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Have inadequate delivery systems hampered the clinical success of inhaled disodium cromoglycate? Time for reconsideration

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Importance of the field: Disodium cromoglycate (DSCG) fits with the perception of a safe drug, but conclusions from questionable meta-analyses reduced its use. In addition, drug delivery aspects, such as hygroscopicity and the poor performance of delivery systems, were not considered to be important determinants of therapeutic failures.

Areas covered in this review: Drug delivery aspects and parameters affecting lung deposition and distribution, important parameters for therapeutic efficacy, are addressed. In addition, the distribution and ratio of mast cell tryptase and chymase-positive phenotypes in the lungs and their role in the prostaglandin and leukotriene pathway are discussed.

What the reader will gain: Information on why *in vitro* data are an excellent tool to understand better therapeutic failures associated with the moisture sensitivity of DSCG and the difficulty in handling and operating DSCG delivery systems in a therapeutically reliable way.

Take home message: Pharmacological efficacy of DSCG has been demonstrated in animals and humans. If the drug is delivered to the site of inflammation in an effective dose, a reliable therapeutic effect can be expected. DSCG has extra properties and potential unspecific antiviral properties and may offer new therapeutic treatment aspects for asthma and viral-induced bronchiolitis in early childhood.

Keywords: aerosol therapy, asthma, children, cromolyn, disodium cromoglycate, inhalation drug delivery

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1. Introduction

Asthma, a major cause of morbidity and mortality in the world, has increased over the past 20 years, especially in children. The pathogenesis of asthma is complex, but there is evidence that chymase-positive mast cells in the distal airways play a crucial role, and targeting of the prostaglandin D_2 (PGD_2) signaling pathways of mast cells could be an option for treatment of severe asthma [1,2]. Asthma exacerbations have been associated with virus infections occurring more frequently in children compared with adults (~ 85% versus 40 – 60%) [3,4]. The direct deposition of pharmacological agents into the lungs by inhalation is the preferred route for the treatment of asthma because it enables high concentrations of drug to be delivered to the site of the disease with minimal systemic side effects. Current treatments of care with inhaled corticosteroids (ICS) (e.g., budesonide (Pulmicort Respules®, AstraZeneca)) and leucotriene modifiers (e.g., montelukast (Singulair®, Merck)) reduced the use of cromones such as disodium cromoglycate (DSCG) and nedocromil as anti-inflammatory drugs [5] and as asthma controller medication, despite their

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Article highlights.

- This review highlights why inhalation of a hygroscopic drug such as DSCG is strongly affected by both the drug formulation and drug delivery system.
- *In vitro* data show that moisture absorption is associated with particle growth and a drop in the respirable dose.
- The impact of devices, formulations and inhalation patterns on urinary excretion and pharmacokinetic is shown, compared and discussed.
- The poor rating of DSCG in Cochrane reviews is critically discussed and put in context with questionable clinical study selection criteria, failures in statistical evaluations and conclusions.
- Mast cell phenotype and distribution including pathophysiological aspects of asthma are outlined to provide information on requirements for successful therapy.
- The paper highlights why therapeutic failures can be explained and why a sufficient dose must be delivered deep into the lung to ensure an anti-asthmatic effect.
- Evidence is provided on why efficient nebulizers producing small droplets and a high and evenly distributed lung dose offer the most promising treatment concept.
- New pharmacological actions of DSCG are discussed, demonstrating that nebulized DSCG could offer new treatment perspectives in viral-induced asthma and bronchiolitis.

This box summarizes key points contained in the article.

proven efficacy and excellent safety profile [6,7]. DSCG, widely used for the treatment of rhinitis, conjunctivitis and primarily allergic asthma in children, lost its position for several reasons, such as inconvenient three to four times daily administration by means of a metered dose inhaler (MDI), dry powder inhaler (DPI) or nebulizer in combination with an irritating hypotonic, DSCG solution (e.g., Intal 20 mg/2 ml water) [8]. Furthermore, meta-analyses [9,10] probably resulted in a change in European guidelines and DSCG is no longer recommended as a first-line therapy. These aspects may have contributed to the worsening reputation of DSCG in many countries [8]. However, DSCG is a very safe drug and this review illustrates why the disputed clinical efficacy of DSCG may be attributed to a questionable statistical interpretation of clinical trials [11]. Furthermore, it has not been investigated to what extent the hygroscopic nature of DSCG and the use of inappropriate drug delivery systems and/or formulations associated with subtherapeutic lung doses may have contributed to negative clinical outcomes [12-18]. This review gives information on: formulation aspects relevant for inhalation solutions, DPIs and MDIs; pharmacokinetics of DSCG; conclusions from pharmacokinetic and lung deposition studies; pathophysiological aspects of asthma; pharmacologic and pharmacodynamic effects of DSCG; the role of DSCG in current therapeutic guidelines for pediatric asthma; meta-analysis dealing with DSCG efficacy in clinical studies; new pharmacological properties of DSCG supporting its value in asthma; and prospects for optimizing DSCG drug delivery and therapeutic efficacy.

2. Formulation aspects relevant for inhalation solutions, DPIs and MDIs

DSCG is a white, tasteless, odorless, hygroscopic, crystalline powder and loses not more than 10% of its weight on drying [19,20]. DSCG is soluble in water up to ~ 10%, but may precipitate over time and forms, in the presence of sodium chloride, concentration-dependent viscous solutions or gels. Micronization of DSCG powder into respirable particles < 5 µm is difficult and requires drying in a high vacuum. The micronized powder spontaneously absorbs water, causing the micronized powder to clump in the Spincaps [21]. The hygroscopic nature of DSCG was utilized to improve the dispersing properties of other drug compounds in MDIs (US patent 6,475,467). DSCG was one of the first pharmacological agents for the treatment of asthma using a propeller device called the Spinhaler. It is operated by inserting and puncturing a capsule containing 20 mg of DSCG either pelletized or dispersed in a lactose powder blend [21]. A deep inspiratory maneuver through the mouthpiece empties the capsule and shear forces generated by the impellor de-agglomerate the drug pellets or scrape off the micronized DSCG particles attached to the surface of the coarse lactose carrier particles (~ 30 – 60 µm), delivering the drug into the lungs [21,22]. However, the de-agglomeration performance and drug delivery efficiency of the Spinhaler are flow rate dependent and strongly affected by the relative humidity [13,18]. It was recognized by several investigators that clumping of the powder delivered with the Spinhaler may negatively affect clinical outcomes [13,18].

DSCG is insoluble in organic solvents such as ethanol, chlorofluorocarbons (CFCs) and hydrofluoroalkanes (HFAs) used as propellants for MDIs. The delivery performance of DSCG MDIs is fairly poor and depends, for example, on the volume of the metering valve, the drug concentration and the shape and size of the actuator [23]. Sales of MDIs containing 5 mg DSCG/actuation (label claim) were ceased owing to clogging issues of the valve associated with moisture ingression. The hygroscopicity of micronized DSCG particles is enhanced by HFA propellants, leading to water ingression over time into the canister. This is associated with a growth of DSCG particles suspended in the propellant, causing a decline of the delivered fine particle dose over time (US patent 6,475,467).

