIC) averaged for samples of Malassezia pachydermatis isolated from the ear canal of dogs was 0.019 mg / m and 0.012 mg / m for samples isolated from the skin (Coutinho, 1997). Ketoconazole had the best in vitro activity against M. pachydermatis, when compared to other antifungal agents (clotrimazole, miconazole, nystatin and pimaricina) with a MIC at a concentration of 0.02 mg / m reaching 11 samples including a standard strain of 42 samples studied (Uchida et al., 1990). For M. pachydermatis in vitro resistance to ketoconazole has been low, ranging between 0 and 6.7%(UCHIDA et al. 1990; Coutinho, 1997).

KETOCONAZOLE AND BENZETHONIUM CHLORIDE SAFETY

Both ketoconazole and benzethonium chloride are very safe at this concentration for topical use in dogs and cats. However, like any other active ingredient used topicaly, both ketoconazole and benzethonium chloride can result in a local allergic reaction. In these cases, it is recommended to stop using the shampoo and wash the region immediately with plenty of water to remove excess product. If necessary, contact your Veterinarian.

SALICYLIC ACID

Salicylic acid is a beta hydroxy acid which, in contact with the skin region, causes the peeling of the skin region where there is hyperkeratosis (Santoro, 2005), which justifies its wide application in cosmetics and dermatology.



MECHANISM OF ACTION

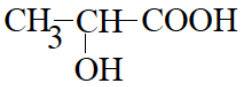
Salicylic acid is keratolytic by lowering the pH of the skin, resulting in increased hydration of the keratin and swelling of the corneocytes. It also solubilizes the intercellular cement substance in the stratum corneum, facilitating desquamation. Salicylic acid does not change the mitotic rate of the basal keratinocytes. It is mildly antipruritic and anti-inflammatory.

BENZOIC ACID

Benzoic acid C7H6O2 (or C6H5COOH), is a colorless crystalline solid and a simple aromatic carboxylic acid. The name is derived from gum benzoin, which was for a long time its only known source. Benzoic acid occurs naturally in many plants[9] and it serves as an intermediate in the biosynthesis of many secondary metabolites. Has been used with salicylic acid as a topical antifungal[10].

LACTIC ACID

Lactic acid (2-hydroxypropanoic acid), also known as milk acid, is a chemical compound that plays a role in several biochemical processes. It has a hydroxyl group adjacent to the carboxyl group, making it an alpha hydroxy acid . In solution, it can lose a proton from the acidic group, producing the lactate ion CH3CH(OH) COO−. It is miscible with water or ethanol.



Lactic acid is chiral and has two optical isomers. One is known as L-(+)-lactic acid or (S)-lactic acid and the other, its mirror image, is D-(-)-lactic acid or (R)-lactic acid. L-(+)-Lactic acid is the biologically important isomer.

Lactic acid is an alpha hydroxy acid used in skin care as a chemical exfoliant. A lactic acid lotion benefits skin by dissolving dead skin that accumulates on the surface of functioning skin. These dead cells lead to dry, dull skin and can prevent your skin treatments from working properly because they can't be absorbed. Removing this accumulation is one of the most important parts of a skin care regimen.

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