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Original article

New antimicrobial tetracycline analogue A (Nitrocycline) designed using chemoinformatics

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ABSTRACT

Background: In every country around the globe, bacterial resistance to antibiotics is a serious problem. Investigating new antibacterial medication sources is necessary in light of this. Tetracycline resistance is brought about through changes to the ribosome binding site and/or the development of mobile genetic elements carrying genes for resistance to tetracycline's.**The objective of the study:**The creation of new tetracycline antimicrobial analogues and testing of their in vitro antibacterial activity. **Methodology:**The current investigation was a screening experimental study. This research determined the minimum inhibitory concentrations (MI-Cs) of growth of several pathogenic microbes in Egypt by evaluating the in vitro antibacterial new tetracycline analog activity semi-synthetically synthesized from Streptomyces species. Tetracycline was purified using aqueous two-phase systems with polyethylene glycol and salts based on cholinium, which were then altered using chemo-informatics. The tetracycline analog A (nitrocycline) was created by adding an electron-withdrawing nitro group at position 7 of the tetracycline. **Results:** Although it showed less bacterial resistance than tetracycline, the antibiotic nitrocycline (tetracycline analogue A) was a more effective bacteriostatic antibacterial agent. When compared to the tetracycline prototype antibiotic, tetracycline analogue A demonstrated MICs of less than 10 micrograms/ml for bacterial growth, reflecting its potent antimicrobial activity. **Conclusion:** The development of a nitrocycline antibiotic with a broad range of activity, which helps in addressing the formidable problem of bacterial resistance, made this study intriguing.

Introduction

A significant worldwide impasse is being created by microorganism resistance to modern antibiotics[Parveen K, Clark M, 2020]. To overcome this situation, it is necessary to look into alternative sources of antibiotics[Caroline S, Zeind G, 2018]. The need for antibiotics is evident given the problem of antibiotic resistance on a worldwide

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scale[Trevor Anthony, Katzung Bertram, Kruidering-Hall Marieke,

2022]. The discovery of antibiotics is one of the most significant medical breakthroughs, and their widespread use has significantly reduced death and morbidity worldwide[Bardal Stan,Waechter Jason, Martin Douglas, 2021]. Unfortunately, due to widespread antibiotic use, multi-drug resistance pathogenic organisms have emerged and the effectiveness of many of the present most potent antibacterials has been diminished[Olson James, 2020]. Stepping up, several negative consequences of antibiotics were recognized, most notably the rising prevalence of inflammatory bowel illness caused by Clostridium difficile.6 Bacterial resistance mechanisms' four main pathways mediate bacterial antibiotic resistance: (i) The antibiotic is rendered inactive by bacterial enzymes (beta-lactamases can render cephalosporins and penicillins inactive by cleaving the antibiotic's beta-lactam ring)[Swanson Larry N, Souney F, Muntnick H, Shargel Leon, 2019]. The resistance to streptomycin can be brought on by a mutant protein in the 30S ribosomal subunit, just as the resistance to erythromycin can be brought on by a methylation 23S ribosomal RNA. (ii) Modified targets are synthesized by bacteria against which the antibiotic has a reduced impact[Fisher Bruce, Champe Pamela, Harvey Richard, 2021].

