Antimicrobial Activity Of Lincomycin and Gentamicin delivered from Chitosan and Chitosan-Gelatin Matrices

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Abstract :
The antimicrobial activity of lincomycin and gentamicin released from chitosan and chitosan-gelatin matrices against gram positive and gram negative bacteria were studied. The inhibition zone diameter were determined After (24,48) hrs of incubation using agar diffusion assay . The results showed that both matrices were very active to deliver the antibiotics.there are significant increasing p<0.05 in inhibition zone after 48 hrs compared with 24 hrs of incubation. Also there is non significant increasing in the antibiotics delivery in chitosan-gelatin matrix. This study suggest to use such matrices in drug delivery system for local (directed) bioavailability of compound antibiotic against gram positive and gram negative bacteria at the same time which is very important in the treatment of some bacterial infections.

Introduction :
The use of polymer in drug delivery systems is widely applied in pharmaceutical studies .Since the discovery of mucoadhesive polymer the research in the drug delivery of these systems was increased. such polymer can be designed for drug delivery in nose,mouth ,vagina, stomah, intestine and rectum(1) Because of the biocompatibility and specificity, of chitosan is widely used in pharmaceutical applications such as drug delivery system( 2,3 ). Chitosan is linear polysaccharide polymer of d-glucos-amine [(1- 4 )-2-amino-2-deoxy-β-D-glucan].Pharmacological and clinical studies showed that chitosan is effective wound healing accelerators in both animal and human tests(4,5),as well as it showed antibacterial activity(6), Several drug delivery systems based on chitosan for other routes of administration are also being investigated. the good muco adhesive properties of chitosan make it a promising candidate for development of intestinal delivery system(7).The biocompatible chitosan was used as potential delivery system for the controlled and localized release of endothelial cell growth factor which is stimulate visualization(8).The other important polymer is Gelatin, is a heterogeneous mixture of proteins derived from animal collagen, Gelatin contains a large numbers of glycine(almost 1 in 3 residues,arranged every third residue),proline and 4-hydroxy proline residues(9,10).Gelatin is very important polymer in pharmaceutical industries. Microspheres containing gelatin and corallin hydroxyapatite were prepared by the dispersion polymerization technique using the antibiotic gentamicin as drug model(11). Gelatin and chitosan matrix were droplet coagulate at low temperature and then cross linked by an ions sulfate,citrate and tripolphosphate(TPP)(12).The present study deals with Lincomycin,an antibiotic derived from cultures of the bacterium Streptomyces lincolnensis ,belong to Lincosamide group. It has been structurally modified to its more commonly known 7-chloro-7 deoxy derivative, Clindomycin, derived by combination of amino acid and carbohydrate
metabolites (13). This antibiotic have primarily gram-positive spectra, including pneumococci staphylococci streptococci and the anaerobic spectra(gram positive and negative bacteria),in susceptible organisms this antibiotic suppresses protein synthesis by binds to 50S subunit of bacterial ribosome(14). The other important antibiotic is ,Gentamicin, an aminoglycoside antibiotic used to treat many types of bacterial infections particularly caused by gram negative bacteria(14). This antibiotic contain 2-amino-sugar residues and a 2-deoxystreptamine unit.Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis (16).There are many studies for develop novel drug localized antibiotic delivery. Drug delivery systems could be designed to deliver drugs locally in the oral cavity, stomach small and large intestine and the rectum, Stomach-specific antibiotic drug delivery, for instance, would be highly beneficial in the treatment of gastrointestinal infection (17). The aim of this study was try to ensure an in vitro delivery of both gentamicin and lincomycin at the same time from chitosan and chitosan-gelatin matrices.

Materials and Methods

1-preparation of lincomycin and gentamicin-chitosan matrices

Chitosan solution was prepared by dissolving (2% w/v) of chitosan powder from(Fluka, switzweland) in 100 ml of 0.1N acetic acid with stirring. Then 20mg of Lincomycin were added with stirring for 1h at room temperature. Glutar aldehyde were then added to the mixture in the ratio 1ml/100ml (18). 100ml of 0.1 M of sodium hydroxide was added to the mixture . The mixture was filtered and washed in distilled water until pH changed to 7 then dried using air dried technique by leaving the matrix in dried hood (17).The same procedure was repeated to prepare gentamicin supported to chitosan matrix and the mixed antibiotic supported to the same matrix.

