PAMAM Dendrimers: novel polymeric nanoarchitectures for solubility enhancement of Ketoconazole

JOBIN JOSE*, R NARAYANA CHARYULU
Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore-575018, Karnataka, India

Clinical use of ketoconazole is limited by lipophilic nature of the drug molecule. PAMAM dendrimer provides an excellent carrier for encapsulating the drugs that has the capacity to attach and discharge drugs through various mechanisms. In this research work, the solubilization mechanism, *in vitro* release studies of ketoconazole- PAMAM complex were investigated. The experimental results suggested that an increase in dendrimer concentration and generation results in an increase in aqueous solubility of ketoconazole. *In vitro* release study revealed the sustained release of the drug from drug dendrimer complex.

(Received September 24, 2015; accepted August 3, 2016)

Keywords: PAMAM Dendrimer, Ketoconazole, Solubilization

1. Introduction

Among new drug delivery systems, nanotechnology has appeared as a novel research ground that mainly involves design, synthesis and formulation of compounds at the molecular level. As polymer technology has advanced over the past two centuries, the peculiar characteristics of macromolecules synthetically available have also developed [1]. The highly branched three dimensional man made polymeric macromolecules are known as dendrimers are the mainly known element of the polymer science. Dendrimers have often been referred to as the "Polymers of the 21st century". Dendrimers have developed a young set of polymers with a versatile architecture and unique chemical structures [2]. The better encapsulating ability of dendrimers makes them excellent carriers for delivery of drugs. Previous research studies has been proved that the capacity of dendrimers especially PAMAM dendrimers in improving the solubility of poorly water soluble drugs and can act as a carrier for biologically active compounds [3]. Among them, the PAMAM dendrimer is one of the well known carriers to improve water solubility and rate of dissolution of drugs, such as ketoprofen [4], ibuprofen [5], aceclofenac [6], and riboflavin [7].

Ketoconazole, an imidazole derivative with potent antifungal activity. Its clinical use is limited by the lipophilicity and poor aqueous solubility. Over the past several years, various efforts have been made to improve the aqueous solubility and *in vitro* dissolution profile of ketoconazole. The versatile architecture of dendrimers, such as their size, branching length, shape and their surface functional groups permit to transform the dendrimers as per the requisites, constructs these macromolecules perfect carriers in drug delivery and enhancing the solubility of low aqueous soluble drugs [8]. Here we made an attempt to incorporate ketoconazole in different generations (G1-G3) of PAMAM dendrimers to explore the potential of PAMAM dendrimers to enhance the solubility of ketoconazole. The solubilization mechanism, *in vitro* release profiles, of ketoconazole-PAMAM complex were investigated. To enhance the efficacy and get better the acceptability profile, extensive efforts were made to reformulate ketoconazole in to a suitable drug delivery system. Thus, the search for an optimal formulation is still of great importance.

2. Materials and methods

2.1 Materials

Ketoconazole was purchased from Micro Labs. (Bangalore, India). PAMAM dendrimer with ethylene diamime(EDA) as core of different generations were received from Sigma Aldrich (USA). S.D. Fine Chemicals (Mumbai, India) provided the rest of the chemicals used.

2.2 Encapsulation of ketoconazole in to PAMAM dendrimers with EDA as core

Results of dendrimer – mediated solubility studies suggested that an increase in dendrimer concentration and generation results in an increase in aqueous solubility of ketoconazole. The optimized G3 dendrimer- based formulations were selected for advanced studies. The ketoconazole was dissolved in dendrimer solutions (2 mg/ml) after dilution with distilled water. The samples were spun for 10 min at 10000 RPM after being vortexed at room temperature for 24 h. These conjugates were used for *in vitro* release studies after filtering through 0.22 μm membrane filter [9].

2.3 Solubility studies

2.3.1 Determination of effect of dendrimer generation and concentration on the solubility of ketoconazole

Equilibrium solubility technique was used to determine the effect of dendrimer generation and concentration on the solubility of ketoconazole [10]. Diluted concentration solutions from 0.05 to 0.2 % w/v of PAMAM dendrimers (G1-G3) were prepared and an excess amount of drug was then added to each of the test solutions. The samples were spun for 10 min at 10000 RPM after being vortexed at room temperature for 24 h. Spectrophotometric measurements of absorbance using UV spectroscopy [11] were taken after filtering through 0.22 µm membrane filter. Drug loaded dendrimer solutions were analyzed in a UV visible spectrophotometer over the UV range to examine the effect of drug loading and solubilization at λ max. Three repeats were conducted. The maximum solubilization and drug loading showed by dendrimer generation was chosen for further studies.