(iii) Changes in porins can reduce the quantity of penicillin that enters bacterial cells, reducing the permeability to an antibiotic and preventing an effective drug intracellular concentration[Dipro Cecily, Schwinghammer Terry, Dipro Joseph, Well Barbara, 2021]. (iv) Using a multi-drug resistance efflux pump, bacteria aggressively export the antibiotics. In an exchange-type process, a multidrug resistance pump (MDR) imports protons while exporting a number of different compounds, including certain medicines like tetracyclines[Golderg Stephen, 2020]. The majority of antibiotic resistance is caused by genetic changes in bacteria, such as chromosomal mutations, plasmid or transposon acquisition, or both[Wilson Golder N, 2019]. Mutations in the ribosome binding site and/or the development of mobile genetic elements carrying tetracycline-specific resistance genes are the two main mechanisms by which bacteria develop resistance to the antibiotic tetracycline[Metting Patricia J, 2019]. a description of tetracycline: Chlamydiae, mycoplasmas, and rickettsiae are only a few of the gram-positive and gram-negative bacteria that the antibiotic tetracycline has a bacteriostatic effect on[B Oliva, G Gordon, P Mcnicholas, G Ellestad and I Chopra, 2012]. By attaching to the 30S ribosomal subunit and preventing aminoacyl transfer RNA (tRNA) from entering the acceptor site on the ribosome, they stifle protein synthesis[Alexey Aleksandrov, Thomas Simonson, 2009]. Due to the similar reduction of in vitro protein synthesis by tetracycline in isolated ribosomes from both human and bacterial cells, the discriminating activity of tetracycline on bacteria is not at the ribosomal level. Tetracycline has a remarkable increased uptake into susceptible microbe cells when compared to human cells, which underlies its selectivity[Jana L et al, 2019]. In the present study, the aim was to design and develop novel chloramphenicol analogs by chemo-informatics to overcome bacterial resistance to tetracycline worldwide.

Ethical statement:

In the present study, we followed All applicable national, international and/or institutional guidelines for the attention and utilization of humans and animals. All processes carried out in study including humans and animals were authorized by the local authorities, Ethical committee for human and animal handling at Cairo university(ECAHCU), at the faculty of Pharmacy, Cairo University, Egypt in agreement with the recommendations of the weathrall report with approval number P-24-7-2021. All efforts were performed to ablate the number of humans and animals utilized and their suffering during study.

Materials:

All chemicals and biochemicals were purchased from Algomhuria and Alnasr pharmaceutical and chemical companies in Egypt. This study was done between January 2022 to July 2022 in the faculty of pharmacy, Cairo University, Egypt.Source of animal models:

They were purchased from the faculty of pharmacy, Cairo University, Egypt.Inclusion criteria for animal models:

I.Adult animals such as rabbit and mice models. II. Can be infected by different bacterial infectious diseases such as Tonsillitis and Pneumonia. III. Obese animals.Exclusion criteria for animal models:

I.Young animal. II. Pregnant female animals. III. Can not be infected by bacterial

infectious diseases such as pneumonia and meningitis. IV.Thin animals.

Type of the study: Screening experimental study.

Methods:

Isolation of Streptomyces rimosus on mineral Streptomyces agar(MSA) selective media:

A total of 50 grassland soil samples were collected from 1–10 cm depth in different locations in Egypt. We prepared these soil samples for the isolation of bacterial strains by the standard serial dilution method. We suspended one gram of each sample in 9 ml of distilled water and

vortex-ed. Then, serial dilutions of each sample were carried out up to 10−3 dilutions. The 100 μL of each aliquot from final dilutions was spread over the surface of MSA containing humic acid dissolved in bacteriological agar 25 g, MgSO4 1 g, Na2HPO4 3 g, CaCO3 0.5 g, humic acid 7 g, KCL 15 g, cycloheximide 6 g, distilled water 1 L. The cultured plates were incubated at 25°C in darkness until sporulations of bacterial colonies for one week. Bacterial colonies were identified based on morphological characteristics by light microscopy. Pure and single colonies of Streptomyces rimosus were picked and preserved at 2–3°C for further evaluation of the antimicrobial activity.

Purification of tetracycline:

Tetracycline was extracted and purified from fermentation broth utilizing aqueous two phase systems involved of polyethylene glycol with average molecular weight of 600 g/mole and cholinium- based salts(cholinium acetate,cholinium chloride and bicarbonate[Jorge Pereira et al, 2013].

Preparation of tetracycline analog by chemo-informatics:

Production of tetracycline analog A(nitrocycline) was finished via the chemical modification of 6-deoxytetracycline at the C7 position with strong electron withdrawing group such as nitro group(NO2). Aromatic nitro tetracycline was synthesized through nitration. Nitration was achieved utilizing a mixture of concentrated nitric acid and sulfuric acid at neutral PH and temperature not exceeding 30 C. The origin of the nitronium ion was via the protonation of nitric acid by sulfuric acid, which brought about the formation of a nitronium ion and a water molecule. It was a great electrophil; hence it was attacked by aromatic rings to bring forth nitrocycline at carbon 7.

