Ketoconazole coated silver nanoparticles-A point antidandruff agent

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SUMMARY

The antidandruff activity of ketoconazole coated silver nanoparticles (AgNp) of 4±2 nm towards the dandruff scales collected from human volunteers by disc diffusion method was investigated. The minimal inhibitory concentration (MIC) of ketoconazole and ketoconazole coated AgNp during incubation with the dandruff causing fungi- *Malassezia furfur* was also studied. Antidandruff activity was highest with ketoconazole coated AgNp when compared to ketoconazole and AgNp individually. MIC was 0.06 mg/ml for ketoconazole, 0.026 for AgNp and 0.0135 mg/ml for ketoconazole coated AgNp. Results revealed the synergistic antidandruff activity of ketoconazole and AgNp. It was concluded that AgNp enhanced the activity of ketoconazole. This is because Ketoconazole acts on fungi at the level of cell wall, while AgNp powerfully penetrates through the membrane leading to complete eradication of the fungi.

Key words : Silver, Nanoparticles, Dandruff, Ketoconazole.

Dandruff is one of the serious problem in the society, characterized by scaling of scalp and skin. Persistence of dandruff may lead to itching and hair loss (Al-waili, 2001). *Malassezia* species is well recognized as a causative organism for dandruff (Squire and Goode, 2002). *Malassezia furfur, Malassezia* sympodialis, *Malassezia sloofia, Malassezia pachydermatis, Malassezia globusa* and *Malassezia restructa* are some examples for dandruff causing fungi (Gupta *et al.*, 2000). Association between *Malassezia furfur* and dandruff in human beings is well recognized (Samuel *et al.*, 2005). Antidandruff shampoos are formulated with azoles like ketoconazole, fluoconazole, and itracocnazole (Odds *et al.*, 2004). Ketoconazole was reported to be effective in the treatment of subjects with severe dandruff (Pierard-Franchimont *et al.*, 2002). In spite of several commercially available ketoconazole based antidandruff shampoo, dandruff recurrence is more frequent. Further, resistance of dandruff to antifungal agent is also of immense interest due to the development of resistant strains. Hence, development of a novel and efficient dandruff agent to prevent recurrence is essential.

Nanotechnology is a rapidly growing science of producing and utilizing nanosized particles that measure in nanometer (1nm= 1 billionth of a meter). Inorganic nanoparticles possess low toxicity and versatile properties like wide availability, rich functionality, good biocompatibility and potential capability of target delivery, driving forces for delivery and controlled release of target drugs (Kim, 2006). Silver in minute concentration is highly toxic to germs with low MIC values, while relatively non toxic to human cells. Microbes are unlikely to develop resistant against silver as they do against conventional and highly targeted antibiotics (Chen *et al.*, 2005, Ping and Zang *et al.*, 2006).

Drug nanoparticle hybrid system have widely been found useful in the enhancement of bioavailability, bioactivity and stability of drugs used in various infections. Drugs like sulphonamide, sulphadiazine and sulphamerazine complexed with silver showed enhanced activities against *Aspergillus* and *Candida* species (Wright *et al.*, 1999). Nanosilver has fastest and broadest spectrum fungicidal activity which makes it a good candidate to eradicate fungal infection without recurrence (Wright *et al.*, 1999). Silver is also reported to enhance the generation of reactive oxygen species which in turn degrade the cell membrane. Silver catalyze the denaturation of disulphide bridge in the cellular protein, thus denaturing the tertiary structure and function of cellular proteins (Fig. 1).

