**Research article** 

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# Pharmacokinetic Evaluation of Wheat Germ Agglutinin-Grafted Nanoparticles of Mometasone Furoate

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# Abstract

The aim of the investigation was to assess the pharmacokinetic parameters of wheat germ agglutinin (WGA)-grafted nanoparticles of mometasone furoate (MF), after intratracheal instillation in rats. PLGA (50:50) nanoparticles (NPs) loaded with MF were prepared by emulsion-solvent evaporation technique. WGA was conjugated to spherical MF loaded nanoparticles by carbodiimide coupling yielding a ligand density of 10-15µg WGA / mg NP. The nanoparticles were characterized for size, zeta potential, entrapment efficiency and in-vitro drug release. Plain drug, MF nanoparticles and WGA-grafted MF nanoparticles were administered intratracheally in rats and at different time intervals drug levels in the lung tissue and bronchoalveolar lavage (BAL) were estimated using HPLC. Cumulative MF-release from unconjugated and conjugated MFnanoparticles after 2 weeks was 68% and 55% of the initial drug loading. From the lung MF concentration vs time plot, AUC for WGA-MF-NPs and MF-NPs was found to be 5.9 and 3.8 times higher than AUC for plain MF. This improvement of intracellular drug uptake by means of WGA-conjugation might be due to bioadhesive nature of the lectin which provides an intimate contact to lung mucosal cells followed by facilitated transcytosis as confirmed by improved pharmacokinetic behaviour. All in all, these findings are expected to contribute for better management of asthma to overcome the limitation of short lived action associated with dry powder inhalers.

# Keywords

Nanoparticles • Mometasone furoate • Poly[D,L-lactic acid-*co*-glycolic acid] • Sustained delivery • Wheat germ agglutinin

# Introduction

Glucocorticoids are the most effective therapy available for patients with asthma. Mometasone furoate (MF) is one of the newer and more potent inhaled glucocorticoids used in the management of persistent mild-to-moderate asthma in adults and adolescents. It has been shown to inhibit sulfidoleukotriene production [1], which are potent eicosanoid mediators and play an important role in the pathophysiology of allergic disease [2].

Asthma is a chronic disease and it is required to sustain the drug levels at the site of action (i. e. the cytoplasm for glucocorticoids) for maximum therapeutic benefits, minimal side effects and reduced frequency of dosing. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules [3]. Site-specific targeting can be achieved by attaching targeting ligands to surface of nanoparticles [4]. Various ligands which have been employed include transferrin, folic acid, lectins etc [5–7].

Local delivery of corticosteroids to the lungs is a desirable strategy to inhibit the factors leading to asthma because oral administration of MF results in extensive hepatic first pass metabolism (around 85%) and local delivery reduces the systemic side effects of corticosteroids [8].

Hence, it was hypothesized that if a ligand is attached to drug loaded nanoparticles and these are administered directly to the lungs as a dry powder, sustained intracellular levels of drug can be established. Consequently, drawbacks associated with currently available dry powder inhaler formulations on the market might be alleviated, such as prevention of nocturnal exacerbations, short lived actions, high frequency of administration, increase in dose with passage time and associated toxicities.

Among the polymers used for the preparation of nanoparticles, poly[D,L-lactic acid-*co*glycolic acid] (PLGA) is highly preferred because it is biodegradable and biocompatible. The drug entrapped in the PLGA matrix is released at a sustained rate through diffusion of the drug through the polymer matrix and by degradation of the polymer matrix [9]. Lectins are a family of natural nonenzymatic proteins/glycoproteins that have cytoadhesive and cytoinvasive properties. They specifically recognize and bind to carbohydrate residues on the cell surface and can initiate vesicular transport processes into the cells [10]. The wheat germ agglutinin (WGA) from Triticum vulgare is of interest for research in drug delivery because it is widely characterized and one of the least immunogenic lectins [11]. It binds specifically to N-acetyl-D-glucosamine residues located at the surface of alveolar epithelium [12].

# Results and discussion

## NP formulation and WGA-conjugation

NPs formulated by emulsion-solvent evaporation technique with a drug: polymer ratio of

1:2 and 2% aqueous PVA as an emulsifier were spherical with smooth surfaces. The amount of WGA conjugated onto the surface of NPs by carbodiimide coupling method was  $10-15 \mu g/mg$  of nanoparticles.