Evaluation of antimicrobial activity:

Antimicrobial activity of Tetracycline analog A was proven by agar dilution technique against enteropathogenic Escherichia coli O157:H7, methicillin-resistant Staphylococcus aureus ATCC 43300, Enterococcus faecium, Pseudomonas aeruginosa, Haemophilus influenza type b, Neisseria meningitides, Streptococcus pneumonia, Bacteroids fragilis, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Rickettsiae infectious bacteria (such as Rickettsia rickettsia, Rickettsia prowazekii, Coxiella burnetii, Analplasma phagocytophilum and Ehrlichia chaffeensis (all Rickettsia were grown in embryonated eggs), Salmonella typhi, Salmonella paratyphi, Chlamydiae trachomatis, and Chlamydiae pneumonae.

(The pathogenic bacterial samples were obtained from the faculty of agriculture at Ain shams university in Egypt) . Minimum inhibitory concentrations (MICs) of growth were determined and compared with a standard chloramphenicol antibiotic.

Formulation of film coated oral nitrocycline drug delivery systems:

Tablets of micro-particles of nitrocycline were prepared by the wet granulation method. Starch was added as a diluent,binder and disintegration agent. Magnesium stearate was added as a lubricant agent.All ingredients were passed through 80# mesh sieve.The film-coated tablets were prepared via the aqueous film coating method (film coating is a single process that involves the deposition of a thin film polymer such as 100-micrometer hydroxypropyl methylcellulose phthalate via spraying coating solution onto the tablet beds in a pan coater followed by immediate drying to form thin, film and enteric coat on the micronized tablets in presence of plasticizer such as polyethylene glycol(200-6000)).

Evaluation tests of oral nitrocycline tablets:

These tests were carried out as per British pharmacopeal specifications.

Compatibility study:

We characterized nitrocycline and different excipients utilized in the preparation of oral tablet formulations by FT-IR spectroscopy and DSC to see the compatibility.

Hardness:

We performed a diametric compression test according to British pharmacopeal technique 2.9.8 utilizing Monsanto hardness tester.A hardness of 2kg/cm2 was acceptable in case of oral nitrocycline tablets according to standard literature.

Friability:

We dedusted, accurately weighed and placed a random sample of the whole tablets corresponding to 6.5 g in the drum of a Roche friability tester.we rotated the drum 100 times and tablets were accurately weighed ,dedusted and removed.1% was considered acceptable as a maximum weight loss.

Wetting time:

Two layers of a rectangular absorbent paper (10cm×7.5 cm)fitted into a petri dish and wetted thoroughly with distilled water were used for carrying out the test for wetting time. Then we placed the tablet at the centre of the plastic dish and recorded the time required for the water to diffuse from the absorbent paper using stop watch.

Determination of water absorption ratio:

We kept a piece of tissue paper folded twice in a petri dish (internal diameter 6 cm)incorporating 7 ml of purified water.Then we settled the tablets on the tissue paper and left to wet wholly. The wetted tablets were separated and reweighed.

Disintegration test:

The test was carried out according to British pharmacopoeia standards.we placed one tablet in each of the six tubes and utilizing distilled water maintained at 370 C;then tablets were observed for disintegration.The basket from the fluid was lifted up and observed for the tablets complete disintegration at the end of the time limit.

Weight variation:

From each batch 20 tablets were chosen randomly and their average weights were calculated utilizing digital weighing balance(Essae Teraoka ltd);then percentage weight difference was estimated and checked with British pharmacopoeia specifications.

Determination of uniformity of drug content:

From each formulation twenty tablets were weighed and powdered;then10mg of the powder was weighed and dissolved in 100 ml of distilled water.we sonicated the mixture for 170 seconds and filtered through Whatman filter paper No. 40.Then the filtrate was diluted with distilled water and the absorbance at 310 NM was estimated.