2.1 The label claim – a poor predictor of the delivered and respirable dose

The label claim (LC) of DSCG inhalation products is misleading as neither the DSCG dose delivered from the device ex mouthpiece nor the dose to the lungs is represented by the LC. Meanwhile, for newly approved MDIs and DPIs,

the delivered dose (DD) ex mouthpiece is specified as LC, but there are still inconsistencies between inhalation products sold in the US and Europe. Dependent on the inspiratory flow rate, the DD for a 20 mg DSCG capsule varied from 15.2 to 16.8 mg, corresponding to 76 – 84% of the LC [17].

2.2 Effects of flow rate on droplet/particle size and respirable dose

The particle or droplet size and the inhalation maneuver are therapeutically most relevant for lung deposition and drug distribution within the airways. The fine particle or respirable dose (RD) corresponds to the mass or fraction of the DD within a particle size range < 5 µm. Generation and delivery of the RD depend on the humidity and the inspiratory flow rate [13,24,25]. The particle size of an aerosol is characterized by the mass median or volume median diameter (MMD/VMD; measured, for example, by laser diffraction) or the mass median aerodynamic diameter (MMAD; measured by cascade impactor), which should always be specified in conjunction with the geometric standard deviation (GSD). They provide information on the broadness of the particle or droplet size distribution. A narrow size distribution corresponds with a low GSD. An idealized monodisperse aerosol would have a GSD of unity.

Differences between nebulizer systems with respect to the RD can be up to 10-fold for a 20 mg DSCG solution, as is apparent from **Figure 1**, which compares the delivery efficiency of a Hudson MicroMist T-piece nebulizer with the PARI LC® SPRINT Junior, a breath-enhanced jet nebulizer and an investigational eFlow electronic nebulizer. The latter generates the aerosol by means of a perforated vibrating membrane [14,26].

Another *in vitro* performance characteristic of nebulizers is represented by the respirable drug delivery rate (RDDR), that is, the mass of respirable droplets delivered per minute. A high RDDR is desirable from a patient adherence perspective because it hints at a shorter inhalation time to deliver the drug into the lungs [27]. For a detailed comparison of nebulizer delivery characteristics, parameters as shown in **Table 1** should be investigated. Frequently information of this kind is missing in scientific publications, making interpretation of clinical outcomes difficult.

2.3 Effects of humidity on the particle and droplet size

The particle size of aerosols on their way to the lungs can increase by about four- to fivefold of the original size owing to the ambient humidity [13,28]. This is particularly relevant for DSCG particles administered by means of a DPI or MDI, which grow quickly in the presence of moisture and thus deposit in the tracheobronchial tree instead of the targeted proximal and distal airways. Measurements with aerosolized DSCG in an inhaled air flow with 90% relative humidity (r.h.) revealed an MMAD of > 9 µm with only 6% expected lung delivery [29]. Hence, it is crucial to understand possible interactions of a drug formulation and its delivery system, particularly with respect to a very hygroscopic drug, such as DSCG [14,20,29]. The influence of hygroscopic particle growth on the RD for DSCG particles < 5 µm and < 3.3 µm is shown in **Figure 2** for Intal MDI and DPI in comparison with a breath-enhanced jet nebulizer (PARI LC SPRINT Junior powered by a PARI Boy N compressor) and an investigational eFlow® 30S electronic nebulizer [14,27].

Contrary to the MDI and DPI, much higher respirable doses were delivered by the PARI LC SPRINT Junior jet nebulizer. The investigational eFlow 30S nebulizer delivered up to 9.5-fold higher fine particle mass (RD < 3.3 µm) compared with the DPI, which decreased, however, by 6 and 19%, respectively, at 95% r.h. versus 50% r.h. Only a negligible amount of respirable DSCG particles below 3.3 and 5 µm (1.7 and 0.7% of LC) was found for the MDI, and this fraction was reduced further at high humidity. Similarly, the DPI delivered only a small fraction of DSCG in particles < 3.3 and 5 µm (8 and 12.5% of LC at 50% r.h.), which was reduced substantially to 1.1 and 0.6% at 95% r.h. conditions, respectively [27].

From the physical properties of DSCG and these *in vitro* data it can be concluded that nebulizers are more suitable than an MDI or DPI for the delivery of DSCG to the lungs. This is supported by the fact that the RD < 3.3 µm was 40 – 156.7-fold higher for the nebulizers tested when a 20 mg DSCG dose was aerosolized. Consequently, clinical study results may have device- and dose-dependent outcomes that have not been adequately addressed, even by the Cochrane meta-analysis of van der Wouden *et al.* [30].

3. Pharmacokinetics of DSCG

3.1 Pharmacokinetics of DSCG in urine and plasma

DSCG measured in the plasma is derived largely from drug delivered and absorbed in the lungs owing to the extremely low oral bioavailability. About equal amounts of the inhaled DSCG dose were recovered in urine and feces (40 and 43%, respectively) [31]. Thus, urine excretion studies were regarded as useful for investigating the bioavailability and lung deposition of inhaled DSCG, as most DSCG deposited in the airways is removed by absorption into the blood rather than by mucociliary clearance or swallowing of the drug [31,32]. Walker *et al.* [31] investigated the kinetics of DSCG after inhalation of 20 mg DSCG by means of a Spinhaler in six asthmatic patients. Maximum mean plasma concentrations of 9.2 ± 1 ng/ml were obtained within 15 min after inhalation and the average plasma alf-life was 81 ± 7 min. Very little drug remained in the blood after 4 h (< 1 ng/ml) and 84% of the total administered dose was recovered in feces over 3 days and no metabolites were detected. After intravenous administration DSCG was eliminated rapidly in both the urine and feces in the unchanged form in about equal proportions. From a 20 mg powdered dose delivered via Spinhaler only 0.8% was absorbed from the gut and 3.2% from the airways