#### Characteristics of the NPs

The NPs were characterized for particle size, zeta potential, encapsulation efficiency (EE) and in-vitro drug release (Table1). The increase in particle size and the shift in zeta potential may be due to the presence of WGA at the NP-surface. The reduction in the % entrapment efficiency may be due to the diffusion of surface drug during the conjugation process. A burst release of MF was not observed which is most likely due to the absence of drug on the surface of conjugated nanoparticles. The images acquired by ESEM indicated spherical particles.

#### In vivo studies

To study the in-vivo effects of nanoparticles and their retention in the lungs, rats were intratracheally instilled with plain drug, unconjugated NPs and WGA-conjugated NPs. At different time intervals the rats were sacrificed to collect the biological samples – BAL and lung.

Formulation	Mean particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)	% cumulative in-vitro drug released in 2 weeks	Poly- dispersity index
MF- NP	255 ± 7	-15.3 ± 1.9	78.0 ± 5.5	68% (after initial burst- release of 25%)	0.09
WGA-MF- NP	340 ± 5	-2.6 ± 2.1	60.0 ± 2.5	55% (no burst release)	0.07

**Tab. 1.** Characteristics of the nanoparticles (Mean  $\pm$  S.D., n = 3)

Following a single intratracheal instillation of MF, MF-NPs or WGA-MF–NPs, (equivalent to 150 µg of MF) the area under the lung MF concentration time curve (AUC) for WGA-MF-NPs and MF-NPs was 195.57 and 127.4 ng/mg/day, respectively, which is 5.9 and 3.8 times higher than AUC for plain MF (Fig 1, Table 2). The site exposure enhancement factor was 3.87 and 5.95 for unconjugated and conjugated NPs, respectively (Table 2). The elimination rate constant was lower and elimination half life was longer in case of WGA-NPs as compared to unconjugated NPs and plain MF (Table 2). Total clearance was higher for plain drug than with nanoparticles. Among the nanoparticles, total clearance was less with WGA-conjugated nanoparticles than with unconjugated nanoparticles. All the pharmacokinetic parameters indicated improved bioavailability of the drug applied as WGA-conjugated nanoparticles. It is suggested that specific binding of WGA to N-acetyl-D-glucosamine residues located at the surface of cells is followed by internalization of NPs, which provides for higher and sustained drug levels. Improved cytoassociation of the NPs grafted with WGA to Caco-2 cells has been reported by Wirth et al. as compared to the unmodified ones [13].

In the BAL, MF was detectable up to 2 days for the group treated with plain MF. The BAL MF levels were  $25.7 \pm 1.5$  ng/ml in the WGA-MF-NPs and  $7.3 \pm 2.6$  ng/ml for MF-NPs treated group after day 7 and day 5 ,respectively. (Fig. 2).



- **Fig. 1.** Lung mometasone furoate levels after intratracheal instillation of MF, MF-NPs and WGA-MF-NPs. (Mean  $\pm$  S.D., n = 3)
- **Tab. 2.** Pharmacokinetic parameters after intratracheal instillation in rats (Mean  $\pm$  S.D., n = 3) AUC= Area under the curve, Kel= elimination rate constant, SEF= Site enhancement factor

Parameter	MF	MF-NP	WGA-MF-NP
AUC (0–7) ng/mg/day	32.85 ± 0.80	127.40 ± 1.40	195.57 ± 2.60
AUC (0–∞) ng/mg/day	35.19 ±1.50	144.22 ± 1.50	204.39 ± 1.40
Kel	1.91 ± 0.20	0.49 ± 0.05	0.64 ± 0.13
(Day⁻¹)			
Elimination half life	0.36 ± 0.04	1.39 ± 0.80	1.07 ± 0.09
Total clearance ng/day	4261.66 ± 4.70	1040.00 ± 1.20	733.57 ± 1.90
SEF		3.87 ± 0.50	5.95 ± 0.85