In vitro drug release profile :

Distilled water was used as the dissolution medium at 37 C and 50 rpm(paddle).We collected samples at 3,6,8,11,16,19,60,120,240 minutes intervals.The amount of nitrocycline released was measured using UV spectrophotometer at 275 NM.

Stability study:

It was carried out for optimized formulation.The storage conditions utilized for stability studies were accelerated conditions 40 C and room temperature 30 C.Optimized formulation tablets were kept,striped and packed in humidity chamber for thirty days on above mention temperature.

Formulation of intravenous nitrocycline drug delivery systems:

We processed IV antibiotic standard solutions of nitrocycline(1000 microgram/ml via solubility of 100 mg nitrocycline standard powder in 100 ml deionized distilled water(DDW).

Study of the pharmacokinetics of tetracycline analog A:

The pharmacokinetics of tetracycline analog A were studied on 50 mice and rabbit animal models in a comparison with standard chloramphenicol.

Study of pharmacodynamics of tetracycline analog A:

The pharmacodynamics of analog were studied on 50 mice and rabbit animal models infected with different infectious bacterial diseases such as meningitis, pneumonia, and soft tissue infections.

Human evaluation of oral and intravenous drug delivery systems of nitrocycline via human clinical trials phases 1/2:

3 groups of adult patients with different bacterial infections were included in our study. Each group consisted of 100 subjects:

Group(1)(negative control group) were administrated graded amounts of the placebo by IV and oral routes of administration.Group(2)(positive control group) were administrated graded amounts of the standard chloramphenicol antibiotic intravenous and oral routes of administration.

Group(3)(test group) were administrated graded amounts of the test antibiotic. The activity of nitrocycline was estimated by the reduction in

bacterimia, septicemia and observation of the clinical signs of infectious disease.

In vivo bio-availability study:

Before dosing IV or oral tablets 0.7-0.9ml of blood samples were withdrawn ,and immediately after dosing at 30,60,120,240 minutes.Blood samples were further refrigerated and centrifuged at 4 C within one hour of sampling.Nitrocycline concentrations were determined using HPLC.HPLC analysis was through a reversed phase column utilizing phosphate buffer(PH 4.4) and acetonitrile(660/340, v/v) as mobile phase with a flow rate 0.9ml/min. The limit of UV estimation of tetracycline concentration in blood was at 275 NM. Area under the curve(AUC) and the % of relative bio-availability were measured. % of relative bio-availability was determined by the following equation:

% Relative bio-availability=(AUC Oral/AUC Intravenous)×(Dose Intravenous/Dose oral)×100%.

Statistical analysis

It was done in triplets for every culture. Means and standard deviation were used in their presentation. The statistical analysis was conducted using an excel spreadsheet and a one-way analysis of variance with a p value less than 0.05. In conducting this investigation, the F-test was used.

Results

In the present study different batches of nitrocycline Oral tablets utilizing various ingredients as starch, sucrose DC , talc… etc were prepared (Table 2).

Isolation of streptomyces rimosus sp producing tetracycline on MSA:

28 pure and single colonies of streptomyces were picked and preserved at 3°C for further evaluation of an antibacterial activity.

Evaluation of antimicrobial activity by standard agar dilution technique:

For tetracycline analog A(nitrocycline): 8.7µg/ml, 5.3µg/ml, 9.4µg/ml, 5.8µg/ml, 7.2µg/ml,4.1µg/ml, 4.7µg/ml,8.4µg/ml,6.2µg/ml, 7.7µg/ml, 5.8µg/ml,9.3µg/ml, 6.5µg/ml, 4.7µg/ml, 5.9µg/ml, 6.1µg/ml, 7.8µg/ml MIC values were observed against Escherichia coli 0157:H7, Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenza type b, Neisseria meningitides,Streptococcus pneumonae, Bacteroids fragilis,Clostridium difficile,Clostridium perfringens, Clostridium tetani, Rickettsia rickettsia,Rickettsia prowazekii, Coxiella burnetii, Analplasma phagocytophilum , Ehrlichia chaffeensis, Salmonella typhi and Salmonella paratyphi respectively.

