Tolerance and Effects of Lupulon in Man*

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Introduction

It is recognized that the antibacterial therapy of tuberculosis has lagged behind that of other infectious diseases. While streptomycin has given clinical evidence of efficacy, this antibiotic alone is not entirely as satisfactory as it is in conjunction with collapse therapy. Antibacterial therapy of mycobacteria differs from that of other infectious diseases primarily because of the chronicity of infection. In addition, the proliferative and destructive changes of host's tissues are definite disadvantages, from the standpoint of its chemical control.

Pathologic changes may prevent adequate access of an antibacterial agent to causative micro-organisms, and further, the tissue changes may be so profound that their reversibility is not possible by means of chemical agents in readily tolerated amounts. At best, suppression of the infectious process appears to be the most easily attainable goal, thus permitting natural defenses of the body to completely overcome the infectious process. It is further recognized that many forms of tuberculosis tend toward recovery with the general medical and surgical procedures now employed.

As stated by Pfuetze and Pyle, the chief impediment to more effective therapy with streptomycin, has been the high incidence of bacterial resistance after the antibiotic has been used for a few weeks or months. Streptomycin is believed to be useful in three ways: as definitive therapy, as palliative treatment, and as an adjunct to surgical procedures. Since it is recognized that streptomycin alone cannot be relied upon for definitive treatment in pulmonary tuberculosis, combined therapy is now being utilized with such agents as promin, diason, and p-aminosalicylic acid. In the continued search for a satisfactory preparation in the

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treatment of this disease, the antibiotic properties of a new agent, lupulon, have undergone scrutiny.

Development

Lupulon is one of two antibiotics derived from the soft resins of hops (Humulus lupulus). According to Wieland and Wollmer, it has the chemical structure:

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(CH3)2C:CH·CH2·HC
      /\       C·CO·CH2·CH(CH3)2
      \        \         \\OH
         C       CH=CH·CH(CH3)2
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It is a colorless, odorless, and tasteless crystalline substance that is lipoid soluble, unsaturated and sensitive to oxidation. It has a melting point of 88 to 92 degrees C. and a solubility in water at pH 5.9 of 12 mgm./l.

The toxicity of lupulon was determined in inbred white mice. Daily intramuscular administration of one-tenth of the acute LD₅₀ as 1.5 per cent lupulon crystals in cottonseed oil over a period of four weeks was tolerated without gross evidence of harmful effect. Histopathologic examination of tissue from these animals revealed small areas of leukocytic infiltration in the liver as well as foci of degeneration in the renal tubules.

In vitro tests with lupulon against tubercle bacilli (H₃₇Rv) in Dubos fluid medium showed consistent inhibition of mycobacterial growth at a dilution of 1:40,000. This was altered neither by the substitution of other wetting agents for the "Tween 80" in Dubos medium nor the presence of sub-bacteriostatic concentrations of other antituberculous substances such as promin, streptomycin, subtilin, and dimercaptopropanol (BAL).

The antituberculous activity of lupulon was tested in vivo using groups of mice infected intravenously with 0.02 mgm. of the H₃₇Rv strain of M. tuberculosis grown in Dubos medium. One-tenth the LD₅₀ was administered by two different routes, intramuscularly as a single daily dose and intragastrically at 12 hour intervals. As determined by histopathologic sections stained with hematoxylin and eosin for lesions and by acid-fast methods for determining the abundance of tubercle bacilli, lupulon exerted considerable suppressive effect on the development of tuberculosis. Treated animals had fewer lesions in all but cardiac tissue; but more
remarkable was the significant reduction in number of microorganisms in the liver (34 to 1), heart (8 to 1), spleen (4 to 1), lungs (3 to 1), though not in the kidneys.

Antituberculous activity both in vitro and in vivo, together with a relatively low toxicity, suggested possible application of this antibiotic to man. The greater solubility in lipoids, with its greater partition coefficient, may serve to make lupulon more available to the waxy coat of mycobacteria. In this respect it may differ from other available antituberculous agents, and thus provide an explanation for its potency against tubercle bacilli. For these reasons, it was deemed desirable to consider the oral application of lupulon to tuberculous patients.

The first 10 patients given lupulon orally ranged in age from 25 to 43 years and included members of both sexes. Nine had moderately advanced pulmonary tuberculosis: There was one with tuberculous laryngitis and one with minimal tracheo-bronchial disease in addition to the pulmonary disease.

Administration and Absorption

Lupulon* was supplied in gelatin capsules each containing approximately 0.5 gram. Each patient was given a daily dose of 8.0 grams regardless of weight. The antibiotic was administered orally in one gram amounts every four hours between 6:00 a.m. and 10:00 p.m.

Of the 10 patients beginning therapy, five completed three months, one two months, two 50 days and two a week or less. The latter four developed complications or side reactions requiring discontinuance of the medication.

The Western Regional Research Laboratory, Albany, California, has developed a spectrophotometric method for the assay of blood levels during therapy. The details of the procedure have not yet been published. It is evident, however, that detectable amounts of the antibiotic occurred in each of the seven patients tested at regular intervals over eight weeks. These ranged from 1.9 to 6.5 gamma per ml., which were beyond the range of control sera obtained from untreated patients.