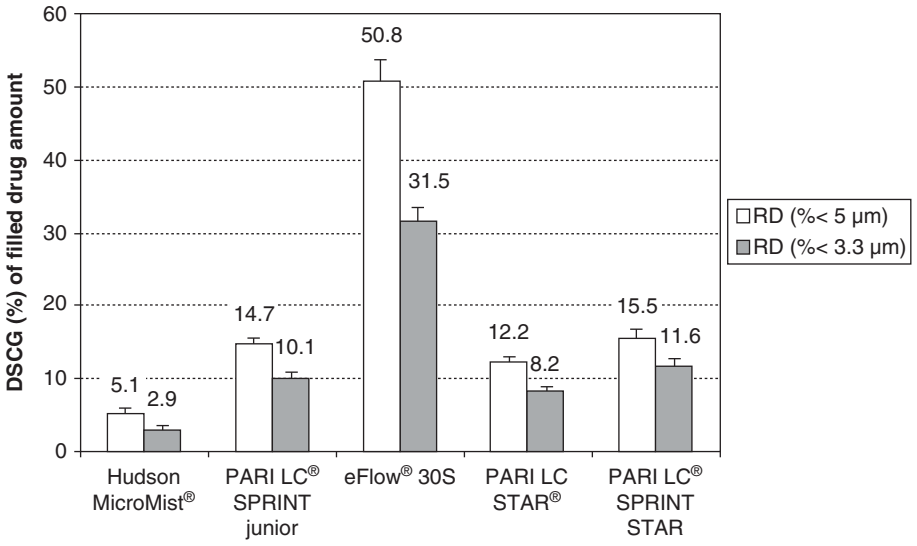

Figure 1. Results are expressed as mean RD < 5 µm (n = 6) calculated from the delivered dose obtained from breath simulation measurements (15 breaths of 500 ml tidal volume, inhalation:exhalation = 1:1) and the respirable fraction < 5 µm calculated from laser diffraction [14,27].

RD: Respirable dose.

Table 1. DSCG aerosol characteristics of different nebulizers*.

Breathing pattern	Hudson MicroMist®/ DeVilbiss Pulmo Aide		PARI LC® SPRINT Junior‡/Boy® S§		Investigational eFlow® 30S	
	10 years	3 years	10 years	3 years	10 years	3 years
MMD (µm)	5.0 ± 0.5	5.1 ± 0.5	3.6 ± 0.1	3.4 ± 0.1	3.1 ± 0.0	3.2 ± 0.0
GSD	1.9 ± 0.0	2.0 ± 0.0	2.2 ± 0.0	2.4 ± 0.1	1.5 ± 0.0	1.5 ± 0.0
RF < 5 µm (%)	50.8 ± 6.2	49.6 ± 6.2	67.9 ± 1.9	70.8 ± 1.5	86.8 ± 0.8	85.1 ± 0.6
RF < 3.3 µm (%)	28.7 ± 4.5	28.5 ± 4.5	44.8 ± 2.0	48.8 ± 1.6	54.6 ± 1.1	52.8 ± 0.6
DD (%)	10.7 ± 0.3	10.3 ± 0.7	22.1 ± 0.9	20.7 ± 0.9	59.6 ± 3.0	59.7 ± 3.7
RD < 5 µm (%)	5.4 ± 0.6	5.1 ± 0.8	15.0 ± 0.3	14.7 ± 0.9	51.7 ± 2.5	50.8 ± 2.9
RD < 3.3 µm (%)	*3.1 ± 0.5*	*2.9 ± 0.5*	*9.9 ± 0.2*	*10.1 ± 0.7*	*32.5 ± 1.5*	*31.5 ± 1.8*
RDDR < 5 µm (%/min)	1.6 ± 0.2	1.5 ± 0.2	3.0 ± 0.1	2.5 ± 0.1	13.6 ± 1.7	13.2 ± 1.6
RDDR < 3.3 µm (%/min)	0.9 ± 0.1	0.9 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	8.5 ± 1.0	8.2 ± 1.0
Nebulization time (min)	3.5 ± 0.1	3.8 ± 0.3	5.2 ± 0.1	5.9 ± 0.1	3.8 ± 0.3	3.9 ± 0.3

*The most important RD for droplets < 3.3 µm is marked in italics. Breath simulation tests were carried out applying breathing pattern as a follows. For 10-year-old children 16 breaths/min, tidal volume 225 ml, inhalation:exhalation ratio 40:60; and for 3-year-old children 24 breaths/min, tidal volume 125 ml, inhalation:exhalation ratio 40:60. MMD, GSD, RF, DD, RD and RDDR were assessed as described by Keller *et al.* [27]. Values expressed as median ± standard deviation.

‡Equivalent to PARI LC® SPRINT 35 nebulizer in the US.

§Equivalent to PARI Proneb® Ultra II compressor in the US.

DD: Delivered dose; GSD: Geometric standard deviation; MMD: Mass median diameter; RD: Respirable dose; RDDR: Respirable drug delivery rate; RF: Respirable fraction.

(see **Table 2** later). These urinary excretion data were supported by investigations carried out by Aswania *et al.* [32] when DSCG was administered orally and by means of inhalation in 11 volunteers. Assuming, that 50% of the total dose administered was excreted via the urine, the results corresponded to a lung deposition of ~ 3 and 1.8%, respectively.

The absorption rate of DSCG can be taken as an indirect measure of the depth to which DSCG penetrates into the lungs: the more peripheral the deposition, the higher should be the absorption rate, and the shorter is the measured absorption half-life. This means that the declining phase of the urinary excretion rate plot represents the absorption, not the elimination. The slow absorption is probably due to the bronchial epithelium, which represents a barrier as it is less permeable to highly polar water-soluble molecules compared with the endothelium [33,34]. This conclusion is supported by

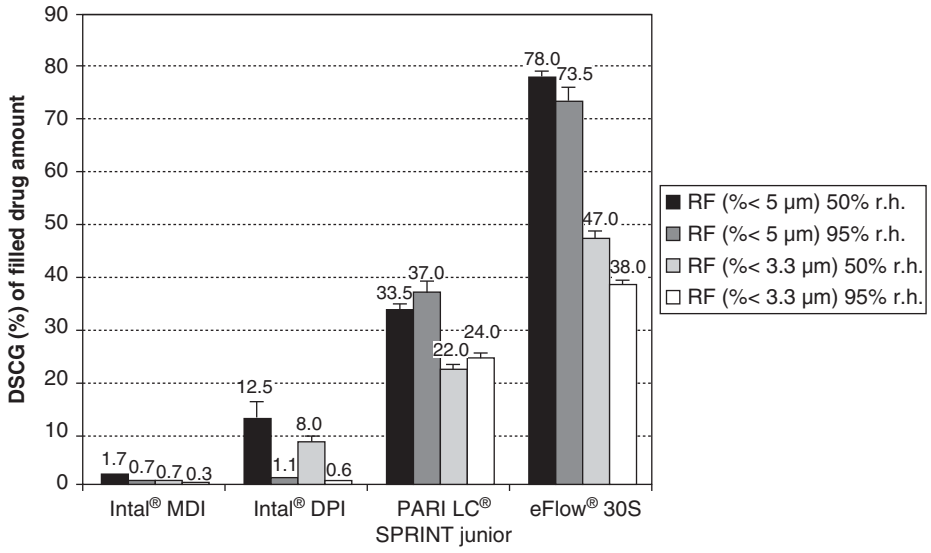

Figure 2. The effects of humidity on the aerodynamic particle and/or droplet size distribution was investigated *in vitro* by an NGI operated in a climate box at 50 and 95% r.h. as published by Keller and co-workers [14,27]**.** Twenty milligrams DSCG each was aerosolized into the NGI from an MDI (Intal® N Aerosol), DPI (Intal®/Fisons Spinhaler®), the PARI LC® SPRINT Junior and an investigational eFlow® 30S electronic nebulizer (clinical trial version) using an isotonic DSCG solution. The RF was calculated as described by Keller *et al.* [27]. The MMAD values increased for the MDI from 5.3 to 8.9 μm and for the DPI from 3.9 to 5.8 μm, at an r.h. of 95 ± 5%, respectively. No such increase was seen for the LC® SPRINT Junior jet nebulizer (all 3.8 μm) and only a slight increase from 3.3 to 3.6 μm was observed for the investigational eFlow® 30S electronic nebulizer. Error bars represent standard deviation.