#### Pulmonary toxicity studies

Changes in cell populations in BAL, AP (acid phosphatase) and LDH (lactate dehydrogenase) levels following intratracheal instillation are presented in Table 3. There was no significant difference (P>0.05) in the cell recovery, neither macrophages, neutrophils nor eosinophils. This was similar in animals instilled with PBS, NPs or WGA-NPs. Similarly, the data indicate that no significant difference in total protein-content (about 24 mg in control animals, 22-25 mg in treated animals), AP-levels (about 16 units in control animals, 14-16 units in treated animals) and LDH-levels (about 105 units in control animals, 98-101 units in treated animals). This suggests that instillation of either NPs or WGA-NPs did not provoke either pulmonary inflammation or lung cellular injury. This finding is true for single administration of NPs.



**Fig. 2.** Mometasone furoate levels in bronchoalveolar lavage. (Mean  $\pm$  S.D., n = 3)

Tab 3	Lung inflammatory	/ narameters	(Mean + S D n=	3)
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Parameter	PBS (Control)	MF-NP	WGA-MF-NP
Total cells count( x 10 <sup>6</sup> )	7.20 ±2.12	5.00 ±1.14	5.30 ±1.53
Macrophages( x 10 <sup>6</sup> )	1.52 ±0.68	0.90 ±0.38	1.10 ±0.32
Neutrophils( x 10 <sup>6</sup> )	2.40 ±1.12	1.48 ±0.77	1.60 ±0.88
Eosinophils( x 10 <sup>6</sup> )	2.50 ±1.44	2.00 ±0.87	1.80 ±1.11
Protein-content (mg)	24.50 ±1.64	25.60 ±3.64	22.80 ±4.16
AP(nmol of 4-nitrophenolate produced min <sup>-1</sup> mg <sup>-1</sup> protein)	15.80 ±0.57	15.20 ±0.86	14.80 ±1.16
LDH (nmol of NADH oxidized min <sup>-1</sup> mg <sup>-1</sup> protein)	105.80 ±3.64	98.50 ±3.57	100.20 ±4.73

# Experimental

## Materials

PLGA (lactide/glycolide ratio 50:50, inherent viscosity 0.45dl/g) was a gift sample from Boehringer Ingelheim, Ingelheim, Germany. Mometasone furoate was donated by Alembic chemicals Itd., Baroda, India. Polyvinyl alcohol (PVA,Mw 125,000; degree of hydrolysis 87-89%) was purchased from S.D. fine Chemicals, town, India. WGA and Bichinconinic acid protein Assay Kit were obtained from Banglore Genei, town, India. 3-Ethyl-1-[(3-dimethylamino)propyl]carbodiimide hydrochloride (EDAC), *N*-hydroxysuccinimide (NHS), glycine, *N*'-(2-hydroxyethyl)piperazine-*N*-ethanesulfonic acid (HEPES) and sodium dodecyl sulfate were purchased from National Chemicals, town, India. Methylene chloride, methanol and glacial acetic acid obtained from Loba Chemicals, town, India, were of HPLC grade. All other reagents used for the present study were of analytical grade. The rats were procured from Cadila Healthcare, Ahmedabad, India. The animal experiments were approved by the Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India, with the permission number 404/01/a/CPCSEA.

## Methods

#### NP formulation and WGA-conjugation

Mometasone furoate loaded PLGA nanoparticles were prepared by an emulsion-solvent evaporation technique as described earlier [14]. Briefly, a solution of MF and PLGA in dichloromethane (DCM) was poured into an aqueous PVA solution (2% w/v) and the resulting mixture was stirred with a high speed homogenizer (Ultra-turrax T-25, Ultrapure Scientific, Mumbai, India) to obtain a primary O/W emulsion. The primary emulsion was passed through high pressure homogenizer (Emulsiflex, C5, Avestin, Canada) for three cycles at 15,000 psi pressure. The homogenized O/W emulsion was immediately added drop-wise to an aqueous PVA solution (2% w/v). This dispersion was further stirred overnight with a magnetic stirrer (Remi Equipments, Mumbai) to evaporate the DCM. NPs were collected by centrifugation for 30 min at 25,000 rpm, washed and lyophilized for 24 hrs.