2.3.2. Determination of pH –dependent solubility of ketoconazole

To determine the effect of pH on solubility of ketoconazole assisted by PAMAM dendrimer, an experiment was carried out under three different pH conditions [Phosphate buffer (pH 7.4), 0.05 M acetate buffer (pH 4.5) and 0.1N HCl (pH 1.2)] [11]. The ionization status of ketoconazole can have an effect on its solubilization process and so the pH values are selected in such a way that it can offer characteristic acidic/basic states. The glass vials containing different concentrations of G3 PAMAM dendrimers in 0.1N HCl (concentration ranges from 0.05 to 0.2 % w/v) are mixed with an excess quantity of drug. An analogous method was practiced for pH 4.5 and 7.4 also. The rest of the method was same as that of methods practiced to establish the effect of concentration of dendrimer on the solubility of ketoconazole.

2.3.3 In vitro release studies

In vitro release studies of ketoconazole -PAMAM G3 dendrimer complex was performed by the dialysis method [12]. The ketoconazole was dissolved in dendrimer solutions to a concentration of 2 mg/ml after diluted with distilled water. Pure ketoconazole was dissolved in a small quantity of dimethyl sulphoxide, then diluted with distilled water and used as a control. Drug dendrimer solution of about 5 ml was filled in dialysis bags (M.W. cut off =1000 Daltons, Himedia, India), which was pretreated with 0.1 N HCl and the dialysis bags were suspended in 100 ml of 0.1 N HCl maintained at 37$\pm$ 0.5°C under constant stirring. A perfect sink condition was achieved by withdrawing 1 ml of the sample from the outer phase, and it was again refilled with same volume of dissolution medium. The quantity of drug released at each time interval for 12h was estimated at 270 nm using UV spectrophotometer.

2.3.4 Effect of temperature on the stability of drug dendrimer complexes

Stability studies of KET-G3 PAMAM dendrimer was performed under dark and light conditions [13]. The dark and light conditions were achieved by keeping 10 ml of sample in amber-colored and in colorless glass vials respectively. The samples were kept at 4$\pm$2°C, 25$\pm$2°C and accelerated temperature (40$\pm$2°C) for about six weeks. The turbidity, precipitation, color change, consistency and leakage of the drug samples were analyzed by taking out and examining the samples at the start and periodically (every week) up to six weeks. The percentage drug loss from the drug- dendrimer complex was estimated by raise in the release rate of drug throughout storage. Drug dendrimer solution of about 5 ml was filled in dialysis bags (M.W. cut off =1000 Daltons, Himedia, India), which was pretreated with buffer and dialyzed against 50 ml of dissolution medium and absorbance of the samples were taken at 270 nm using UV spectrophotometer and up to 6 weeks the above method was duplicated weekly. The obtained data were examined to find out any degradation of drug samples at specified storage conditions and also to identify the right storage condition for the drug samples.

3. Results and discussions

3.1. Solubility studies

To examine the influence of PAMAM dendrimer generation with EDA as core (G1, G2, and G3) and concentration on the solubility of KET a sequence of solubility studies were conducted. The results in Fig.1 showed that the solubility of ketoconazole (KET) carried out in presence of PAMAM dendrimer at room temperature. In association with PAMAM dendrimers aqueous solubility of KET has been significantly improved than in distilled water. A linear relationship can be observed between the solubility of KET and dendrimer concentration. The enhancement of solubility of KET was due to the internal architecture that are offered to incorporate KET molecules (host- guest interaction) and theses versatile features make them excellent carrier for drug delivery applications[14]. The different generations of PAMAM dendrimers had a strong influence on the solubility of KET. As the generation of PAMAM dendrimer increases the solubility of KET also increases [15]. Higher generation of PAMAM particle has a greater affinity to absorb and interact with the ketoconazole molecule when compared with the lower ones because of its increased number of surface amino groups. An increase in dendrimer concentration and generation results in an increase in solubility of ketoconazole. The internal cavities present in PAMAM dendrimers incorporates KET molecules which sequentially increases the solubility of

KET and makes them an excellent carrier for drug delivery applications [16]. From the Fig. 1, it was clear that the solubility of KET was influenced by different generations of PAMAM dendrimers. Higher generation dendrimers has a greater effect of size and surface amino groups on solubility and thus solubility is enhanced. [17]. Solubility enhancement of KET with an increase in dendrimer concentration is shown in Fig. 1. The solubility of KET enhanced in a linear fashion with increasing concentration of the dendrimer. Results showed that the solubility of ketoconzole is enhanced to increase in dendrimer generation as well as concentrations. Micellar structure of dendrimers remains unchanged even at higher concentrations of solvents. Hydrogen bonding, hydrophobic interactions and electrostatic attraction between the drug and surface functional groups of the dendrimers results in the solubility enhancement of dendrimer drug complexes.