DPI: Dry powder inhaler; DSCG: Disodium cromoglycate; MDI: Metered dose inhaler; MMAD: Mass median aerodynamic diameter; NGI: Next-generation cascade impactor; RF: Respirable fraction; r.h.: Relative humidity.

urinary excretion data after administration of [^{14}C] DSCG by means of a bronchoscope in two patients [35] and after intravenous administration in two volunteers showing a terminal $t_{1/2}$ of 76 and 100 min, similar to that seen after inhalation [31].

3.2 Effect of devices on pharmacokinetics and lung deposition

The results from various pharmacokinetic studies are outlined below and summarized and compared in **Table 3**. Moss *et al.* [22] reported a mean total urinary excretion of 4.2 ± 1.9% of the nominal 80 mg dose with lung deposition of 7.5% of the nominal dose. Benson *et al.* [36] reported that the total urinary excretion appeared to correlate with the peak inspiratory flow rate.

The relative bioavailability of the generic Cromogen MDI alone and in combination with a large volume spacer was assessed versus the Cromogen Easibreathe alone and with the Optimizer, a 50 ml spacer [37]. A corresponding lung deposition of ~ 3.6, 12.2, 2.8 and 4.3%, respectively, was calculated from 24 h urine sampling in 9 healthy volunteers. In another urine excretion study by Aswania *et al.* [38], lung depositions were estimated to be 3.8% for the Intal MDI (4 puffs, each 5 mg), 11.9% with an attached Volumatic large volume spacer (~ 750 ml) and 10.4% for the Spinhaler.

Auty *et al.* [21] conducted a pharmacokinetic study in healthy (6) and asthmatic subjects (10) measuring DSCG plasma concentration and urinary excretion after inhalation of pelletized DSCG and a DSCG:lactose mixture (1:1) via the Spinhaler. Urinary excretion in asthmatics was low and highly variable, with 0.4 ± 0.5 mg for the Intal lactose blend and 0.5 ± 0.4 mg for pelletized Intal, accounting for a mean of 4.1% of the administered drug dose corresponding to a lung deposition of ~ 4 and 5%, respectively. Mean urinary excretion in healthy subjects was twice as high (1.1 ± 0.4 mg) and all urinary excretion data correlated well with individual plasma AUC levels.

A lung deposition of ~ 14% for the Multisonic compact nebulizer (Schill) and ~ 10% for PARI LC PLUS in 10 cystic fibrosis (CF) patients aged 9 – 21 years was reported by Köhler *et al.* [39]. The shorter absorption half-life observed with the ultrasonic compared with the jet nebulizer (84 ± 14 versus 101 ± 19 min, p = 0.005) suggests a more peripheral deposition, and this assumption was supported by a smaller MMD of 3.3 versus 3.6 μm for the ultrasonic nebulizer. As the absorption rate depends on the site of deposition in the lungs, a more peripheral DSCG lung deposition would be linked with a more rapid drug absorption into the systemic circulation [40]. Köhler *et al.* conducted another study in 10 CF patients (8 – 19 years) with and without a nose clip

Table 2. Comparative plasma levels (C_{max}, T_{max}, AUC) of DSCG obtained after administration of DSCG via Spinhaler DPI, MDI, or nebulizer.

Subjects	Study design	Delivery route/ system	DSCG dose (mg)	Administration mode	C_{max} (ng/ml)	T_{max} (min)	Terminal $t_{1/2}$ (min)	AUC 0 – 240 min (ng/ml min)	Authors
6 asthmatics	Single dose	DPI (Spinhaler)	1 × 20 capsule	According to leaflet	9.2 ± 1.0	15	81 ± 7		Walker *et al.* 1972 [31]
4 healthy volunteers	Single dose	DPI Spinhaler	1× 20 capsule	According to leaflet	36 ± 4	15	71		Neale *et al.* 1986 [40]
6 healthy volunteers (25 – 42 years)	Single dose	DPI Spinhaler (lactose)	1 × 20 capsule	According to leaflet	42 ± 26	8 ± 7	113 ± 65	4880 ± 2400	Auty *et al.* 1987 [21]
10 asthmatics (18 – 77 years)	Single dose	DPI Spinhaler (lactose)	1 × 20 capsule	According to leaflet	16 ± 15	17 ± 8	96 ± 20	1920 ± 1800	
10 asthmatics (20 – 74 years)	Single dose	DPI Spinhaler (pellets)	1 × 20 capsule	According to leaflet	27 ± 17	15 ± 9	116 ± 32	2640 ± 1440	
11 patients 19 – 51 (mean age 32 years)	Single dose, randomized, placebo double-blind crossover	MDI dose per puff Wright nebulizer	2 × 1 = 2 2 × 5 = 10 4 × 5 = 20 20/2 ml	According to leaf let	1.3 ± 0.2 3.4 ± 0.9 6.2 ± 1.1 14.2 ± 1.6	15 15 15 45			Patel *et al.* 1986 [16]
9 healthy volunteers (31 – 37 years)	Single dose crossover	MDI (dose per puff) Omron NE-U07 Intal inhalation sol. 20 mg + 5 ml saline 20 mg + 5 ml saline + 0.3 ml procaterol (0.1 mg 7 ml)	1 × 2 = 2 20/2 ml 20/7 ml 20/7.3 ml	Slow inhalation Face mask Face mask Face mask	1.5 ± 0.7 8.8 ± 6.2 17.2 ± 16.3 24.5 ± 11.9	30 30 5 10	246 ± 117	 2680 ± 2160 4950 ± 4400 4830 ± 2840	Kato *et al.* 1999 [15]
13 healthy volunteers, 21 – 25 years	Single dose crossover	 DPI Spinhaler	 1 × 20	Inspiratory flow rate (l/min) 184 ± 14 101 ± 4 57 ± 3	 12.6 ± 3.0 5.6 ± 1.0 3.3 ± 0.5	 12 ± 3 18 ± 3 20 ± 4	 126 ± 23 176 ± 36 194 ± 27	 1245 ± 220 657 ± 90 413 ± 69	Richards *et al.* 1987 [17]
10 asthmatics (mean age 22.9 years)	Placebo-controlled single dose crossover	Inspiron Mini-neb driven by compressed air at a flow of 9 l/min DD: 6.8 ± 0.4 mg ~ 22.7% of LC MMD: 4.2 μm	30/3 ml	60 breaths of tidal breathing over 6 min Unconstricted Constricted	 7.7 ± 1.0 10.6 ± 1.3	 31 ± 8 23 ± 5	 166 ± 35 120 ± 20	 1687 ± 266 1918 ± 480	Richards *et al.* 1988 [45]

DD: Delivered dose; DPI: Dry powder inhaler; DSCG: Disodium cromoglycate; LC: Label claim; MDI: Metered dose inhaler; MMD: Mass median diameter.