Oral dosage:

The recommended oral dose for adults was 0.35-0.65 g three times per day and 30-40 mg/kg/d for children older than two years old for swiftly expelled nitrocycline. For severe systemic infections, the greater dosage is advised—at least during the first several days. As its absorption is not significantly affected by meals, nitrocycline may be the best oral tetracycline to use. The use of nitrocycline with ferrous sulphate, milk, or antacids should be avoided since these substances have a metal affinity. Nitrocycline should not be administered to children under the age of eight or to pregnant women to prevent deposition in developing bones or teeth. According to FT-IR and DSC studies, there is no chance that nitrocycline and excipients will interact.

The tablets' hardness was measured, and the results ranged from 3.79 to 3.98 kg/cm2.All formulations' weight variations were estimated and found to be within the acceptable range according to the British Pharmacopoeia.In the limit of our ability, we discovered percentage friability in the range of 0.71 to 0.89%. All formulations' water absorption ratios were found to range from 38.23 to 39.67.All formulations' wetting times were calculated to be between 19 and 24 seconds. We evaluated the in vitro disintegration time of the oral tablets.In vitro disintegration times for formulations F1 through F5 were found to be between 7 and 9 minutes.

The formulation F2 showed a 7-minute rapid disintegration period.This is due to the burst effect and the quick absorption of water by the medium. Between 98.83 and 99.67 percent of nitrocycline was found in all formulations, which is within the normal range. The controlled release tablets' release times varied from 98.19% to 99.13 at 4-6 hours at 37 0C and 50 rpm in contrast to the immediate release tablets' release times, which ranged from 98.71% to 99.34% at 2 hours at 37 0C and 50 rpm. The medication was released more quickly from Batch F2 than from any previous batch.Batch F2 showed 98.45% total drug release in 240 minutes at 37 0C and 50 rpm. 180 minutes were noted to be Batch F2 t50%.Batch F2 was suggested as an optimized formulation due to its quick disintegration time and dissolving profile. Using 10 mg of sucrose DC and 17 mg of starch, batch F2 was

created. Nitrocycline oral tablet (batch F1 to F5) storage was shown to be best between 2 and 8 0C.

Par-enteral dosage:

Nitrocycline was administered intravenously in dosages of 0.2–0.6 g every 12 hours. Because of the discomfort and irritation at the injection site, intramuscular injection is avoided.

Pharmacokinetics:

Nitrocycline had an absorption rate of around 70% following oral dosing. An oral dosage of nitrocycline contained a component that was excreted in the faeces after altering the intestinal flora and corp-sing in the gut lumen. The superior small intestine was where absorption occurred most, and it was inhibited by alkaline pH, divalent cations (Ca2+, Fe2+), Al3+, and antacids. Nitrocycline solutions specifically buffered for intravenous delivery may be created.About 60% of nitrocycline was bound by serum proteins. Nitrocycline oral doses of 250 mg per 8 hours resulted in peak blood levels of 5-7 mcg/mL. Nitrocycline levels from intravenously administered doses were moderately high. Nitrocycline's steady-state peak serum concentrations at the recommended dose were 0.5 mcg/mL.

With the exception of the cerebrospinal fluid, where concentrations were 9–17% of those in serum, nitrocycline was distributed widely across tissues and bodily fluids. The meningococcal carrier status might be eliminated thanks to the high amounts of nitrocycline found in saliva and tears. The drug nitrocycline was excreted in milk and passed across the placenta to the developing foetus. Nitrocycline was attached to developing teeth and bones as a result of chelation with calcium, which caused harm to them. The half-life of nitrocycline was shortened by 50% by the stimulation of hepatic enzymes that break down the medication phenytoin, carbamazepine, persistent alcohol use, and barbiturates.Urine and bile were the main elimination pathways for nitrocycline. Concentrations in bile were seven times higher than in serum. Only a small portion of the medication excreted in bile was reabsorbed from the colon (enterohepatic circulation) and contributed to serum level maintenance. 80 percent of the drug, mostly through glomerular filtration, was excreted in the urine. Faeces included 20% of the drug's excretion. In renal failure, nitrocycline dose may need to be adjusted. A classification of intermediate-acting was given to nitrocycline based on its 6–8 hour serum half-life.