WGA was conjugated onto the surface of MF-nanoparticles by two-step carbodiimide coupling method [14]. Briefly, 1.0 ml 7.0% w/v solution of EDAC and 1.0 ml of 0.3% w/v NHS solution in 20 mM HEPES/NaOH buffer, pH 7.0 were added to a suspension of NPs in the same buffer. After 2 hr of incubation at room temperature, excess EDAC and NHS were removed by centrifugation. The pellet was resuspended in WGA solution containing 200 µg lectin in 20mM HEPES/NaOH buffer pH 7.0 and incubated for 18 hr. Excess WGA was removed by centrifugation. To saturate the free coupling sites, 1.0 ml 20 % glycine solution in 20mM HEPES/NaOH buffer pH 7.4 was added and incubated for 1 hr. Finally, the particles were washed and lyophilized for 24 hrs. To estimate the amount of WGA conjugated to the surface of MF-NPs, the amount of lectin in the supernatant and the washings was subtracted from the amount of lectin added.

#### NP characterization

Plain and WGA-grafted NPs were characterized for **Particle size and zeta potential** by laser light scattering (Nano-ZS, Malvern Instruments, UK). The **entrapment efficiency** of MF in the nanoparticles was determined by extracting and quantifying the encapsulated MF (14). **For in-vitro drug release studies** a suspension of NPs containing 500µg MF in PBS was placed in a dialysis bag, suspended in 15ml PBS and incubated at 37°C. Sampling was done at predetermined time intervals and the volume was adjusted with fresh PBS. To determine the MF-content, the samples were extracted with methylene chloride for 30 min. The methylene chloride was evaporated and the residue was reconstituted with the mobile phase, centrifuged and injected into the HPLC column [15]. **Surface morphology** was analyzed using EDAX (Energy dispersion analysis by X-ray) SEM (ESEM-mode).

#### In vivo-studies

Animal experiments were approved by Social Justice and Empowerment Committee, Ministry of Government of India, New Delhi, India, with the permission number of 404/01/a/CPCSEA.

#### Animals and Treatment

Wistar rats weighing 200-250 g were housed in polypropylene cages with free access to pelletized chow and tap water. The animals were exposed to alternate cycles of 12 hours

light and darkness. The temperature was maintained at approximately 26°C to 28°C. Three rats of either sex were used in each group at every time interval. Animals had free access to water and rat feed but were fasted overnight prior to each experiment.

#### Intratracheal instillation

The intratracheal instillation of saline, drug solution, plain-MF-NP and WGA-MF-NPs were carried out according to a well adapted method [16]. Prior to the experiment the rats were anesthetized by intraperitoneal administration of ketamine (50mg/kg) and intramuscular injection of diazepam (5 mg/kg). Anesthetized animals were placed in supine position on a 45° slanted support, and a small middle incision was made over the trachea. The trachea was exposed by blunt dissection of the sternohyoideus muscle and a small midline incision was made over the trachea. A small hole was made in trachea between the fifth and the sixth tracheal ring using as 20-gauge needle. The trachea was cannulated with a 10-15 cm long PE50 tubing with the tip positioned approximately at the tracheal bifurcation. Doses equivalent to 150 µg of MF as a solution or nanoparticles suspended in sterile saline were slowly instilled over a 1min period using a 1-mL syringe attached to the PE50 tubing. Following instillation, the tubing was withdrawn and a small drop of cyanoacrylate adhesive was placed over the hole to seal the opening. The skin was clothed with 3-0 Dexon sutures. Intraperitoneal Ampicillin (10 mg/kg) and Diclofenac sodium (1 mg/kg) were administered to the rats to combat infection and pain of the animals. Following anaesthesia, the animals were allowed to recreate under a heating lamp. After recovery, animals were housed in individual plastic cages with free access to food and water for the remainder of the study. At the end of each time point biological samples were collected and the animals were sacrificed.