Table 3. Comparative urine excretion of DSCG administered either via Spinhaler DPI, MDI (Intal or Generic) with or without a large or small volume spacer, or nebulizer*.

Subjects	Study design	Delivery route/ system	Application mode in mg DSCG	Total urine excretion μg/6 – 24 h	% With respect to LC	% Estimatedlung deposition	Year of publication	Authors
15 healthy volunteers	Single dose	DPI (Spinhaler)	4 × 20	3400 ± 1480	4.2 ± 1.9	7.4	1971	Moss *et al.* [22]
12 asthmatics	Single dose	DPI (Spinhaler)	1 × 20	468 ± 153	2.34	4.7	1973	Benson *et al.* [36]
6 healthy volunteers 10 asthmatics 10 asthmatics	Single dose Single dose Single dose	DPI Spinhaler (lactose) DPI Spinhaler (lactose) DPI Spinhaler (pellets)	1 × 20 1 × 20 1 × 20	1100 ± 400 400 ± 500 500 ± 400	5.5 2 2.5	11 4 5	1987	Auty *et al.* [21]
11 healthy volunteers	Single dose crossover	Oral MDI MDI + charcoal	20 4 × 5 4 × 5	83.7 ± 71.1 305.6 ± 82.3 184.9 ± 98.4	0.42 1.53 0.92	0.84 3.06 1.84	1999	Aswania *et al.* [32]
9 healthy volunteers 33 ± 7.3 years 71.8 ± 11.8 kg 174.9 ± 6.6 cm	Single dose crossover	MDI MDI + L-Spacer Easybreathe MDI EB MDI + S-Spacer	4 × 5 4 × 5 4 × 5 4 × 5	364.7 ± 266.2 1227.0 ± 459.0 280.2 ± 155.4 429.5 ± 176.7	1.82 6.13 1.40 2.15	3.6 12.2 2.8 4.3	2001	Aswania *et al.* [37]
10 healthy volunteers 31 ± 6.6 years 73 ± 11.6 kg 174.4 ± 6.8 cm	Single dose crossover	MDI MDI + L-Spacer DPI	4 × 5 4 × 5 1 × 20	388.7 ± 199.5 1194.4 ± 657.0 1041.8 ± 723.2	1.9 5.96 5.21	3.8 11.92 10.42	2002	Aswania *et al.* [38]
10 CF patients 9 – 21 years	Single dose crossover	PARI LC PLUS Master compressor Schill Multisonic compact	20 + 2 mg Salbutamol/ 2.4 ml	1040 ± 460 1430 ± 470	5.2 7.15	10.4 14.3	2003	Köhler *et al.* [39]
10 CF patients 9 – 21 years inhaling without and with noseclip	Randomized single dose crossover	PARI LC PLUS Master compressor	20/2 ml	930 ± 380 990 ± 490	4.65 4.95	9.3 9.9	2004	Köhler *et al.* [41]
17 preterm infants 36 ± 2 weeks, 2201 ± 162 g	Randomized three period single dose crossover	PARI LC STAR + Master compressor LS 290 ultrasonic Projet ultrasonic, all connected via a 0.6 m tube to a PARI face mask 0	20/2 ml	89 ± 36 55 ± 19 46 ± 25	0.445 0.275 0.23	0.89 0.55 0.46	2008	Köhler *et al.* [42]

*Lung deposition was estimated assuming that urine excretion corresponds to 50% of the nominal dose delivered into the lungs.
DPI: Dry powder inhaler; DSCG: Disodium cromoglycate; LC: Label claim; MDI: Metered dose inhaler.

inhaling 20 mg DSCG dissolved in 2 ml of water (= Intal) via a PARI LC PLUS powered by a PARI MASTER compressor [41]; 85.1 ± 7.8% of the total DSCG dose excreted within 12 h could already be measured after 4 h, corresponding to an absorption half-life of 93 ± 25 min (without nose clip) and a corresponding lung dose of ~ 10%.

Köhler *et al.* [42] also investigated DSCG lung deposition in 17 preterm infants, measuring urinary excretion over 12 h. *In vitro* aerosol measurements of three nebulizers tested revealed MMDs and respirable fraction (RFs) of 3.2 µm and 20.3% for the PARI LC STAR®, 5.04 µm and 6.4% for the LS 290® and 3.45 µm and 6.4% for the Projet® ultrasonic nebulizers, respectively. Assuming equal biliary and urinary excretion, the corresponding lung deposition could be estimated with ~ 0.89, 0.55 and 0.46%, respectively. These data clearly demonstrate that lung deposition in preterm infants is 11 – 21-fold lower compared with adults when the same DSCG dose is nebulized and administered by means of a face mask (**Table 3**).

It can be summarized from these studies that lung deposition in healthy and asthmatic adults ranged for MDIs without spacer from 1.8 to 3.8% and with spacers from 4.3 to 12.2%, for the DPI from 4.1 to11% and for nebulizers from 9.3 to 14.3%, respectively. In general, lung deposition was lower for asthmatic patients.

3.3 Parameters affecting pharmacokinetics of DSCG in plasma

Results from various pharmacokinetic studies assessing DSCG in the plasma are outlined below and summarized and compared in **Table 2**. Plasma C_{max} varied from 3.3 to 36 ng/ml with a T_{max} of 12 – 20 min post-inhalation of 20 mg DSCG by means of the Spinhaler. T_{max} was delayed to ~ 23 – 45 min when DSCG was inhaled from a nebulizer. In general, terminal half-lives ($t_{1/2}$) varied from 71 to 194 min and seemed not to be device related.

3.3.1 *Effect of the inspiratory flow rate on the delivery efficiency of the Spinhaler*

Richards *et al.* [17] investigated DSCG plasma concentration in 13 healthy volunteers based on different inspiratory flow rates. This flow rate-dependent drop in C_{max} from 12.6 to 3.3 ng/ml was associated with an increase in the terminal $t_{1/2}$ from 126 ± 23 to 194 ± 27 min, respectively, although the delivered DSCG dose varied for all three flow rates only in a range of 15.2 – 16.8 mg (**Table 2**). However, at a flow rate of 140 l/min ~ 50% of DSCG particles expelled from the Spinhaler had a diameter of 5 µm or less, whereas at 40 l/min this fraction dropped to < 5%. Peak concentration and the amount of DSCG reaching the circulation (as assessed by AUC) was related directly to the flow rate, which disperses the particles for inhalation. Thus, even with a maximum inspiratory maneuver of 184 l/min, only ~ 1.5 mg or 7.5% of a nominal capsule dose reaches sites within the lung from which it was absorbed. Based on corresponding AUC values ~ 4 and 2% lung deposition can be calculated for flow rates of ~ 100 l/min and 60 l/min, respectively.