Table 1. List of instruments:

Instrument	Model and manufacturer
Autoclaves	Tomy, japan
Aerobic incubator	Sanyo, Japan
Digital balance	Mettler Toledo, Switzerland
Oven	Binder, Germany
Deep freezer -70 ^{0}C	Artikel
Refrigerator 5	Whirpool
PH meter electrode	Mettler-toledo, UK
Deep freezer -20 ^{0}C	whirlpool
Gyratory shaker	Corning gyratory shaker, Japan
190-1100 nm Ultraviolet-visible spectrophotometer	UV1600PC, China
Light(optical) microscope	Amscope 120 X-1200 X, China

Table 2. It shows batch formulation of oral tablets of nitrocycline(tetracycline analog A) F1-F5 by wet granulation technique:

Ingredients(mg/tablet)	**F1**	**F2**	**F3**	**F4**	**F5**
Nitrocycline(Tetracycline analog A)	250	250	250	250	250
Starch	15	17	16	12	14
Sucrose DC	11	10	11	13	10
Talc	1	3	1	3	2
Mg stearate	3	1	2	2	4
Total weight(mg)	280	280	280	280	280

Table 3. It represents MICs of Chloramphenicol(C) and tetracycline analog A(Nitrocycline):

	C	A
MIC	13	9.6

Figure 1. I t shows tetracycline chemical structure

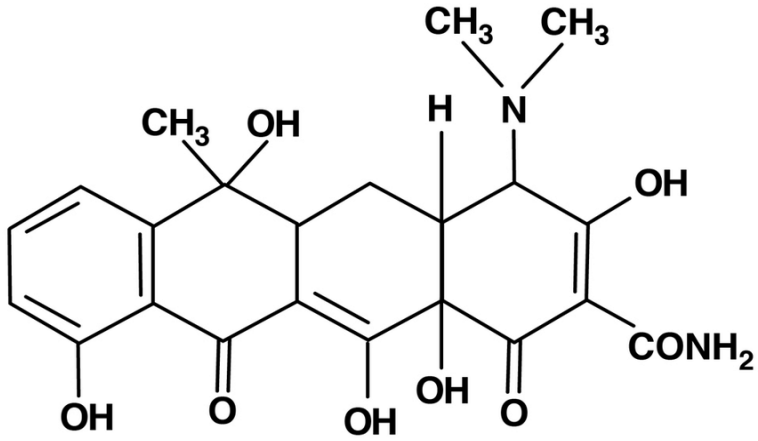
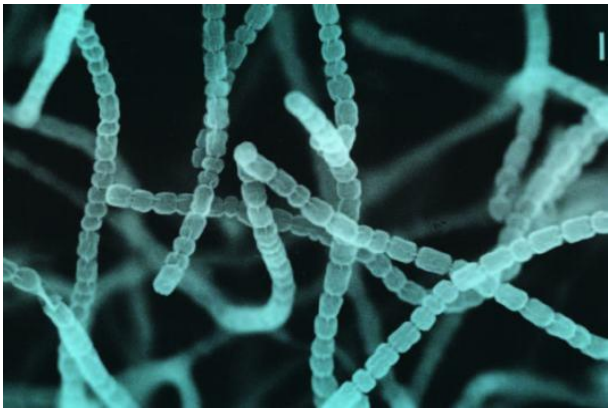
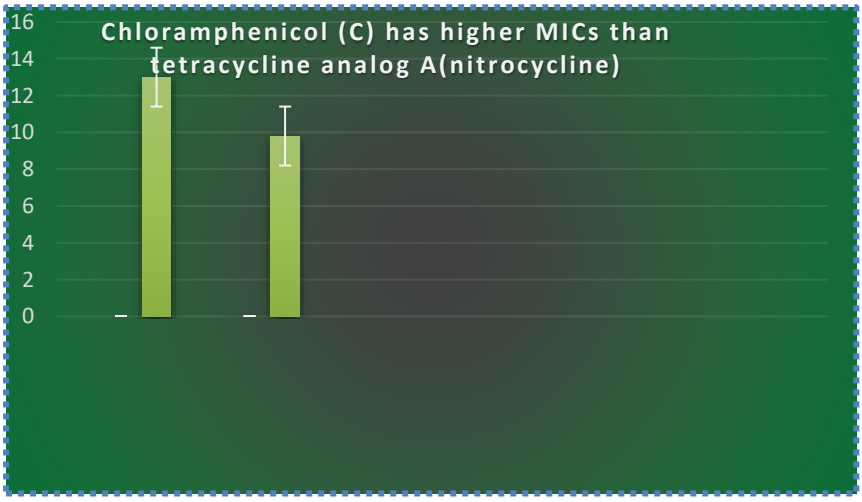


