IMPROVED ACTIVITY OF AMINOGLYCOSIDES ENTRAPPED IN SILICA NETWORKS AGAINST MICROBIAL STRAINS ISOLATED FROM OTOLARYNGOLOGICAL INFECTIONS

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Abstract

The purpose of this study was to investigate the efficiency of natural and synthetic zeolites as carriers for aminoglycoside antibiotics,by using an adapted diffusion method and microbial strains isolated from otolaryngological infections. The results showed that the natural and synthetic zeolites proved to be excellent carriers for aminoglycosides, exhibiting a strong potentiator effect on the activity of kanamycin and streptomycin, especially against the Gram-positive bacterial strains, as demonstrated by the drastic increase of the growth inhibition zone diameters induced by the antibiotics entrapped in the silica networks. These results are demonstrating the utility of silica networks in the pharmaceutical industry for the identification of new ways to improve the bioavailability and dissolution of the currently used antibiotics.

Rezumat

Scopul acestui studiu a fost de a investiga eficacitatea zeoliților naturali și sintetici ca transportori pentru antibioticele aminoglicozide, prin utilizarea unei metode difuzimetrice adaptate şi tulpini microbiene izolate din infecţii otolaringologice. Rezultatele au arătat că zeoliții naturali și sintetici au dovedit a fi transportori excelenţi pentru aminoglicozide, manifestând un efect puternic potenţiator asupra activității kanamicinei și streptomicinei, mai ales față de tulpinile bacteriene gram-pozitive, fapt demonstrat de creșterea dramatică a diametrelor zonelor de inhibiție induse de antibiotice imobilizate în rețelele de silice. Aceste rezultate demonstrează utilitatea rețelelor de silice în industria

farmaceutică pentru identificarea de noi modalități de a îmbunătăți biodisponibilitatea și dizolvarea antibioticelor utilizate în prezent.

Keywords: kanamycin, streptomycin, zeolite, growth inhibition zone diameter, carrier

Introduction

Aminoglycosides are the most used broad-spectrum antibiotics, being traditionally used for the treatment of serious Gram-positive and Gram-negative infections [1-3]. Isolated in 1943, streptomycin is the most widely known aminoglycoside antibiotic, being also the first effective remedy used for the treatment of tuberculosis [4]. Streptomycin inhibits the growth of bacteria by targeting protein biosynthesis [5]. Kanamycin is an aminoglycoside antibiotic very effective against a broad spectrum of Gram-positive and Gram-negative bacteria [6]. It can be administered orally or parenterally as a second line antibiotic, both in human and veterinary medicine. The occurrence of aminoglycoside-resistant bacteria has significantly reduced their clinical applications [7]. Studies revealed that in recent years many bacteria acquired resistance to streptomycin, including *M. tuberculosis*. Since this pathogen grows slowly and is hardly amenable to genetic manipulations, *Mycobacterium smegmatis* and *Escherichia coli* were used as ideal hosts for the genetic resistance investigations [8]. Strains of the genera *Streptomyces*, *Bacillus*, and *Pseudomonas* also proved enhanced streptomycin resistance rates [9]. Kanamycin resistance was identified in many isolates of *Staphylococcus aureus, Streptomyces fradiae*, *Klebsiella pneumonia, Salmonella typhimurium* and *E.coli* [10].

Taking into account the above mentioned facts, it could be concluded that the improvement in the delivery of the respective antibiotics to the microbial target would favor the occurrence of an early and more efficient microbicidal effect. In this context, the development of new delivery systems for the release of therapeutic agents in active form has received significant attention [11-24].

