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DENDRIMERS: POTENTIAL TOOL FOR ENHANCEMENT OF ANTIFUNGAL ACTIVITY

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ABSTRACT: Nystatin and Terbinafine are antifungal drugs with well-known antifungal properties. In the present study we investigated the potential of PAMAM and PPI dendrimers of different generations as tool for enhancement of antifungal activity of selected two antifungal drugs. Microbilogical study showed that PAMAM and PPI dendrimers could enhance the antifungal activities of Nystatin and Terbinafine against *Candida albicans, Aspergillus niger* and *Sachromyces cerevasae*. Results showed increase in the antifungal activity of Nystatin and Terbinafine in dendrimer solution compared to pure Nystatin and Terbinafine dissolved in DMSO (dimethylsulfoxide). The antifungal activity studies indicated that PAMAM and PPI dendrimers as potential tool for enhancement of antifungal activity of Nysatin and Terbinafine.

Keywords:Antifungal drugs, Nystatin, PAMAM, Polyamidoamine dendrimers, , Polypropyleneimine dendrimers, PPI, Terbinafine.

INTRODUCTION

Dendrimers are the new artificial macromolecules which have the structure like a tree. They are hyperbranched and monodisperse three-dimensional molecules, and have defined molecular weights and host–guest entrapment properties[1]. Compared with traditional linear polymers, dendrimers offer several featured advantages as drug carrier candidates. These advantages include: 1) high density and reactivity of functional groups on the periphery of dendrimers make multifarious bioactive molecules to be easily modified on to the surface. 2) Well defined globular structure, predictable molecule weight and monodispersity of dendrimers ensure reproductive pharmacokinetics.3) Controllable size (generation –dependent) of dendrimers satisfies various biomedical purposes. 4) High penetration abilities of dendrimers through the cell membrane cause increased cellular uptake level of the drugs complexed or conjugated to them[2]. Antibacterial drugs like nadifloxacin, prulifloxacin, sulfamathxazole still display their antibacterial activity against *E. coli* in the presence of PAMAM dendrimers[3,4]. When equal amounts of free prulifloxacin and prulifloxacin-G4 PAMAM dendrimer are considered, prulifloxacin-PAMAM dendrimer is definitely more potent than free prulifloxacin dissolved in DMSO[3]. As pure G4 PAMAM dendrimer displayed antibacterial activity against *E.coli* at much higher concentration, the enhanced antibacterial activity should not be contributed to dendrimer itself. As PAMAM dendrimers with primary amine surface functional groups could penetrate through cell membrane. It was conclude that the enhanced antibacterial activity was contributed to the dendrimers which might favor the interaction of the drug with its target or help drugs with penetration through the bacterial membrane[4]. It was well known that during prolonged treatment

several antifungal drugs involve hepatic abnormalities or kidney damages. Ketaconazole was experimently shown to be teratogenic at high doses in rats[5]. Nystatin is a polyene antifungal antibiotic. It is similar to amphotericin B in antifungal action and other properties. However, because of higher systemic toxicity, it is used only locally and generally preferred over amphotericin B[6]. Hence the aim of present work was to investigate antifungal activities of Nystatin and Terbinafine in presence of PAMAM and PPI dendrimers.

MATERIALS AND METHODS

PAMAM G3 (polyamidoamine dendrimer generation 3), PAMAM G4 (polyamidoamine dendrimer generation 4), PPI G3 (polypropyleneimine generation3), PPI G4 (polypropyleneimine generation 4) were gift samples from Govt. College of pharmacy, Aurangabad. Nystatin and Terbinafine were gift samples from Cipla Ltd, Kurkumbh. Agar-agar powder, Peptone, Sodium chloride, Glucose, Dimethyl sulfoxide (DMSO) Conc.H2S04, Barium chloride were purchased from Loba Cheme Pvt. Ltd., Mumbai. *Candida albicans, Aspergillus niger, Sachromyces cerevasae* were purchased from IMTECH Chandigarh

Preperation of 0.5 McFarland standard of BaSO4 solution[7,8]:

To standardize the inoculum density for a susceptibility test, a BaSO4 turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), was used. A BaSO4 0.5 McFarland standard was prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L BaCl2 (1.175% w/v BaCl2. 2H2O) was added to 99.5 ml of 0.18 mol/L H2SO4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard was verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at used in growing or diluting the bacterial inoculum.
3. The Barium sulphate suspension was transferred in 4to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum
4. These tubes were tightly sealed and stored in the dark at room temperature.
5. The $BaSO_4$ turbidity standard was vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard was replaced. Latex particle suspensions was mixed by inverting gently, not on a vortex mixer
6. The $BaSO_4$ standards were replaced or their densities verified monthly.

Standardisation of inoculum preparation of *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger* at 0.5 McFarland[7,8]

2 ml seed culture of each fungus species was taken and absorbance was recorded at 625 nm. 0.5 Mc Farland turbidity standard was maintained by diluting inoculums by saline solution (0.85% Nacl in sterile distilled water) and absorbances were adjusted in between 0.008 to 0.10.

Preliminary screening for antifungal activity of dendrimers against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*

a) Prepeartion of dendrimer solution:

PAMAM and PPI dendrimers were dissolved in required quantity of sterile distilled water to produce 800µg/ml solution of both dendrimers.

Agar diffusion method[9]:

Sabouraud Agar Media was prepared according to formula and poured in petri plates. The test was performed by agar diffusion method. Saboraud glucose agar plates containing $2x10^5$ spores for fungi were used. Previously prepared dendrimer solutions (100µlit) were placed in wells. Plates were incubated at $32\text{-}34^0$ C for 72 hours. Sensitivity was recorded by measuring the clean zone of inhibition on surface of saboraud glucose media.