Auty *et al.* [21] investigated in healthy (6) and asthmatic subjects (10) DSCG plasma levels after inhalation of pelletized DSCG and a DSCG:lactose mixture (1:1) via the Spinhaler. Pleak plasma concentrations (16 – 42 ng/ml) and AUC values (32 – 80 ng/(h ml)) were highly variable and about twofold higher in volunteers compared with asthmatics. The authors concluded that most of the inter-subject variability was a result of differences in the inhalation technique, particularly with regard to the inspiratory flow rate and duration of breath hold after inhalation.

It can be concluded from the studies that the flow rate generated by inspiration through a Spinhaler is an important determinant of the amount of DSCG reaching the airways and being absorbed into the circulation.

3.3.2 *Device related differences in plasma DSCG concentrations (C_{max})*

C_{max} in 11 asthmatic patients was 14.2 ± 1.6 ng/ml after 45 min when 20 mg were inhaled through a nebulizer compared with only ~ 6.2 ± 1.1 ng/ml after 15 min, when the same dose was inhaled from an MDI. Only 3.4 ± 0.9 and 1.3 ± 0.2 ng/ml were obtained when 2 × 5 mg and 2 × 1 mg were administered from the MDI [16]. Furthermore, when a 20 mg dose was inhaled the corresponding AUC values of the nebulizer treatment were 2.2-fold higher compared with the MDI treatment using an equivalent dose, and maximum DSCG plasma concentration did correlate with clinical efficacy in protecting patients with asthma against exercise-induced bronchoconstriction [16]. As delivery efficiency of the jet nebulizer was at least twofold higher than the MDI, it was speculated by the authors that nebulizer treatment may provide a better and longer acting effect, indicating that potentially the frequency of administration could be reduced.

Kato *et al.* [15] conducted a crossover pk study in 9 healthy volunteers comparing plasma concentration after inhalation of DSCG by means of an MDI (2 puffs per 1 mg) and as an inhalation solution (20 mg) using an Omron NE-U07 ultrasonic nebulizer with a face mask and normal tidal breathing. DSCG was inhaled either as a hypotonic solution alone or mixed with 5 ml isotonic saline and in addition with 0.3 ml procaterol (0.1 mg/ml). C_{max} was ~ 5.9-fold higher after inhalation of the nebulized solution compared with the MDI, which is mainly a dose effect. More important was the difference in C_{max} after inhalation of the hypotonic (8.8 ± 6.2 ng/ml), the isotonic (17.2 ± 16 ng/ml) and the procaterol-containing DSCG solution (24.5 ± 11.9 ng/ml). Although data were highly variable, the study design not strictly parametric and the use of a face mask questionable, the data indicate a better lung deposition for the isotonic solution alone and in combination with a beta-agonist. This is no surprise owing to dilatation of the airways by the beta-agonist, enabling droplets to travel deeper into the lungs. However, it can be concluded from these data that smaller droplets can be

deposited more effectively into the lungs and that an insufficient therapeutic effect will be related to a subtherapeutic DSCG lung dose. Thus, the authors' conclusion is that measuring DSCG plasma concentration provides valuable information on adequate compliance and inhaler technique, and switching the patient to another DSCG formulation should be considered [15].

3.3.3 *Effect of particle size and breathing pattern on lung deposition*

The effect of humidity on inhalation and deposition of nebulized DSCG in two different particle size distributions was investigated in asthmatic children by Nerbrink *et al.* [24] using a Hudson Updraft II nebulizer characterized by a delivered dose of 14.4% of the LC (20 mg/2 ml). Nine children with a mean age of 10 years inhaled the DSCG solution at two different relative humidities of 13% and > 90% r.h., which resulted in two different droplet size distributions. Lung deposition can be estimated to be ~ 3.5 and 5% for droplets with an MMAD of 1.2 µm and 5.9 and 7.2% for droplets with an MMAD of 2 µm at 400 and 800 ml tidal volume, respectively. These data may allow the conclusion that very small droplets will be exhaled and that droplets ~ 3 µm are more suitable for this age group [43,44].

4. Conclusions from pharmacokinetic and lung deposition studies

Results from different urine excretion studies, summarized in **Table 3** [21,22,32,36-39,41,42], and plasma concentrations, summarized in **Table 2** [15-17,31,40,45], indicate that only ~ 3% of a 20 mg DSCG dose delivered by means of an MDI will be deposited into the lungs. If a large volume spacer is used this fraction can be increased to ~ 12%. Not surprisingly, lower and more variable lung deposition data were obtained from the Spinhaler (~ 2.0 – 10.4%) in asthmatic patients and the correct handling of the Spinhaler, including inhalation technique, plays a crucial role [16,17,21,45].

With nebulized DSCG administration lung deposition depends on the delivery performance of the nebulizer as well as the breathing pattern and droplet size. Hence, the nebulizer type and driving gas flow or compressor type must be specified to understand the device-related impact on lung deposition. Thus, information on the aerosol performance characteristics as outlined in **Table 1** and **Figure 1** is important to understand and interpret clinical outcomes [45]. Lung deposition of nebulizers used for DSCG in children and adults ranged from ~ 3.2 to 14%. However, much higher lung depositions of 30 – 40% of the nominal dose were reported for the investigational eFlow, an electronic perforated vibrating membrane nebulizer [26], on aerosolization of drugs such as Budesonide [44], Tobramycin [46], or liposomal Ciclosporin A [47].

Contrary to lung deposition studies, measurement of C_{max} and AUC is the fastest, easiest and most sensitive way to obtain information on successful delivery of DSCG into the lungs dependent on the delivery system, drug formulation, inhalation technique, patient-handling skills and compliance [15,21]. The poor reputation of DSCG could have been overcome if pharmacokinetic data had been used to monitor therapeutic failures. This aspect has shifted the mindset of regulatory bodies demanding pK studies to demonstrate the safety and efficacy of inhaled products as a surrogate measure of bioequivalence (IPACT-RS Conference, Frankfurt, Germany, 12 and 13 November 2010).