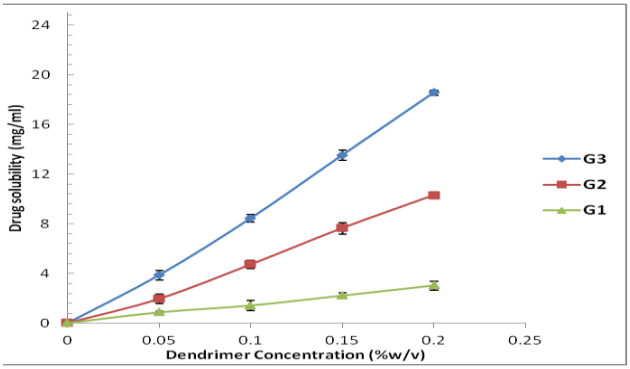

Fig. 1. Solubility of ketoconazole in the presence of Increasing concentrations of PAMAM dendrimers

The influence of pH condition on the solubility of ketoconazole using PAMAM G3 dendrimer was examined and results are shown in Fig. 2. From the above results, it is concluded that the solubility of ketoconazole was pH dependent. The solubility of KET was enhanced by the third generation of PAMAM dendrimer (0.2% w/v) at pH 1.2 and 4.5, than at pH 7.4. The solubility of KET increased in the order of pH 1.2 > 4.5 > 7.4 in 3.0G based dendrimer formulations. Surface amine groups of dendrimer molecule and functional groups present in the drug molecule are interfaced to enhance the solubility. The combined effect of the ionization state of the drug and the PAMAM dendrimers is responsible for the difference in solubility of drug in different pH solutions.

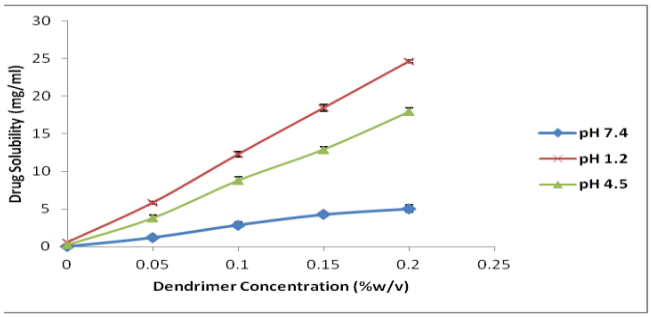

Fig. 2. Solubility profile of ketoconazole at different pH with increasing concentration of PAMAM G3 Dendrimers

3.2. *In vitro* release studies

The *In vitro* release studies of ketoconazole -PAMAM G3 complex carried out in 0.1 N HCl of pH 1.2 shows the potency of the drug – dendrimer complex [18]. Pure ketoconazole was released (59.11%) in 6 h, whereas KET-dendrimer complex exhibited the delayed release of the drug (Fig. 3). After 10h, 71.63 % release was accomplished for the pure KET while 40.07 % release was shown by KET-G3 PAMAM complex. In comparison with the pure ketoconazole the release profile of KET from the drug dendrimer complexes was in a sustained manner. This is probably due to hydrophobicity of KET which permit them to reside little longer in the hydrophobic pockets of the dendrimers. It is clear from these results that electrostatic interaction plays an inevitable role in release of drugs from dendrimer complexes.

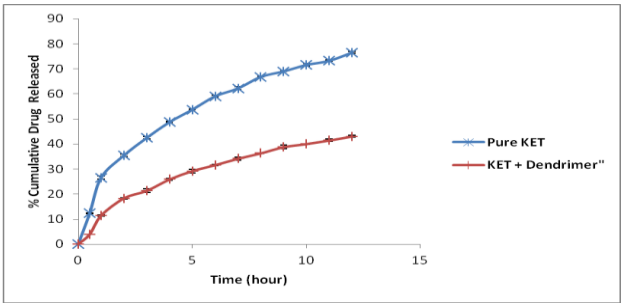

Fig. 3. Comparative In vitro release of KET in G3 PAMAM dendrimer solution compared with the pure KET (ketoconazole) release behavior

3.3. Effect of temperature on the stability of drug dendrimer complexes

The stability studies of KET-G3 PAMAM dendrimer was carried out under different storage conditions. There was no alteration in color, consistency and turbidity was observed in the drug dendrimer complex placed in the dark condition at 4±2°C and 25±2°C. Percentage drug leakage was determined and the results are shown in Fig. 5. The percentage drug leakage was found to be maximum at

40±2°C in the light. Based on the stability studies, it was concluded that the drug dendrimer complex should be stored in dark place at cold temperature.