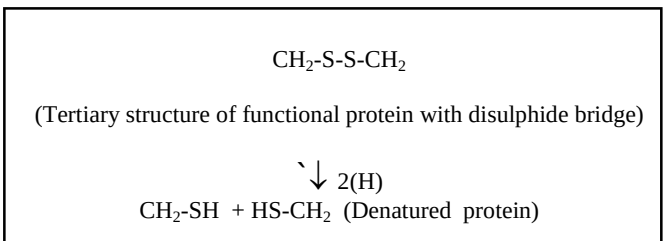

Fig. 1 : Denaturation of protein by silver induced reduction

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In vitro antibacterial activity of streptomycin, gentamycin and neomycin have been enhanced by coating the drugs to nanoparticles (Sheehan *et al.*, 1999). Assuming that the action of ketoconazole could also be enhanced by coating on to nanoparticles, we investigated the antidandruff property of ketoconazole coated silver nanoparticle (AgNp) against the dandruff causing organism-*Malassezia furfur* isolated from scalp scrapings of male and female volunteers. Previous studies have also used pure ketoconazole as a reference drug for investigating the antifungal property of various test drugs (Sheehan *et al.*, 1999). The aim of this study is investigate the effect of ketoconazole coated silver nanoparticles on dandruff scales isolated from human volunteers and to fix the minimal inhibitory concentration of the drugs.

MATERIALS AND METHODS

Silver nanoparticles (AgNp) was prepared as described by Murphy (2000).

Disc diffusion methods:

Dandruff scales were collected from the scalp of volunteer belonging to the age group of 20-30 years. This was done using sterile forceps/comb. Scales were inoculated into Petri plate containing Sabouraud Dextrose Agar medium using a sterile cotton swab. The medium was incorporated with streptomycin (50mg/ml) and coconut oil. Sterile filter paper discs of 5mm diameter were impregnated with 10 µl of the ketoconazole or AgNp or ketoconazole coated AgNp and placed over the medium. The plates were incubated at 35ºC for 2 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 are expressed as average diameter.

Minimal inhibitory concentration(MIC)

Minimal inhibitory concentration of ketoconazole and AgNp were determined by serial tube two fold dilution method as described by Rajarajan *et al.* (2002).

A standard concentration of ketoconazole (5mg/ml) was serially diluted with distilled water in a row of 6 tubes. The concentration in the tubes in mg/ml was 5, 2.5, 1.25, 0.625, 0.3125 and 0.156. From each tube, 0.1 ml of the solution was transferred to another set of 6 tubes followed by 0.9ml of the *Malassezia furfur* culture broth inoculum.

The above procedures were also followed for ketoconazole (5mg/ml) coated AgNp. AgNp was mixed with ketoconazole solution and stirred vigorously for 2 hours in a magnetic stirrer. The tubes were then serially diluted with distilled water in a row of 6 tubes. The ketoconazole concentration in the tubes in mg/ml was 5, 2.5. 2.15, 0.625, 0.3125 and 0.156. From each tube, 0.1ml of the solution was transferred to another set of 7 tubes followed by 0.9 ml of the *Malassezia furfur* culture broth inoculum. A seventh tube containing 0.1 ml distilled water and 0.9 ml of the inoculums served as control.

All the tubes were incubated at 35ºC for 24 hours. Then, the test tubes were observed for presence or absence of turbidity in comparison to that of control. The lowest concentration of the drug inhibited the growth of fungus was inferred by the lack of visual turbidity and recorded as MIC value of the drug.

RESULTS AND DISCUSSION

Antidandruff activity of silver nanoparticles (AgNp) and ketoconazole measured as zone of inhibition is shown in Table 1.

Table 1 : Antidandruff activity of ketoconazole coated silver nanoparticles (AgNp)

Drug tested	Diameter of zone of inhibition (mm)
Ketoconazole (5mg/ml)	8
AgNp	11
Ketoconazole (5mg/ml)+AgNp	15
Ketoconazole (2.5mg/ml) + AgNp	15

Diameter of zone of inhibition was in the order:

Ketoconazole (5mg/ml)+AgNp> Ketoconazole (2.5mg/ml) + AgNp >AgNp >Ketoconazole (5mg/ml).

The diameter of the inhibition zone was greatest with Ketoconazole (5mg/ml)+AgNp. The zone of inhibition was similar for AgNp coated with 5mg/ml and 2.5 mg/ml of ketoconazole. The zone of inhibition was lesser when ketoconzole and AgNp were used individually.