5. Pathophysiological aspects of asthma

Asthma is a chronic inflammatory disorder of the lung and often involves infiltration of the airway by inflammatory cells such as activated lymphocytes and eosinophils, denudation of the epithelium, deposition of collagen in the sub-basement membrane area and mast cell degranulation. These inflammatory cells release chemical mediators, resulting in altered airway physiology, structural abnormalities (remodeling) including swelling of the mucosa and submucosa, thickening of the basement membrane, enlargement of smooth muscles and peribronchial fibrosis, as well as excessive production of bronchial secretion [48]. During the past few years evidence has been provided that distal airways from the 8th to the 24th generation, including terminal bronchioles, play a crucial role in the pathogenesis of asthma and are the major determinant of airflow obstruction [49]. Across disease groups, the median number of tryptase-positive mast cells in the bundles of airway smooth muscle was significantly higher in asthma patients compared with bronchitis patients and normal subjects ($p < 0.001$), thus highlighting an important difference in the inflammation process between eosinophilic bronchitis and asthma. Balzar *et al.* [2] showed that the number of inflammatory cells in tissues from 20 severe asthmatics generally increased towards the lung periphery. Mast cell number, percentage and the chymase-positive phenotype increased in the small airways regions, and the latter may be protective for lung function in severe asthma, supporting a pivotal role in modulating disease severity. It was shown in another paper by Balzar *et al.* [1] that the mast cell population in severe asthma is dominated by the chymase-positive phenotype in the submucosa and more uniquely in the epithelium. This is associated with an increased PGD_2 level in bronchoalveolar lavage, and a targeting of the PGD_2 signaling pathway may be an option for the treatment of severe asthma.

Several studies on lung tissue specimens from asthmatic patients have demonstrated that more severe inflammatory and structural changes occur in the distal airways < 2 mm in diameter and in the lung parenchyma of asthmatic patients [50]. Hamid *et al.* [51] observed in a clinical study with lung specimens from asthmatic and non-asthmatic subjects that based on the accumulation of T cells and eosinophils, a similar but more severe inflammatory process

is present in the periphery versus the central airways in patients with asthma. Another study came to the conclusion that cells expressing IL-5 mRNA were significantly elevated in distal airways compared with proximal airways [52].

These findings strongly suggest that asthma drugs should be delivered into the deep airways.

6. Pharmacologic and pharmacodynamic effects of DSCG

6.1 Protective effects of DSCG after bronchoconstriction – effect on prostaglandins and leukotrienes

Brannan *et al.* [53] investigated in a crossover placebo-controlled study in 14 asthmatics the protective effect of DSCG administered 15 min before mannitol-induced bronchoconstriction. The maximum fall in forced expiratory volume in 1 s (FEV_1) of 32 ± 10% on placebo was reduced by 63% on inhalation of 40 mg DSCG by means of the Inhalator Ingelheim DPI. Following placebo there was an increase in median urinary 9α,11β-prostaglandin F_2 concentration from 61 to 92 ng/(mmol creatinine), but no significant increase in the presence of DSCG (66 versus 60 ng/(mmol creatinine). These results support the hypothesis that the DSCG effect on airway response to mannitol is a result of the inhibition of mast cell prostaglandin D_2 release.

Anderson *et al.* [54] investigated in a randomized, double-blind, double-dummy, placebo-controlled crossover trial the protective effect in 14 asthmatics of 40 mg DSCG 15 min before inhaled mannitol bronchoconstriction. Administration was conducted by means of identical DPI devices (RS-01, Plastiape, Italy) either alone or in combination with 10 mg montelukast, which was given orally the night before and 3 – 5 h before the study. The protection by DSCG alone on the percentage drop in FEV_1 was 64 ± 21.0% versus 65.8 ± 62.8% for the combination. The protection on the area under the 30 min FEV_1 time curve for DSCG was 81.8 ± 14.0% versus 89.3 ± 9.8% for the combination ($p > 0.001$) compared with placebo. It was concluded that the addition of montelukast to DSCG provided only a small extra benefit against airway response to mannitol being a stimulus for the release of histamine, prostaglandins and leukotrienes.

A similar therapeutic effect was also observed by Kippelen *et al.* [55] carrying out a study to clarify the role of DSCG during hyperpnea. Eleven athletes with exercise-induced bronchoconstriction (EIB) and 11 without performed a eucapnic voluntary hyperpnea (EVH) test (a surrogate for exercise) 10 min after inhalation of a placebo or 40 mg DSCG inhaled by means of a MDI. In the EIB group, the maximum drop in FEV_1 of 20.3 ± 3% on placebo was reduced to 11.5 ± 1.9% following DSCG administration ($p = 0.003$). The increase in the urinary excretion of 9-alpha, 11-beta-PGF_2 and LTE_4 was abolished by DSCG but not by the placebo. The authors concluded that mast cell activation with release of bronchoconstrictive mediators, for example PGD_2, following hyperpnea in athletes is inhibited by DSCG.

6.2 DSCG – influence on other mediators and biomarkers

In clinical studies evaluating the protective effect of DSCG with regard to infiltration of inflammatory cells, Diaz *et al.* [56] showed in a placebo-controlled study with 36 atopic asthmatics that the percentage of eosinophils in bronchial mucus and bronchoalveolar-lavage (BAL) fluid was significantly less after administration of DSCG. In addition, a significant decrease in the concentration of total IgA in BAL fluid after a 28-day treatment with DSCG was observed.

The effect of DSCG (8 puffs per 1 mg/day administered for 12 weeks) was investigated in a biopsy-proven study in comparison with beclomethasone and ketotofen in a total of 32 patients with atopic asthma. A significant reduction was shown in the DSCG cohort (nine patients) for the inflammation parameters eosinophils, mast cells, T-lymphocytes, macrophages, adhesion molecules ICAM-1, VCAM-1 and ELAM-1, $EG2^+$-activated eosinophils, $AA1^+$ mast cells and $CD3^+$, $CD4^+$, $CD8^+$, T-lymphocytes [57,58]. Although not placebo controlled, all nine DSCG patients also had a significant improvement in FEV_1, vital capacity (VC), symptom score, bronchial responsiveness, peak expiratory flow (PEF) and diurnal variation compared with baseline [58].

Larsson *et al.* [59] investigated in a randomized placebo-controlled study the protective effect of 20 mg DSCG (20 MDI puffs) in 32 healthy volunteers exposed to organic dust. The increase in neutrophils, IL-6, TNF-α, myeloperoxidase and soluble intracellular adhesion molecule-1 (ICAM-1) was significantly reduced in BAL fluid by DSCG.

Pharmacological actions show that DSCG blocks the activation and subsequent mediator release from a variety of inflammatory cells in addition to mast cells, including eosinophils, neutrophils, monocytes, alveolar macrophages and lymphocytes [5,7]. Hence, a therapeutic effect can be expected if the drug is delivered in an effective dose to the site of inflammation.