Preliminary screening for antifungal activity of DMSO against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*:

Antifungal activity of DMSO was determined by using Agar diffusion method as described above.

Preliminary screening for antifungal activity of Nystatin, Terbinafine and against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*:

Nystatin, and Terbinafine and were dissolved in DMSO to produce 300 µg/ml solution. Sabouraud agar media and petri plates were prepared as described above. Antifungal activity of both drugs was determined by using agar diffusion method as described above.

Determination of antifungal activity in presence of Dendrimers:

Dendrimers were dissolved in sterile distilled water to produce 600 µg/ml solution. 1ml dendrimer solution was mixed with 1ml solution of same concentration of drugs solution. All solutions were sonicated and 100 µl dendrimer : drug complex was added to wells prepared on saboraud glucose agar plates containing $2x\ 10^5$ spores of fungi. Plates were incubated at $32\text{-}34^0$ C for 72 hours. Sensitivity was recorded by measuring the clean zone of inhibition on surface of saboraud glucose media.

RESULTS

Standardisation of inoculum preparation of *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger* at 0.5 McFarland:
All three species were standardized at 0.5 McFarland turbidity standard. During further experiments standardized species were used.

Fungal species sample (2ml)	Absorbance at 625 nm without dilution	Absorbance at 625 nm with dilution
Candida albicans	1.7803	0.0938
Sacharomyces serevasae	1.7559	0.0975
Aspergillus niger	0.2764	0.0624

Preliminary screening for antifungal activity of dendrimers against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*
Dendrimers like PAMAM G3, PAMAM G4, PPI G3, PPI G4 did not show antifungal activity against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger.*

Preliminary screening for antifungal activity of dimethyl sulfoxide (DMSO) against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*
Dimethyl sulfoxide (DMSO) did not show antifungal activity against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger.*

Preliminary screening for antifungal activity of Nystatin, Terbinafine against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger*
Nystatin was active against *Candida albicans, Sacharomyces serevasae* and *Aspergillus niger* and *terbinafine* was active against *Aspergillus niger*
Antifungal activity in presence of Dendrimers:
Both Nystatin and Terbinafine showed increased antifungal activity in presence of all four dendrimers. (shown in Table No. 1)

DISCUSSION

To check whether the antifungal drugs Nystatin and Terbinafine still exhibit their antifungal activities in the presence of PAMAM and PPI dendrimers, the antifungal activity of antifungals (Nystatin, Terbinafine), dendrimer and Nystain-dendrimer, Terbinafine-dendrimer solutions were investigated. The results presented in **table 1** indicate that Nystatin and Terbinafine still display their antifungal activities against selected fungal species in the presence of both PAMAM and PPI dendrimers. Interestingly, when equal amounts of free Nystatin, Terbinafine and Nystatin-dendrimer, Terbinafine-dendrimer are considered (the actual amount of Nystatin, Terbinafine in the dendrimer solution was equal to the free drug used), Nystatin-dendrimer and Terbinafine-dendrimer are definitely more potent than free Nystatin and Terbinafine dissolved in DMSO. As pure dendrimers did not show antifungal activity at much higher concentration (data shown in table 1). It is known that antifungals exert their effects by interfering with the fungal synthesis of ergosterol, a constituent of cell membranes, as well as certain enzymes[6]. As dendrimers could penetrate through cell membrane[10], we could presume that enhanced antifungal activity was contributed to dendrimers which might favor the interaction of the drug with its target or help Nystatin and Terbinafine with penetration through fungus membrane. The precise reason for this increased activity is unclear at present. Further investigations are necessary with respect determination of minium inhibitory concentration of antifungal drugs in presence of dendrimers.

CONCLUSION

Although dendrimer drug delivery is in its infancy[11,12], it offeres several attractive features. It provides a uniform platform for drug attachment that has the ability to bind and release drugs through several mechanisams[13,14]. Our work demonstrated that complexation of antifungals with dendrimers leads to excellent antifungal activity with pure drugs themselves. In future we are planning to determine minimum inhibitory concentration of antifungals in presence of dendrimers and to evaluate the potential of dendrimers as carrier for antifungal drugs. Although toxicity problem may exist, modification of the structure of dendrimers should resolve this issue.

Table No.1- Comparative antifungal activity of Drug, dendrimers and Dendrimer: Drug complex

Sr.No.	Drug or Dendrimer or Dendrimer : Drug complex	*Candida albicans* (ZOI)	*Sacharomyces serevasae* (ZOI)	*Aspergillus niger* (ZOI)
1	Nystatin	6 mm	9 mm	12 mm
2	Terbinafine	No	No	9 mm
3	PAMAM G3	No	No	No
4	PAMAM G4	No	No	No
5	PPI G3	No	No	No
6	PPI G4	No	No	No
7	Nystatin: PAMAM G3	15 mm	18 mm	22 mm
8	Nystatin: PAMAM G4	17 mm	22 mm	24 mm
9	Nystatin: PPI G3	17 mm	20 mm	23 mm
10	Nystatin: PPI G4	20 mm	24 mm	26 mm
11	Terbinafine: PAMAM G3	No	No	15 mm
12	Terbinafine: PAMAM G4	No	No	18mm
13	Terbinafine: PPI G3	No	No	23 mm
14	Terbinafine: PPI G4	No	No	25mm

ZOI: zone of inhibition

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