Inorganic materials have been regarded as a prospective medium as drug delivery system due to their excellent mechanical, thermal, and chemical properties [25]. Silica has gained much attention in the scientific research because of its easy preparation and its wide range of industrial, as well as biological applications [26]. Among the wide spectrum of existing solid supports [27-29], silica exhibits some advantages related to the fact that it does not swell in general and presents a high thermal, chemical, and mechanical stability. In addition, the active silanol groups dispersed on the surface provide an easy chemical modification with functional compounds

[30]. In the past two decades, various highly ordered mesoporous silica materials with high surface area, large and versatile pore size and large pore volume have been reported [31]. Extensive researches are in progress in order to apply these materials for the development of new heterogeneous catalysts, drug delivery systems and adsorbents [32].

Entrapped drugs within silica networks provide advantages when using hydrophobic, water insoluble, anticancer drugs by incorporating them into the matrix [33-35]. This loading also prevents premature release and degradation of drugs before the drugs reach the target lesion [36-38].

In this respect, the present study reports the efficiency of natural and synthetic zeolites as kanamycin and streptomycin carriers.

Materials and Methods

Fabrication of hybrid materials

Commercially available ZSM-5 (synthetic zeolite – SZ) and permutit (natural zeolite – NZ) were used to prepare hybrid materials through absorption of the antibiotics onto nanopores. The amount of the antibiotic adsorbed on the zeolites support was 3 %. The zeolite and the respective antibiotic (kanamycin, streptomycin) to be adsorbed were introduced in a grinding mortar. The mix was ground with 2 mL of ultrapure water until the latter completely evaporated at 40^{o}C.

Characterization of hybrid materials

FT-IR. A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to the software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25^{o}C). FT-IR spectra were collected in the frequency range of 4,000–650 cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were rationed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

Biological assay. An adapted diffusion method was used in order to assess the potentiator effect of natural and synthetic zeolites on the efficiency of kanamycin and streptomycin against Gram-positive (*Enterococcus fecalis, Staphylococcus aureus, Bacillus subtilis*) and Gram-negative (*Pseudomonas*

aeruginosa and *Escherichia coli*) strains isolated from otolaryngological infections. Qualitative screening of the susceptibility of different microbial strains to NZ/ATB and SZ/ATB has been accomplished on Mueller Hinton solid medium previously seeded with a bacterial inoculum adjusted to a density corresponding to 0.5 McFarland standard [29-41]. In this purpose, 5 μL from a stock solution of the tested product, containing 30 μg of antibiotic, as well as the antibiotic control used at the same concentration, were distributed in spots on the Petri plates. The results' reading was performed by measuring the bacterial growth inhibition zones' diameters around the spots. The used solvent, dimethyl sulfoxide (DMSO) [42-43], was comparatively tested for its potential antimicrobial activity. The plates were incubated for 24h at 37°C, and the differences between inhibition zones diameters were quantified [44-46].

Results and Discussion

In the recent years, considerable research has been focused on the development of porous carriers as controlled drug delivery matrices. They exhibit several advantages, due to their features such as stable uniform porous structure, high surface area, versatile pore size and well-defined surface properties [47]. Silica is a mesoporous adsorbent exploited for pharmaceutical purposes, e.g. to improve the oral bioavailability of poorly water soluble drugs, to increase the dissolution of relatively insoluble powders and conversion of crystalline state to amorphous state [41-52].

Synthetic and natural silica networks were characterized in previous published papers [53-54]. Also, a preliminary study was reported in order to highlight their capacity to improve the delivery of antimicrobial agents with different chemical structures and microbial targets. Neomycin, polymyxin, norfloxacin, cefotaxime and penicillin were used to highlight the potential of silica network to improve the anti-infective activity of the above mentioned therapeutic agents [53]. Here, we report some silica networks combined with aminoglycoside antibiotics (kanamycin and streptomycin) in order to evaluate their capacity to improve their antimicrobial activity. Firstly, the stability of the therapeutic agents entrapped in the silica networks was evaluated by FT-IR analysis (Figure 1). According to figure 1, the adsorption processes were successfully performed without changing the structure of selected drugs.