2. Preparation of lincomycin and gentamicin- chitosan-Gelatin matrices

Chitosan 1.6 gm were dissolved in 100 ml of 0.1 N acetic acid of gelatin polymer .0.4 mg were added to the mixture with stirring. Then 100 mg of lincomycin were added with stirring until the mixture mixed very well for 1hr at room temperature. of gluteraldehyde 1/100ml was added. Then 100 ml of 0.1N sodium hydroxide was added to the mixture . The mixture was filtered and washed in distilled water until pH changed to 7 then dried using air dried technique (18). The same procedure was repeated to prepare gentamicin supported to chitosan matrix and the mixed antibiotic supported to the same matrix

3- Antimicrobial activity (antibacterial testing).

Five species of bacteri E.coli, Pseudomonas aerogenosa ,Klebsiella Bacillus subtilus ,Staphylococcus aureus were used in this study obtained from the Department of Microbiology,Medicine college,kufa university.Bacterial species were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth for 24hrs(19).the antimicrobial activity tests were then carried out by agar diffusion assay (5), wells (6mm diameter)cotaining 50 mg weight of chitosan and chitosan gelatin matrices with 2mg of lincomycin or gentamicin or both antibiotic and control for each matrix without antibiotic were impregnated in spreaded agar with test organisms (for each species ).negative controls were prepared using standard lincomycin and gentamycin. Then the plats were incubated at 37C°.Antimicrobial activity was evaluated by measuring the inhibition zones diameter after 24 and 48 hrs of incubation (each assay in this experiment was repeated triple times) the analysis of variance ANOVA using spss program microsoft company were used for the statistical analysis of the results .

Results and discussion :

The results in figure(1,2,4,5,7,8,9) showed that both matrices were very active to release both antibiotics, lincomycin and gentamicin . This released indicated by the antibacterial activity of both matrices from inhibition zones diameter measurements. The results display a significant differences p<0.05 between the antibiotics supported to the matrices against gram positive and negative
bacteria which may reflect the activity of each antibiotic against either gram positive or gram negative bacteria (21,22). The released may be due to the higher swelling rate of both matrices, which lead to increasing the drug releases because of increasing the distance between the polymer chains (20,17). Figures. (1,2, 4,5,) appeared a significant increasing $p<0.05$ in inhibition zone diameter for both matrices after 48 hrs of incubation compared with 24hrs and control group fig.(3,6). These increasing in the inhibition zones may related to the higher released of antibiotic from these matrices which may form hydrogel compound when absorbed the water. Previous studies found that gelatin formed hydrogel compound in liquid medium (23,24). also the increasing of inhibition zone after 48 hrs may be due to the continuous delivery of both antibiotics from the matrices(25,26). Figures (2,5) showed non significant increasing in inhibition zones diameters in chitosan-gelatin matrix supported to either lincomycin or gentamicin compared with lincomycin or gentamycin supported to chitosan matrix figures(1,4). on the other hand the differences in inhibition zone diameter between two matrices may also be due to the percentage of chitosan in the matrix and the presence of gelatin in the second matrix which may increased the swollen rate which lead to increased drug released (27). Its indicated from figures (7,8) that both matrices were released the antibiotics successfully which may be useful for treatment of some bacterial infection which needs the presence of gram positive and negative antibiotics simultaneously.

![Graph showing antimicrobial activity](image)

Fig.(1) Antimicrobial activity of lincomycin supported to chitosan (chi) matrix after (24,48) hrs of incubation.
Fig. (2) Antimicrobial activity of lincomycin supported to chitosan-gelatin (chi-ge) matrix after (24,48) hrs of incubation.

Fig. (3) Antimicrobial activity of standard lincomycin after (24,48) hrs of incubation.
Fig. (4) Antimicrobial activity of gentamicin supported to chitosan (chi) matrix after (24,48) hrs of incubation.

Fig. (5) Antimicrobial activity of gentamicin supported to chitosan-gelatin (chi-ge) matrix after (24,48) hrs of incubation.
Fig.(6) Antimicrobial activity of standard gentamicin after (24,48)hrs of incubation

Fig.(7) Antimicrobial activity of lincomycin-gentamicin supported to chitosan (chi) matrix after (24,48) hrs of incubation.
Fig. (8) Antimicrobial activity of lincomycin-gentamicin supported to chitosan-gelatin (chi-ge) matrix after (24,48) hrs of incubation.
Fig( 9) Antimicrobial activity of the antibiotics supported to the matrices
1- gentamicin supported to chitosan and chitosan-gelatin matrices.
A.1=gentamicin standard
B.1= gentamicin supported to chitosan matrix
C.1=gentamicin supported to chitosan-gelatin matrix
2-lincomycin supported to chitosan and chitosan-gelatin matrix.
D.2= lincomycin standard
B.2= lincomycin supported to chitosan matrix
C.2= lincomycin supported to chitosan-gelatin matrix
Reference

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