RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 1 : 44-46 (April to September, 2009)

Activity of ketoconazole coated gold nanoparticles against dandruff causing fungi

T. DEVASENA[1] AND T. RAVIMYCIN[2]
[1]Department of Botany, Annamalai University, Ambattur Study centre, CHENNAI (T.N.) INDIA
[2]Department of Biotechnology, Mother Teresa Women's University, KODAIKANAL (T.N.) INDIA

(Accepted : January, 2009)

We investigated the antifungal property of gold Nanoparticles coated with ketoconazole against the dandruff causing organism - *Malassezia pachydermatis* in comparison with pure ketoconazole by disc diffusion method. The diameter of the inhibition zone is greater for gold Nanoparticles coated with ketoconazole than for pure ketoconazole. This shows that gold Nanoparticles enhances the bioactivity of ketoconazole. Zone of inhibition increased with increase in concentration of gold Nanoparticles (0.1 mM<0.2 mM<0.3 mM). We conclude that ketoconazole coated gold Nanoparticles prevent the growth of the dandruff causing organism. The inhibitory power is greater than the pure drug. Thus, gold Nanoparticles can be incorporated along with ketoconazole and formulated into an effective antidandruff shampoo.

Key words : Nanoparticles, Gold, Dandruff, Ketoconazole, *Malassezia*

INTRODUCTION

Nanotechnology offers unique approaches to probe and control a wide variety of biological process that occur at nanometer length (Kulkarni, 2007; West and Halas, 2000). Controlling the structure of a drug precisely at nanoscale dimenstion can enhance its solubility, biocompatibility and bioconjugation. Drug-nanoparticle hybrid systems have widely been found useful in the enhancement of bioavailability, bioactivity and stability of drugs used in various infections. Gold Nanoparticles have well developed surface chemistry (Prime and Whitesides, 1991), controllable geometry (Sun and Xia, 2002), rigidity and stability (Boyen *et al.*, 2002). Thus, gold Nanoparticles could function as versatile drug carriers and hence be used in the treatment of several medical and biological problems.

Dandruff is one of the serious problem in the society. *Malassezia* species is well recognized as a causative organism for dandruff. It is characterized by scaling of scalp and skin in humans (Gupta *et al.*, 2000). *Malassezia furfur, Malassezia sympodialis, Malassezia sloofia, Malassezia pachydermatis, Malassezia globasa, Malassezia obtusa* and *Malassezia restricta* are some examples for dandruff causing fungi (Hammer *et al.*, 2000). Most of antidandruff shampoos are formulated using ketoconazole (a triazole). In spite of several commercially available antidandruff shampoos, dandruff recurrence is more frequent. Hence, a novel therapy is needed to eradicate and prevent recurrence of dandruff.

Antimicrobial agents in the form of Nanoparticles act with higher efficacy against bacteria (Grace and Pandian, 2006). *In vitro* antibacterial activity of streptomycin, gentamycin and neomycin have been increased by coating the drugs to Nanoparticles (Grace and Pandian, 2006). Assuming that the action of ketoconazole (commercial antidandruff agent) could also be enhanced by coating onto gold Nanoparticles, the antifungal property of gold Nanoparticles coated with ketoconazole against the dandruff causing organism - *Malassezia pachydermatis* in comparison with pure ketoconazole was investigated. Previous studies too have used pure ketoconazole as a reference drug for investigating the antifungal property of various test drugs (Hammer *et al.*, 2000; Jonson *et al.*,2004; Pierard *et al.*, 1991).

MATERIALS AND METHODS

Chemicals:

Gold chloride and trisodium citrate were purchased from Sigma Chemicals. Ketoconazole was purchased from Hi media. All other chemicals and reagents were of analytical grade. Double distilled deionized water was used for the experiments.

Citrate capped gold Nanoparticles:

Citrate capped gold Nanoparticles were prepared as described by Grace and Pandian (2006). Gold Nanoparticles of 12-15 nm with the concentration of 0.5 mM was initially prepared. 50 ml of each 0.1mM, 0.3mM and 0.5mM gold Nanoparticles was mixed separately with

5 ml of ketoconazole (5mg/ml) and stirred effectively for two hours.

Disc diffusion method:

Malassezia pachydermatis was inoculated in Sabourd Dextrose Agar (SDA) medium in a Petriplate using a sterile cotton swab. Sterile filter paper discs of 5mm diameter were impregnated with 10 microlitrre of the drug and placed over the medium. Disc impregnated with 2% DMSO was used as negative control. The plates were incubated at 35^0C for two days. After incubation, the plates were examined for inhibition zones. Diameter of the zones was measured in millimeter using a transparent ruler. Experiments were carried out in quadruplicate.

RESULTS AND DISCUSSION

Inhibitory action of gold Nanoparticles coated with ketoconazole and pure ketoconazole towards *Malassezia pachydermatis* is shown in Table 1.

Table 1 : Effect of ketoconazole and gold nanoparticle on growth of dandruff causing fungi- *Malassezia pachydermatis*

Drug tested	Concentration of gold nanoparticles	Diameter of the zone of inhibition (mm)
Ketoconazole (5mg/ml) (Positive control)	-	9
Gold nanoparticle	0.1 mM	10
	0.2 mM	11
	0.3 mM	12
Gold nanoparticle coated with ketoconazole	0.1 mM	11
	0.2 mM	12
	0.3 mM	15
2% DMSO (Negative control)	-	-

The diameter of the inhibition zone was greater for gold Nanoparticles coated with ketoconazole than for pure ketoconazole. This showed that gold Nanoparticles enhanced the bioactivity of ketoconazole. Zone of inhibition increased with increase in concentration of gold Nanoparticles (0.1 mM<0.2 mM<0.3 mM).