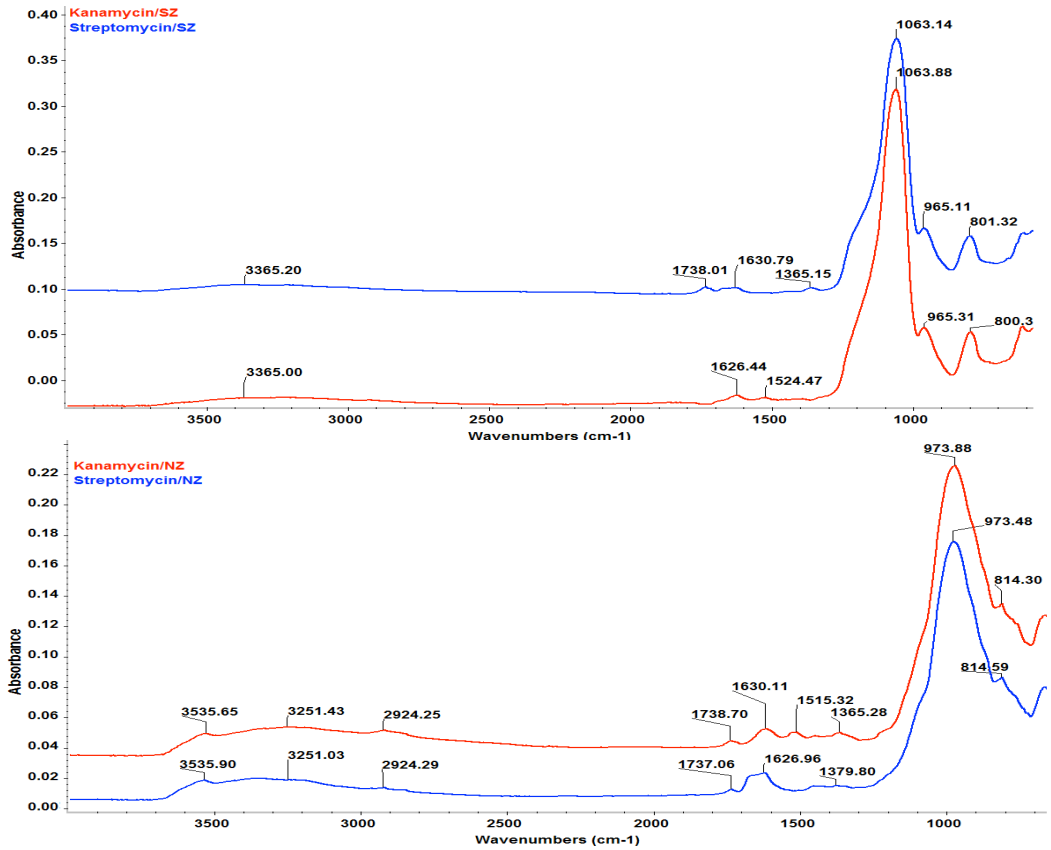

Figure 1

FT-IR spectra of kanamycin and streptomycin entrapped in natural (NZ) and synthetic zeolites (SZ)

The biological assays have clearly demonstrated the efficiency of the natural and synthetic adsorbents in potentiating the antimicrobial activity of two of the most used aminoglycoside antibiotics.

The most significant increase in the microbial growth inhibition diameters were observed for the Gram-positive strains, both for streptomycin and kanamycin, although aminoglycosides have been primarily used in the treatment of infections produced by Gram-negative strains. The most potent activity against Gram-negative strains could be explained by the fact that these cationic agents displace bivalent ions Mg^{2+} and Ca^{2+} stabilizing the lipopolysaccharides of the outer membrane, inducing lesions at this level, favoring the loss of the intracellular content on one side and an enhanced antibiotic uptake, on the other side [55].