#### **Biological sampling**

Broncho alveolar lavage (BAL) was performed on anaesthetized animals with PBS, prewarmed to  $37^{\circ}$ C. At this, the Hamilton syringe connected to the PE50 tubing was replaced by a 3-way stopcock attached to two 20ml syringes. The tubing was reinserted through the cannula and advanced till the tracheal bifurcation. Fluid (PBS) was slowly injected into the lung via one syringe and then BAL withdrawn by gentle aspiration via the other one [17]. This BAL yielded between 7 to 11 ml liquid which was centrifuged at 4.38 x  $10^3$  x g for 5 min. The supernatant was extracted with DCM and assayed by HPLC method for MF. In addition, the lungs were excised and homogenized in 10ml PBS. The lung homogenate (LH) was extracted with DCM and assayed for its MF-content by HPLC.

For drug targeting or drug delivery in general it is important to be able to quantitatively assess the site-targeting effectiveness [18]. A site enhancement factor was calculated as follows:

Site exposure enhancement factor =

AUC (drug delivery system)

AUC (drug alone)

#### Mometasone furoate HPLC assay

To determine the amount of MF in LH and BAL, respectively, a solution of beclomethasone was added as an internal standard and mixed thoroughly. The drug was extracted from the biological matrix with DCM. The DCM extract was evaporated to dryness and the dried residue was reconstituted with the mobile phase (methanol:water, 65:35, v/v). After

vortexing for 1 min. and centrifugation at 12,000xg for 5 min., the supernatant was injected on to an HPLC system (Dionex Softron GmbH, town, Germany). The HPLC system consisted of a pump (P-680, Dionex), a 10-µl loop injector (Rheodyne 7125), a 14 cm Kromasil C 18 150-4.6 HPLC column (Merck, Germany, particle size 5 µm) and a UVvisible spectrophotometric detector (UVD 170U, Dionex). The mean run time of the assay was 30 min. and the retention time of MF was 12 min. During the assay, MF was eluted isocratically at a mobile phase flow rate of 1 ml/min and monitored with a detector operating at 254 nm. Chromatographic runs were performed at room temperature and the data was analyzed using the Chromeleon 6.5 software (Dionex).

## Pulmonary Toxicity studies

Pulmonary toxicity was assessed by analysis of cellular and fluid components of BAL. BAL from animals instilled with sterile PBS was used as a control. To obtain a volume of about 15 ml BAL, the airways and lungs were washed three times with 5.0 ml of PBS each. The recovered BAL was centrifuged and the supernatant removed. The parameters examined were the total protein-content indicating transudation of serum proteins across the capillary barrier, lactate dehydrogenase (LDH)-activity as an parameter indicatinggeneral cell injury and acid phosphatase (AP)-activity, which represents alysosomal enzyme that is released during phagocytosis and/or damage of macrophages and neutrophils. Increased levels of these enzymes indicate cell injury. The pelletized cells were re-suspended in PBS and counted using ahemocytometer. Cytocentrifuge preparations were stained with Leishmann's stain for differentiation of white blood cell types.

The examination of cell populations such as total cells, macrophages, neutrophils and eosinophils is a good indicator of potential pulmonary damage. Biochemical assay in BAL, such as LDH and AP activities were assayed by the methods of Wotton and Moss [19], respectively.

#### Statistical Analysis

All data are expressed as the mean  $\pm$  SD or the mean  $\pm$  SE of the mean, and comparison of the mean values was performed using either Student's *t*-test or ANOVA. Statistical significance was set at *P* < 0.05.

# Conclusion

Nanoparticles, either plain or WGA-grafted, improved the bioavailability of mometasone furoate as compared to plain drug. Among the NPs, WGA-MF-NPs showed better pharmacokinetic behaviour than lectin-free MF-NPs. It is supposed that the bioadhesive and bioinvasive characteristics of WGA enhance the intracellular uptake of WGA-NPs which leads to prolonged release of drug in the cytoplasm. This resulted in higher AUC and less clearance of drug form the lung tissues altogether improving the bioavailability of drug in the lung.

Thus, WGA-MF-PLGA NPs formulated as a dry powder and administered by an inhaler, can play promising role in alleviating problems associated with asthma treatment, such as short lived action, nocturnal exacerbations and hospitalizations of patients, increase in dose with time. However, prior to clinical application, the presented NP need some further thorough and extensive toxicological studies in animals.

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## **Authors' Statements**

#### Competing Interests

The authors declare no conflict of interest.

#### Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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