Figure 2. It shows soil Streptomyces rimosus producing tetracycline in Egypt.



Graph 1. represents a comparison between MICs of chloramphenicol and tetracycline analog A. MICs of chloramphenicol were greater than 10µg /ml, while MICs of tetracycline analog A were less than 10 µg/ml, thus nitrocycline had higher antimicrobial activities than standard chloramphenicol.



Discussion

The discovery of the new antibiotic Nitrocycline was part of the current investigation. Tetracyclines were first discovered in the 1940s and have been effective against a variety of microorganisms, including gram-positive and gram-negative bacteria, Chlamydiae, Mycoplasma, Rickettsiae, and protozoan parasites. However, the emergence of mutant-resistant bacteria against them has created a massive global crisis. According to [Ian Chopra et al study, 2001], this was the case. This study's discovery of nitrocycline was shown to be completely free of these resistance issues.

Major targets of inhibition by antibiotics like tetracyclines within the cell include ribosome and protein synthesis. Due to the emergence of bacterial resistance, MI-Cs of tetracycline were found to be larger than 10 mcg/ml. the various ways that bacteria develop tetracycline resistance, including efflux, drug modification, target mutation, and the use of specialized ribosome protective proteins. This was demonstrated by[the 2014 study by Fabian N et al]. In contrast, it was discovered that the MI-Cs of nitrocycline were less than 10 mcg/ml, indicating that nitrocycline has a stronger biocide impact than tetracycline since bacterial resistance to it is less common.

Estimation of biological activity of nitrocycline as antimicrobial agent:

Nitrocycline(tetracycline analog A) possessed a structure analogous to tetracyclines and held the identical mechanism of action like tetracyclines; that is to say, they impeded protein synthesis of pathogenic bacteria by linking to the 30S subunit of the ribosome. It showed a bacteristatic action. Also a correspondent range of inauspicious effects was demonstrated by nitrocycline. Nitrocycline was efficacious in the remedy of infections caused by group A and group B streptococci, vancomycin-resistant enterococci, E. coli, Bacteroides fragilis, methicillin-sensitive and methicillin-resistant S. aureus; as well as treatment of complex

intra-abdominal infections crusaded by a assortment of anaerobic and facultative bacteria.Tetracycline analog A showed more antimicrobial activity than standard chloramphenicol but showed less bacterial resistance. Nitrocycline showed MICs of less than 10 μg/ml for the bacterial growth of pathogenic bacteria while chloramphenicol showed MICs greater than 10 μg/ml. Nitrocycline was a potent inhibitor of bacterial protein synthesis. It bound reversibly to the 30S bacterial ribosome subunit and stamped down the peptidyl transferase step of protein synthesis. Nitrocycline as a bacteriostatic broad-spectrum antibiotic that was active against both aerobic and anaerobic gram-positive and gram-negative organisms. It was active also against Rickettsia genera . Most gram-positive bacteria were inhibited at concentrations of 4–10 mcg/mL, and many gram-negative bacteria are inhibited by concentrations of 4–9.5 mcg/mL. H influenzae, N meningitidis, and some strains of Bacteroides were highly susceptible, and for them, mitrocycline might be bactericidal. Neither chloramphenicol nor its analogs showed bacterial activity against Chlamydiae infections while nitrocycline showed excellent activity against different Chlamydia infections. NO possibility of interaction between excipients and nitrocycline was unconcealed by the FT-IR and DSC study. Starch events as a disintegration agent and a diluent . Sucrose DC events as a sweetener . Many excipients showed water solubility and thus had better a patient acceptability. Our study was prosperous in terms of decreasing cost,manufacturing difficulties and stipulating an effective medication with better patient compliance. Direct reciprocity between the disintegration time and wetting time was present. Batch F2 showed less disintegration than all other formulations. Optimized formulation was well advised to be batch F2.Fri-ability and hardness of batch F2 were too good.In vivo and stability studies were carried out on batch F2.No change occurred after one month as was informed by stability study. Batch F2 demonstrated a good uniformity of the drug content, dissolution profile, disintegration time and boost a good in vivo absorption profile and stability. Bio-availability of nitrocycline has been improved by oral tablets formulation as was indicated via in vivo studies.