The streptomycin-synthetic silica network induced an enhanced inhibitory effect on the growth of *P.aeruginosa* strain (the growth inhibition diameter being three times higher), as compared with the natural one

(growth inhibition diameter two times higher) (figure 2). Both streptomycin-natural and streptomycin- synthetic silica networks were very efficient in inhibiting *S. aureus* growth, the inhibition zone diameters being increased from 4 to more than 5 times (figure 2).

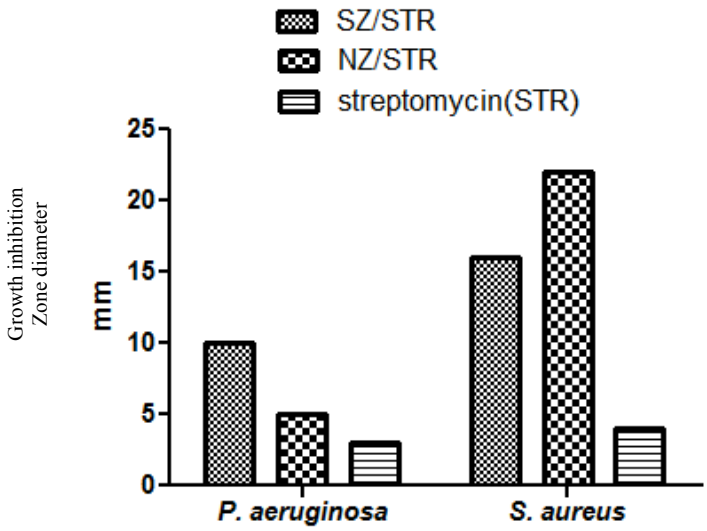

Figure 2
The graphic representation of the growth inhibition zone diameters obtained for streptomycin entrapped in the silica networks.

Kanamycin entrapped in synthetic and natural silica networks exhibited a significantly improved antimicrobial effect against *S. aureus, B. subtilis* and *E. coli* strains, the growth inhibition zone diameters increasing from 3 to 9 times (Figure 3).

However, the superior values obtained for the synthetic networks, as compared to natural ones, in all cases with one exception, could be explained by the differences in pore size and volumes of the synthetic and respectively natural zeolites, features which are very important for drug loading [56]. ZSM-5 forms intermediate size pores of 0.5-0.6 nm, suitable for adsorption of organic substances, as compared to the natural zeolite, permutite yielding larger pores. Smaller pores provide more surface area and expose more sites for attack of aqueous media, yielding greater dissolution rates [47].

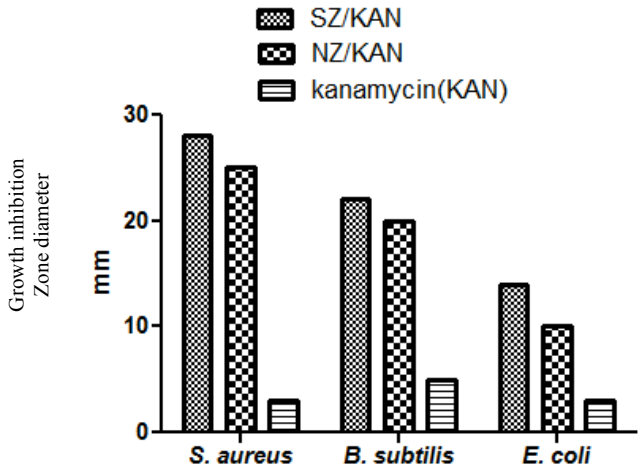

Figure 3
The graphic representation of the growth inhibition zone diameters obtained for kanamycin entrapped in the silica networks.

Conclusions

The natural and synthetic zeolites proved to be excellent carriers for aminoglycoside antibiotics, exhibiting a strong potentiator effect on the activity of kanamycin and streptomycin, especially against Gram-positive microbial strains, demonstrating their usefulness in the pharmaceutical industry for the identification of new ways to improve the bioavailability and dissolution of these antibiotics.

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