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RECENT INNOVATIONS I OF DELIVERY SYSTEMS FOR ANTIMICROBIAL SUSCEPTIBILITY STUDY OF CEFTRIAXONE BIODEGRADABLE FORMULATIONS FOR POST OPERATIVE INFECTION PROPHYLAXIS

Mahmoud Mahyoob Alburyhi*[1], Abdalwali Ahmed Saif[1], Maged Alwan Noman[1] and Randa Mohammed Saif[2]

[1]Professor of Pharmaceutics and Industrial Pharmacy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

[2]B.SC, Department of Pharmaceutics, Faculty of Pharmacy, Aden University, Aden, Yemen.

***Corresponding Author: Mahmoud Mahyoob Alburyhi**

Professor of Pharmaceutics and Industrial Pharmacy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen.

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ABSTRACT

Staphylococcus Aureus is a pathogen that frequently causes postoperative infections. Systemic injection of antibiotics is an intrinsically poor method for achieving high local tissue drug concentrations because the circulatory system equally distributes medications throughout the entire body. In the present study was to determine how well the biodegradable, lipid-based antibiotic Ceftriaxone tablet formulations combat the primary cause of postoperative infection. The molding technique was employed to produce recent innovation delivery system formulation tablets for Ceftriaxone. The microbiological studies were carried out in a laminar flow condition, and Muller Hinton agar was used as a medium to replicate body tissue. Zones of inhibition for all Ceftriaxone formulations were lower than those attained using standards. At p 0.05, every Ceftriaxone formulation deviates significantly from the reference. Based on findings, the result was deemed satisfactory because it was within the acceptable quality control standards for antibiotics. In conclusion *Staphylococcus Aureus* is regarded susceptible to antimicrobial developed Ceftriaxone formulations, so they could be used in the prevention of post-operative infection (POI).

***KEYWORDS*:** Ceftriaxone, Delivery systems, Recent innovations, Formulations, Biodegradable lipid base tablets, Zone of Inhibition, *Staphylococcus Aureus*, Antimicrobial susceptibility.

INTRODUCTION

Formulation of a new dosage form is one of the most challenging aspects in pharmaceutics for its importance in designing and developing a new dosage form. Postoperative infection (POI) is one of the numerous global health issues that must be prevented by adhering to a number of strategies and procedures; otherwise, it may result in a significant and, in some cases, fatal issue. The primary cause of POI is gram-negative bacteria, and because of the nature of these bacteria, the widespread use of antibiotics, some poor social behaviors, and other factors, the bacteria develop antibiotic resistance, which may result in failure to protect against these types of infection and cause a string of infections and health issues that can result in patient loss of a body part or, in severe cases, mortality.[1-3] The Centers for Disease Control and Prevention (CDC) describe surgical site infections (SSIs) as an infection that happens within 30 days of an operation or within a year in the case of material implantation.[3] These infections can also be divided into superficial infections (skin and subcutaneous tissues surrounding the incision) and deep infections (the fascia, muscle, bone, or implant). The most frequent SSI-causing pathogen is *Staphylococcus Aureus* (*S. Aureus*), which is similar to the skin's natural flora.[4] Under normal situations, healthy soft tissue and bone are very resistant to infection. But following the tissue trauma inherent in surgical treatments, the affected tissue's resistance to infection significantly declines. Devitalized tissue that is more prone to infection might result from the disruption of the blood flow during surgery and the following impairment of the microcirculation afterward. These devitalized tissues provide the best culture medium for bacterial growth when joined with the equipment and the nearby hematoma.[5]

SSI, which affects up to one-third of surgical patients, was the most often reported and observed healthcare-associated infection in low- and middle-income nations. Although SSIs are far less common in high-income nations, they are nevertheless the second most common type of infection related with healthcare in Europe and the USA.[1,6,7]

A transient but excessive drug concentration can occasionally result in harmful side effects from a direct delivery, like the injection of a medication solution. Additionally, excessive drug concentration is not required in the case of time-dependent medications because it is therapeutically necessary to keep the drug concentration above the effective lower limit for a long period.[8-10]