The efficacy of gold nanoparticle coated with ketoconazole against the dandruff causing organism-*Malassezia pachydermatis* was investigated. Earlier investigations have reported the use of antibiotic coated gold nanoparticle against *Streptococcus aureus*, *Micrococcus luteus*. *E. coli*, and *Pseudomonas aeruginosa* (Grace and Pandian, 2006). Present results show that ketoconazole exhibit enhanced activity when coated onto gold Nanoparticles.

It is suggested that gold Nanoparticles increase the bioactivity and bioavailability of the drug and triggers fungal cell death. This could be due to the cytotoxic effect of gold Nanoparticles. Other workers (Mini and Kilbanov, 2003; Messsori *et al.*, 2003) have also reported the cytotoxic effect of gold Nanoparticles of 12 nm and 18nm size.

Gold Nanoparticles have smaller size and larger surface area (Nakamura *et al.*, 2008; Grace and Pandian, 2006) due to which the drug molecules adsorb onto the surface of the Nanoparticles, thus each nanoparticle act as a core being surrounded by a number of functional moieties acting as a single group.

Also, gold Nanoparticles have high penetrating power (Nakamura *et al.*, 2008; Grace and Pandian, 2006) due to which they penetrate the outer membrane of the fungal cell. Gold, being the most electronegative metal, prefers the negatively charged vicinity in the nucleic acid strands (Aslanoglu *et al.*, 1998). This leads to inhibition of cell division and cell growth creating zone of inhibition.

Conclusinon:

It is concluded that ketoconazole coated gold Nanoparticles prevent the growth of the dandruff causing organism. The inhibitory power is greater than the pure drug. Thus, gold Nanoparticles can be incorporated along with ketoconazole and formulated into an effective antidandruff shampoo.

REFERENCES

Aslanoglu, M., Houlton, A. and Horrocks, B.R. (1998). Functionalised monolayer for nucleic acid immobilisation on gold surfaces and metal complex binding studies. *Analyst*, **123** : 753–757.

Boyen, H.G., Kästle, G., Weigl, F., Koslowski, B., Dietrich, C., Ziemann, P., Spatz, J.P., Riethmüller, S., Hartmann, C., Möller, M., Schmid, G., Garnier, M.G. and Oelhafen, P. (2002). Oxidation-Resistant Gold-55 Clusters. *Sci.*, **297**: 1533-1536.

Grace, M. and Pandian, K. (2006). Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles—A brief study, *Colloids and Surface*, **10**: 24.

Gupta, A.K., Kohli, Y., and Summerbell, R.C. (2000). Molecular Differentiation of Seven *Malassezia* Species. *J. Clin. Microbiol.*, **38** (5): 1869–1875.

Hammer, K.A, Carson, C.F. and Riley, T.V. (2000). *In vitro* Activities of Ketoconazole, Econazole, Miconazole, and Melaleuca alternifolia (Tea Tree) Oil against *Malassezia* Species. *Antimicrob Agents Chemother,* **44** (2): 467–469.

Johnson, M.D., Dougall, C.M., Ostrosky-Zeichner, L, Perfect, J.R. and Rex, J.H. (2004). Combination Antifungal Therapy. *Antimicrob Agents Chemother*, **48** (3) : 693–715.

Kulkarni, R.P. (2007). Nano-Bio-Genesis: tracing the rise of nanotechnology and nanobiotechnology as 'big science. *J. Biomed Discov. Collab.*, **2**: 3.

Messori, L., Marcon, G., and Orioli, P. (2003). Gold(III) Compounds as New Family of Anticancer Drugs. *Bioinorg. Chem. App.*, **1**(2): 177–187.

Mini, T. and Klibanov, A.M. (2003). Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. *Proc. Natl. Acad. Sci., U S A*, **100**(16): 9138–9143.

Nakamura-Tsuruta, S., Kishimoto, Y., Nishimura, T., and Suda, Y. (2008). One-Step Purification of Lectins from Banana Pulp Using Sugar-Immobilized Gold Nano-Particles. *J. Biochem.*, **143** : 833 - 839.

Pierard, G.E., Pierard, F.C., Van Cutsem, J., Rurangirwa, A., Hoppenbrouwers, M.L. and Schrooten, P. (1991). Ketoconazole 2% emulsion in the treatment of *Seborrhoeic dermatitis*. *Internat. J. Dermatol.*, **30** : 806–809.

Prime, K.L. and Whitesides, G.M. (1991). Self-Assembled Organic Monolayers: Model Systems for studying adsorption of proteins at surfaces. *Science*, **252** : 1164-1167.

Sun, Y. and Xia, Y. (2002). Shape-Controlled Synthesis of Gold and Silver Nanoparticles. *Science*, **298**: 2176-2179.

West, J.L. and Halas, N.J. (2000). Applications of Nanotechnology to Biotechnology - Commentary. *Current Opinion in Biotechnology,* **11** : 215.