Pharmacokinetics:

Variability of oral absorption existed, and might be impaired by multivalent cations (calcium, iron, aluminum)and foods. Nitrocycline crossed the placental barrier and held a broad tissue distribution. It underwent enterohepatic cycling. Nitrocycline was excreted secondarily in feces; but was eradicated. chiefly in the urine.

Antibacterial Activity:

Nitrocycline was a wide-spectrum antibiotic with activity against

gram-negative and gram-positive bacteria, some protozoa, species of Mycoplasma, Chlamydia, and Rickettsia. Nevertheless, resistance existed but less than other members of tetracycline family. Resistance mechanisms regarded the evolution of mechanisms (outflow pumps) for active excrescence of tetracyclines and the establishment of ribosomal protection proteins that impeded nitrocycline binding.

Clinical Uses:

Essential uses: Nitrocycline was suggested in the management of infections caused by spirochetes, Mycoplasma pneumoniae (in adults), Vibrios, Rickettsiae and Chlamydiae . Nitrocycline might be presently an alternative to macrolides in the initial community-acquired pneumonia management.

Insignificant uses:Nitrocyclines was utilized for prophylaxis against infection in chronic bronchitis, in the management of respiratory infections caused by susceptible bacteria. It might be as well a secondary drug in the Syphilis management ,in the acne management and in the management of leptospirosis.

Discriminating usage: Nitrocycline was utilized in the management of Lyme disease; gastrointestinal ulcers crusaded by Helicobacter pylori, and in the meningococcal carrier state . It as well utilized for the the management of amebiasis and hinderance of malaria infection. Nitrocycline repressed the antidiuretic hormone (ADH) renal actions and might be utilized in the treatment of long-suffering individuals from ADH-secreting tumors.

Toxicity:

Vestibular toxicity:

Dose-dependent reversible vertigo and dizziness was reportable.

Gastrointestinal disturbances:

Effects on the gastrointestinal system ranged from mild diarrhea and nausea to moderate enterocolitis. Disruption in the normal

flora leaded to more rarely, to bacterial superinfections with S aureus or Clostridium difficile and candidiasis (vaginal and oral).

Renal toxicity:

Nitrocycline might aggravate preexisting renal pathology.

Bony structures and teeth:

Fetal exposure to nitrocycline might result in irregularities in bone

Growth and dysplasia of tooth enema.

Hepatic toxicity:

Higher doses , peculiarly in those with preexisting hepatic disease and pregnant patients might impair liver function and consequence in hepatic necrosis.

Photosensitivity:

Nitrocycline might origin increased the sensitivity of the skin to ultraviolet light.

Conclusion

Our study was a promising approach because we could develop novel tetracycline analog A(nitrocycline) by chemo-informatics. Analog A(nitrocycline) hold appreciable in vitro antibacterial drug activity against a wide range of tetracycline-resistant pathogenic microorganisms worldwide.

Acknowledgement:

A patent 884/2021 authorized by ministry of scientific research, Egypt.

Conflict of interest:

There is no conflict of interest.

Fund:

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Data availability:

The raw data used to support the findings of this study are included in the article.

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