Furthermore, when a continuous supply of a medicine is only needed for a few days or nearly a week, a slow release over weeks to months is insufficient because the drug release is retained extended over such a time period and not supplied properly. For individuals who have trouble taking medications orally, a continuous I.V. infusion is utilized in therapeutic settings, however it can be painful and burdensome for the patients. In fact, implants with a week-long continuous release are thought to be useful for patients who have trouble taking medications orally or lack gastrointestinal absorption because of an esophageal ulcer, digestive system surgery, etc. Many medications are frequently recommended for a week or less. Additionally, I.V. infusion confines patients to their beds during treatment, preventing them from engaging in their typical daily activities, whereas implant-based therapies free patients from this limitation. For example, implants are thought to exhibit superior qualities over intravenous infusion in terms of quality of life, even for patients receiving treatment for a week.[11]

Due to the vascular system's uniform distribution of antibiotics throughout the entire body and the fact that only a small portion of a given dose reaches the infection site, systemic administration of antibiotics is an inherently ineffective method for achieving high local tissue drug concentrations.[12]

Lipid base formulations are one of the dosage forms utilized to maintain effective concentrations for a long period, and the base materials must meet biocompatibility standards. Polymers, both biodegradable and not, are frequently used as basis materials.[11-22]

The variety and adaptability of pharmaceutical-grade lipid excipients and drug formulations, as well as their compatibility with liquid, semi-solid, and solid dosage forms, are the main causes of the rapidly expanding use of lipid-base formulations.[23, 24]

Ceftriaxone sodium (CTX) with empirical formula $C_{18}H_{16}N_8Na_2O_7S_3$, $3.5H_2O$ & molecular weight [MW¼662 g/mol] belongs to the third spectrum cephalosporin's which are broad-spectrum β-lactam antibiotics.[25] One of the most highly regarded cephalosporin medications is CTX. Like other third-generation cephalosporin's, it has a substantially wider spectrum of activity against gram-negative organisms but is much less effective against gram-positive bacteria than first-generation medicines.[26] Numerous infections brought on by susceptible organisms, such as those of the bone and joints, abdomen, lower respiratory tract, meninges, pelvic region, skin and soft tissue, and urinary tract can be successfully treated with CTX. Septic arthritis, bacteremia, and gonorrhea, which are brought on by organisms susceptible to the antibiotic, can also be effectively treated with it. Additionally, it aids in preventing postoperative infections.[27]

POI requires a much procedures to avoid and prevent, hence the effectiveness of CTX from the third generation of cephalosporins in the prevention of such infection and because of the medicine are available in different dosage form but they don't give higher concentration at the site of infection, and possible side effect associated with systemic administration of such drugs, these study concern in estimation of antibacterial activity of Ceftriaxone developed lipid base biodegradable formulations for inhibit the growth of main cause of postoperative infection.

MATERIALS AND METHODS

CTX (Gift from Medical Union Pharmaceuticals - Egypt), Glyceryl monostearate (GMS), Polyethylene glycol (PEG) 6000, tween 20, Mueller Hinton Agar, *S. Aureus ATCC 29213* (Gift from Medicines Sans Frontiers - Aden), Autoclave (Daihan labtect, England, LAC-5060SD), and Incubator (LTE scientific LTD, Great Britain, IP 250).

Preparation of Ceftriaxone Formulations

A different of Ceftriaxone (CTX) antibiotic biodegradable lipid-based formulations,[28] were prepared in the laboratories of research and development in Modern & Global Pharma Companies Sana'a, and the microbiological tests were carried out under laminar flow in the microbiological laboratory of the Supreme Board of Drug & Medical Appliances Aden. Different formulations were prepared as represented in the table (1).[28 -30]

Table 1: Composition of Different Ceftriaxone Biodegradable Lipid Base Formulations.