Clinical and Laboratory Studies

In an effort to determine the action and possible toxicity of lupulon, in addition to complete physical examination and chest roentgenograms, the following procedures were performed on each

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patient: complete blood count, urinalysis, measurement of the daily sputum volume, determination of the erythrocyte sedimentation rate and the concentration of non-protein nitrogen and creatinine in the blood and protein in the serum, albumin-globulin ratio, icterus index, cephalin flocculation and thymol turbidity. Liver function was also determined by the hippuric acid test and renal function by the phenolsulfonphthalein and Mosenthal tests. Electrocardiograms were obtained routinely and bronchoscopies were routinely done in most of the cases.

Tubercle bacilli were present by direct smear in the sputum of all patients prior to onset of therapy. These were verified by positive cultures in all but two of the cases.

During therapy the following procedures were carried out at regular intervals; daily measurement of sputum volume, urinalyses every second day, and weekly complete blood counts, chemical analyses of the blood and cultures and smears of three-day sputum concentrates. The erythrocyte sedimentation rate, phenolsulfonphthalein test and electrocardiograms were repeated at approximately monthly intervals.

The progress of the disease was followed during and after therapy by means of frequent physical and laboratory examinations and chest roentgenograms.

Clinical Observations: While under therapy with lupulon, three patients had significant decreases in the daily volume of sputum and in the frequency and intensity of their cough. Three had significant weight losses, ranging from 9 to 5½ pounds while that of the others remained essentially unchanged.

All but one of the patients were essentially afebrile when lupulon therapy was instituted. No alteration in body temperature occurred in these individuals. One patient had a daily temperature elevation to 101 degrees F. prior to the institution of lupulon therapy which continued throughout the period of therapy. Another demonstrated an increase in extent and severity of an ulcerative tuberculous laryngitis while receiving lupulon.

One patient had significant roentgen evidence of improvement of the pulmonary lesion. None of the others showed changes which could be attributed definitely to the medication.

Repeated laboratory studies failed to reveal any alteration in hepatic or renal function during or after therapy. Serial electrocardiograms demonstrated no change during or after therapy.

Bacteriologic studies have shown to date that the concentrated sputa of three patients became negative for tubercle bacilli on smear and culture after therapy. The others have shown little change in bacteria present in the sputum.

Toxicity: No toxicity of liver, kidney, bone marrow, or myocar-
dium was evidenced either clinically or by laboratory tests.

Every patient experienced some degree of gastro-intestinal irritation while receiving lupulon. Characteristically there was noted epigastric sensation of burning and lower abdominal cramping which occurred from five minutes to six hours after taking the first dose of the drug. These symptoms were sometimes associated with watery diarrhea which was not completely controlled by bismuth subcarbonate or other agents. Nausea and vomiting, when present, continued for the first two days to one and a half weeks of therapy. Lupulon was discontinued in two cases during the first seven days because of gastrointestinal disturbance. Patients continued to be mildly anorexic after the cessation of these symptoms.

Two patients developed transient mild frontal headaches. Two complained of light-headedness. No neurologic changes have been observed. The causal relationship between lupulon and the above complaints is difficult to assess. Two patients noted slight somnolence during the first week of therapy.

Hypersensitivity reactions were uncommon, and the relationship of these reactions to the drug is not clear. One patient developed an erythematosus macular rash lasting two days and a generalized myalgia of five days' duration. Two individuals demonstrated a transient eosinophilia.

COMMENT

The evaluation of the antituberculous effect of any antibiotic requires a period of months to years. Thus, conclusions from this investigation are not warranted. However, there is a suggestion of therapeutic activity at a daily oral dose of 5.0 grams. In one patient there was definite laboratory and clinical improvement, three were freed of the mycobacterium during therapy, and a similar number had significant reduction in sputum and cough. This evidence together with minimal toxicity, with the exception of gastro-intestinal irritation, affords stimulus for continued study.

An appraisal of the possible development of drug resistance with lupulon is now being made. The effective dose range and most suitable method of administration are yet to be established.

Lupulon in its present form has no place in the treatment of tuberculosis other than purely for investigative purposes.

COMENTARIO

La estimación del valor antituberculoso de cualquier antibiótico requiere un período de meses o años. Así las conclusiones de esta investigación no son sólidas. Sin embargo, hay una sugestión de efecto terapéutico con una dosis diaria de 5 gms. orales. En un enfermo hubo mejoría definida según el laboratorio y la clínica.
Tres se libraron de sus bacilos durante el tratamiento y un número similar ha tenido reducción de la tos y de los esputos. Esta evidencia además de la toxicidad mínima, con excepción de la irritación gástrica, alienta para continuar el estudio.

Se trata ahora de averiguar si se desarrolla resistencia al lupulón.

Aún no se ha establecido el alcance efectivo de la droga ni el más adecuado método de administración. El lupulón en su forma presente no tiene lugar aún en la terapéutica de la tuberculosis, sino con fines de investigación.

REFERENCES