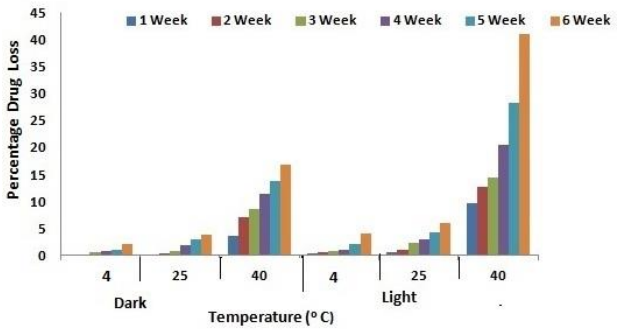

Fig. 5. Drug leakage from KET- G3 PAMAM complex under diverse storage conditions

Table 1. Effect of temperature on the stability of KET -G3 PAMAM Dendrimer Complex

Parameters	Time period	Dark			Light		
		4±2°C	25±2°C	40±2°C	4±2°C	25±2°C	40±2°C
Color change	After six weeks	-	-	+	-	+	++
Turbidity	After six weeks	-	-	+	-	+	++
Precipitation	Days after which first ppt. appeared	-	-	26	-	19	13
Change in consistency	After six weeks	-	-	+	-	+	++

(-) No change; (+) little change; (++) significant change

4. Conclusion

In the present study an attempt was made to incorporate ketoconazole in amine terminated PAMAM dendrimers and to evaluate its potential to improve the aqueous solubility of ketoconazole. The experimental results suggested that an increase in dendrimer concentration and generation results in an increase in aqueous solubility of ketoconazole. It is concluded that the solubility of ketoconazole was pH dependent. *In vitro* release study revealed the sustained release of the drug from drug dendrimer complex. Although toxicity problem may exist, modification of the structure of dendrimers should resolve these issues.

Acknowledgements

Supports from Nitte University, Mangalore and National Institute of Immunology, Delhi were highly appreciated.

References

[1] C. L. Cameron, A. M. John, M. F. J. Jean, C. S. Francis, Nat. Biotechnol. **23**, 1517 (2003).
[2] D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, Polym. J. **17**, 117 (1985).
[3] U. Gupta, H. B. Agashe, A. Asthana, N. K. Jain, Biomacromolecules **16**, 640 (2006).
[4] Y. Y. Cheng, T. W. Xu, R. Q. Fu, Eur. J. Med. Chem. **40**, 1390 (2005).
[5] O. M. Milhem, C. Myles, N. B. McKeown, D. Attwood, A. D. Emanuele, Int. J. Pharm. **197**, 239 (2000).
[6] J. Patel, K. Garala, B. Basu, M. Raval, A. Dharamsi, Int. J. Pharm. Invest. **1**, 135 (2011).
[7] A. Filipowicz, S. Wołowiec, Int. J. Pharm. **408**, 152 (2011).
[8] M. Liu, J. M. J. Frechet, Pharm. Sci. Technol. Today **2**, 393 (1999).
[9] M. Minglu, C. Yiyun, X. Zhenhua, Eur. J. Med. Chem. **42**, 93 (2007).
[10] T. Higuchi, A. Connors, Phase-solubility techniques. In Advances in Analytical Chemistry and Instrument, John Wiley, New York (1965).
[11] D. Bharathi, A. H. Ronald, L. Wilna, Int. J. Pharm. **304**, 193 (2005).
[12] N. Man, Y. Cheng, Y. Ding, Eur. J. Med. Chem. **41**, 670 (2006).
[13] N. P. Ram, K. T. Rakesh, G. Umesh, Mol. Pharm. **6**, 940 (2009).
[14] Y. Hu, J. M. Joseph, T. L Stephanie, Colloids. Surf. B. **273**, 148 (2004).
[15] C. J. Hawker, K. L. Wooley, J. M. Frechet, J. Chem.

Soc. Perkin. Trans. **1**, 1287 (1993).
[16] J. Jobin, R. N. Charyulu, Ind. J. Res. Pharm. Biotech. **3**, 171 (2015).
[17] G. R. Newkome, C. N. Moorefield, G. R. Baker, M. J. Saunders, S. H. Grossman, Angew. Chem. Int. Ed. **30**, 1178 (1991).
[18] S. Stevelmens, J. C. M Hest, J. F. G. A Jansen, D. A. F. Boxtel, E. W. Miejer, J. Am. Chem. Soc. **118**, 7398 (1996).

*Corresponding author: jjmattam07@gmail.com