Formulation	API % CTX w/w	GMS %w/w	PEG6000 %w/w	Tween20 %w/w
CTX1	20	40	35	5
CTX2	20	40	30	10
CTX3	20	40	25	15
CTX4	20	40	20	20
API= Active Pharmaceutical Ingredient				

GMS, PEG 6000, and tween 20 in the precise amounts as shown in table (1) were heated to 70 °C on a water bath while being stirred with a glass rod. The API was evenly distributed throughout the weighed quantity. A 10 mL syringe was used to extract the molten mass, which was then poured into a stainless-steel mold that was round. The mold was allowed to cool at 2 –8˚C. Various formulations were made. The same process was used to create a blend without API.[28 -30]

Activation and Identification of Bacterial Strain

In laminar flow condition, an aliquots of *S. Aureus ATCC 29213* was de freeze at room temperature and by using a sterile loop streaked a plate of blood agar, Vogel-Johnson agar, MacConkey agar, nutrient agar, and nutrient broth. After incubation at 37˚C for 24 h, the growth of bacteria was recognized.

Antimicrobial Activity

Preparation of Muller Hinton Agar

A 29 g of powder was suspended in 500 ml of distilled water. Heat to boiling to dissolve the medium totally. Sterilized by autoclaving at 15lbs pressure (121°C) for 15 min. Mixed well before pouring into a sterilized petri dish.

Preparation of Discs

Whatman filter paper no.1 was used to prepare discs approximately 6 mm in diameter by strongly packing 4 papers together and located in a heavy paper for the night, these discs were placed in a petri dish and sterilized by autoclaving at 15lbs pressure (121°C) for 15 min. Using sterile micropipette tips, these discs take 0.005 ml (5 µL) of antibiotic solution.[31]

Antibacterial Activity of Different Ceftriaxone Formulations

The antibacterial effects of the different Ceftriaxone biodegradable lipid base formulations were assessed by disk inhibition zone. In the disk inhibition zone method, the Mueller-Hinton agar medium was prepared and after the solidification of the agar, they are incubated for 24 h. On the second day, the agar was streaked with a sterile loop with recently prepared cells of *S. Aureus* that were prepared in NaCl 0.9% solution and compared with 0.5% MacFarland opacity standard solution to yield a lawn of growth, and a number of sterilized disks were located onto the agar plate (for each plate one disk was blank controls, three disks were wetted with 5 µL of Ceftriaxone antibiotic. For each formula, three replications were done. After incubation at 37˚C for 24 h, the antibacterial activity was measured as the diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using standard prepared discs of CTX (5µg).[32,33]

RESULTS AND DISCUSSION

Evaluation of Prepared Ceftriaxone Formulations

The result of the preparation of Ceftriaxone biodegradable lipid base formulations for post-operative site delivery is represented in figure (1).

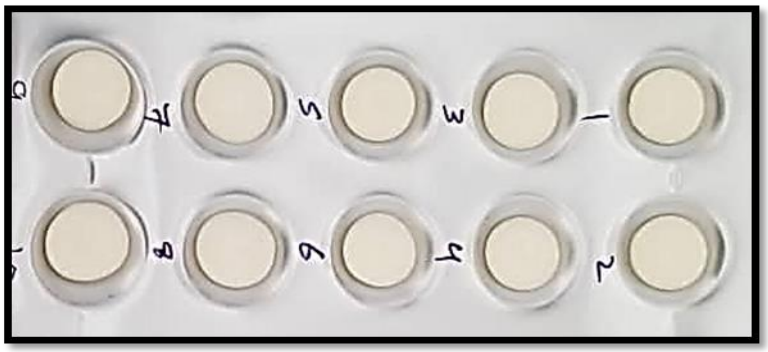

Fig. 1: Molding Technique for Prepared Ceftriaxone Biodegradable Lipid Base Formulations.

The Ceftriaxone biodegradable lipid base formulations were prepared by molding method. In this method, the prepared tablets were circular in shape as represented in figure (1). The color of CTX tablet was pale yellow. The diameter of Ceftriaxone formulations was 12.77±0.14 whereas the thickness of formulations was 4.93 ± 0.13.

Activation and Identification of Bacterial Strain

The results of activation of bacteria on blood agar, Vogel-Johnson agar, MacConkey agar, nutrient agar, and nutrient broth were positive after incubation at 37˚C for 24 h. According to these results the bacteria will be used for testing the sensitivity of *S. Aureus* to different Ceftriaxone biodegradable lipid base formulations. Based on the results of bacterial identification, the growth of the colony was recognized by a different color on different agars plate. *S. Aureus* appears as a brown, black, yellow and white colony on blood, Vogel-Johnson, MacConkey, and nutrient agars respectively. The bacterial growth in nutrient broth media will render it opaque.