The inhibitory acvtivity and the minimum inhibitory concentration (MIC) of ketoconazole and silver nanoparticles (AgNp) are shown in Table 2.

MIC of pure ketoconazole towards *Malassezia furfur* was 0.062 mg/ml. Whereas, the MIC of AgNp and ketoconazole coated AgNp was low and found to be 0.026 and 0.0135, respectively. These findings suggest that AgNp coated with ketoconazole exhibited more antifungal activity with low mic when compared to ketoconazole and AgNp.

The efficacy of ketoconazole coated silver nanoparticles (AgNp) against dandruff scales was investigated. Present results showed that ketoconazole

Table 2 : *In vitro* inhibitory activity of ketoconazole coated silver nanoparticles (AgNp) on *Malassezia furfur*

Antifungal drugs	Concentration of the test drugs in µg								
	1	2	3	4	5	6	7	8	MIC
Ketoconazole	0.5	0.25	0.125	0.062	0.031	0.015	0.007	C	-
	+	+	+	+	-	-	-	-	0.062
AgNp	0.424	0.212	0.106	0.053	0.026	0.013	0.0065	C	-
	+	+	+	+	+	-	-	-	0.026
Ketoconazole coated AgNp	0.924	0.462	0.231	0.115	0.057	0.028	0.0135	C	-
	+	+	+	+	+	+	-	-	0.0135

"+" Complete inhibition "-" No inhibition "C" Control

exhibited enhanced activity when coated onto AgNp. Thus, the effect was synergistic. The activity of ketoconazole coated AgNp was equally enhanced even when its concentration was reduced to half (2.5mg/ml). Earlier investigations that showed enhanced activities of antibiotic coated nanoparticles supported present findings (Grace and Pandian, 2006).

Silver is equally powerful as chemical antimicrobial agent. This property coupled with its relative harmlessness to higher form of life may give it a great potential as antifungal agent (Fowler and Nordberg, 1986). Ketoconazole blocks the synthesis of ergosterol, a fungal cell wall component. Consequently, the integrity of the cell wall is lost with an increase in permeability (Wlodkowski and Rosenkranz ,1973; Berger, 1976). Silver nanoparticles have high penetrating power and large relatively surface area increasing their contact with fungi. Silver is also reported to enhance the generation of reactive oxygen species which in turn degrade the cell membrane. Silver catalyze the denaturation of disulphide bridge in the cellular protein, thus denaturing the tertiary structure and function of cellular proteins (Fig. 1) suppressing the basal metabolism of the electron transfer system. As human cells is tissue type, they are unaffected by these action (Wlodkowski and Rosenkranz, 1973; Berger, 1976). Therefore, it is suggested that the antidandruff property of AgNp could be due to its large surface area, high penetrating power, denaturation of functional proteins and suppression of cellular metabolism and respiration.

It has been reported that ketoconazole have cutaneous side effects like itching, stinging, irritaatiion, contact dermatitis, Stevens Jhonsons syndrome and other effects like head ache, dizziness and drowsiness (Pahan *et al.,* 1995). This could be reduced if the concentration is reduced.

Hence, we have also investigated the antidandruff activity of ketoconazole at half of its standard concentration (2.5 mg/ml) by disc diffusion method. Even when the standard concentration (5mg/ml) was reduced to half (2.5mg/ml) the antifungal activity was not changed in the presence of AgNp. The MIC of ketoconazole was reduced from 0.06 mg/ml to 0.015 mg/ml by AgNp. All these findings suggest that the synergistic effect of silver nanoparticles could be beneficial in the effective control of dandruff.

Conclusion:

We conclude that the inhibitory action of ketoconazole against dandruff was enhanced by silver nanoparticles. This synergistic effect was still retained even when the concentration of the drug is reduced to half. The MIC of ketoconazole was reduced in the presence of silver nanoparticles. Thus, Silver nanoparticles not only act as a better antidandruff agent but could also reduce the side effects of ketoconazole by providing a chance to reduce the concentration of the later.

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