Antimicrobial Activity

The result of the antibacterial activity of different formulations was represented as a zone of inhibition in mm and shown in table (2) and figure (2).

Table 2: Zone of Inhibition of Different Ceftriaxone Formulations.

Formulation	Zone of inhibition (mm)
STD CTX	30.67± 0.58
CTX1	28.67 ± 0.58
CTX2	28.33 ± 0.58
CTX3	28.67 ± 0.58
CTX4	28.67 ± 0.58
STD = Working Standard	

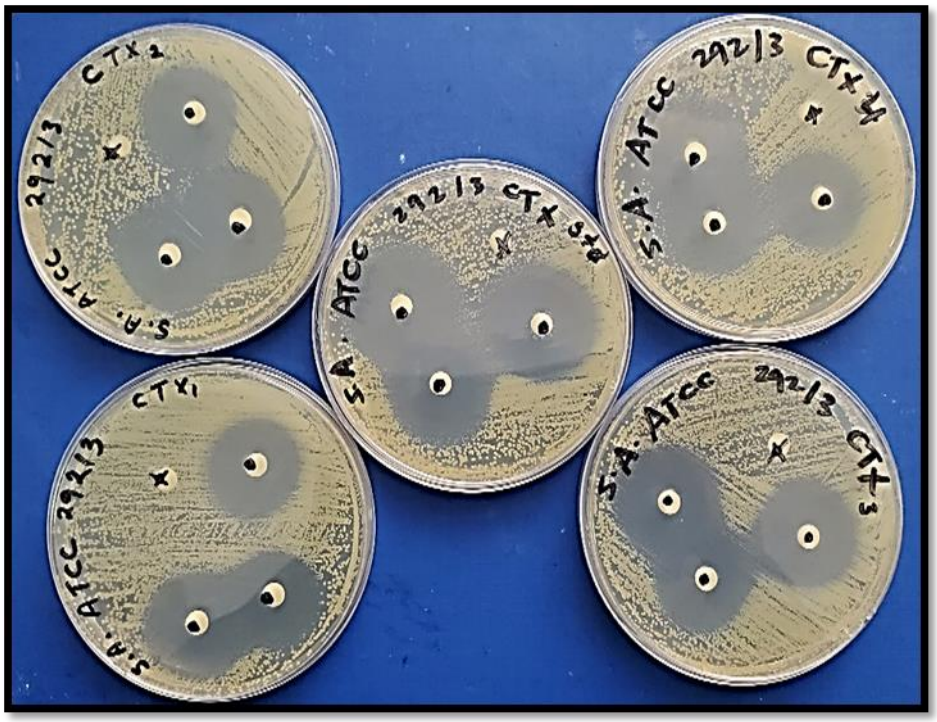

Fig. 2: Zone of inhibition of Ceftriaxone formulations and standard.

All Ceftriaxone formulations had a zone of inhibition less than that obtained by standard. The data were tested by ANOVA using SPSS version 20. All Ceftriaxone biodegradable lipid base formulations show a significant difference from the standard at p of 0.05, the significant value was 0.004. The result obtained was regarded satisfactory and acceptable since it was in the range from quality control limits for antibiotic, based on results obtained using Mueller Hinton agar. There was no significant difference in the zone of inhibition between formulations of CTX. *S. Aureus* bacteria was highly sensitive to all developed Ceftriaxone formulations as shown in figure (2) and table (2) these results achieved by all developed Ceftriaxone formulations.

CONCLUSION

The formulations of lipid base biodegradable Ceftriaxone antibiotic was developed successfully by molding technique. The results of microbiological tests indicated a susceptibility of *S. Aureus* to the prepared Ceftriaxone biodegradable lipid base formulations these gives evidence for the use of biodegradable lipid base formulations in the prevention of surgical site infection. In conclusion, *S. Aureus* is regarded susceptible to antimicrobial developed Ceftriaxone formulations, so they could be used in the prevention of post-operative infection (POI).

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