6.3 Dose response studies

Patel *et al.* [60] investigated in a single-blind dose response study the protective effect of nebulized DSCG versus saline in 10 asthmatic patients (29 ± 3.2 years), measuring the percentage drop in FEV_1 after 8 min of treadmill exercise. A slight bronchodilatation as evident from the increase in baseline FEV_1 (5.7%) was observed only for 40 mg DSCG administered by means of a Wright's nebulizer driven by compressed air at a flow rate of 9 l/min. After exercise the mean percentage drop in FEV_1 after 30 min was 37.3 ± 4.7% after inhalation of saline compared with 17.3 ± 4.1%, 10.0 ± 3.3%, 7.6 ± 2.4% and 12.0 ± 2.9% after inhalation of 2, 10, 20 and 40 mg DSCG, respectively. Results were highly significant for all DSCG concentrations ($p < 0.001$).

The mean FEV_1 returned to baseline values within 15 min only for the 20 and 40 mg doses and a small bronchodilator effect was noted at 30 min. These findings were in agreement with those by Jones and Chung [61].

Tullett *et al.* [18] carried out a placebo-controlled double-blind study in 11 asthmatic patients, investigating the effect of 2, 10 and 20 mg DSCG delivered via an MDI with 1 and 5 mg/puff compared with a nebulized dose of 12 mg administered with a Wright nebulizer at a flow rate of 9 l/min. The protective effect of DSCG was dose-dependent and a 20 mg DSCG dose delivered by means of the MDI was comparable to a 12 mg dose administered from the Wright nebulizer.

6.4 Animal studies supporting a long-lasting therapeutic effect of DSCG

In an asthma mouse model with inhaled DSCG, albuterol and hydrocortisone, the early- and late-phase reaction after allergen challenge was suppressed with a maximum effect at 15 min and 3 – 12 h, respectively [62]. Correlated with the late-phase reaction, the eosinophil density in tissue (not from bronchoalveolar lavage) was significantly increased 3 h after allergen challenge. In this model, the early-phase reaction was suppressed by DSCG and albuterol, the late-phase reaction by DSCG and hydrocortisone.

Moon *et al.* [63] evaluated in guinea-pigs the effect of nebulized DSCG (10 mg/ml) on the early and late bronchoconstriction and leukocyte infiltration after allergen challenge with ovalbumin. Administration of DSCG 15 min before and 6 h after challenge reduced the accumulation of neutrophils, retrieved from BAL, at 17 h and the influx of eosinophils at 72 h with comparable efficacy as dexamethasone (5 mg/ml). The inhibitory activity of DSCG on polymorphonuclear leukocytes (PMN), eosinophils and lymphocytes was significant 24 and 72 h post-administration and partly superior to dexamethason. A superior effect over dexamethason was also observed on histamine and protein contents in BAL, whereas the effect on the phopholipase A_2 specific activity was comparable to dexamethasone. These data show that a sustained effect of DSCG at a dose of 10 mg/ml could be observed even 24 h after administration, indicating that drug effects may exceed serum half-time significantly, which may be important for reducing dosing intervals.

6.5 Dose duration effects of nebulized DSCG in humans

Patel and Kerr [64] investigated in another single-blind placebo-controlled study in eight asthmatic patients (26.5 ± 3.1 years) the dose duration effect of DSCG. The mean drop in FEV_1 after running 8 min on a treadmill was monitored after 20, 150 and 270 min post-inhalation of DSCG via a Wright's nebulizer driven by compressed air at a flow rate of 9 l/min carried out at tidal breathing for 5 min each. DSCG was effective after 20 and 150 min in all doses tested. In the third exercise test after 270 min a partial protection in the exercise-induced fall in FEV_1 was observed for the 20 and 40 mg doses, whereas the effect of DSCG 2 mg/ml had worn off by this time. The protection of the lowest dose does not last beyond 2 h, indicating that higher doses may require less frequent drug administrations. Other studies confirm that a reduction of dosing frequencies from four times to three times or twice daily in a step-down approach is feasible to maintain asthma control [65-67].

6.6 Effect of formulation tonicity on tolerability and efficacy

Three clinical studies with isotonic versus hypotonic DSCG solution have been performed, as it is well recognized that non-isotonic solutions are a stimulus to airway narrowing and might cause the provocation of asthma attacks. It is believed that this effect is due to the mediator release from mast cells in the airway lumen and submucosa [68,69]. Weiner *et al.* [12] and Soferman *et al.* [69] reported a significantly increased protection against exercise- and methacholine-induced bronchoconstriction with isotonic compared with hypotonic DSCG in asthmatic children. By contrast, no such protection difference was found by Kano *et al.* [70]. As already discussed, Kato *et al.* [15] did find a higher C_{max} and AUC after inhalation of an isotonic compared with a hypotonic DSCG solution.

6.7 Effect of nebulized DSCG on lung function parameters in asthmatic children

In 2008, Moeller *et al.* presented data of a study conducted in 28 stable atopic asthmatic children (11.9 ± 2.8 years, 12 girls) inhaling either an isotonic DSCG solution (3 × 20 mg/2 ml saline) over 6 months by means of an investigational small droplet eFlow electronic nebulizer (MMAD ~ 3.3 µm) or inhaled steroids twice daily by means of an MDI with spacer [71]. The mean asthma score decreased significantly in both treatment groups as follows: for DSCG from 5.6 ± 1.5 to 3.2 ± 1.9 ($p < 0.001$) and for ICS from 5.2 ± 2.5 to 3.07 ± 2.2 ($p < 0.021$). No statistical differences were found for FVC, FEV_1, MEF_{50}, FeNO, eosinophils and β_2-mimetic use, and the authors concluded that 3 × 20 mg isotonic DSCG delivered by the investigational eFlow nebulizer per day was therapeutically equally efficient as twice daily administered inhaled steroids.

7. Role of DSCG in therapeutic guidelines for pediatric asthma

The Global Initiative for Asthma (GINA) has published an update on the management and prevention of asthma in children [72]. In the treatment of childhood asthma, ICSs are regarded to be a first-line therapy, whereas DSCG is regarded as less valuable and not recommended anymore.

In the National Asthma Education and Prevention Program (NAEPP) Expert Panel Report, DSCG at a dosage of 3 – 8 mg/day (MDI) or 60 – 80 mg/day (nebulizer) is mentioned as an alternative treatment in children with mild,

persistent asthma [73]. In the British Thoracic Society (BTS) asthma guidelines, DSCG is recommended as another preventive therapy with some benefit in adults but inconclusive recommendations for the treatment of childhood asthma [74], mainly based on the negative DSCG meta-analyses [9,10].

In contrast to US and European guidelines, the English summary of the Japanese asthma guidelines associates a higher therapeutic value with DSCG for the treatment of childhood asthma [75]. Two milligrams per day (MDI) and 40 mg/day (nebulizer) DSCG is recommended in the group of mediator release inhibitors as monotherapy (6 – 15 years of age, mild persistent asthma), or in combination with other medications in children < 2 to 5 years in mild, moderate or severe persistent asthma.

8. Meta-analyses dealing with DSCG efficacy in clinical studies

Tasche *et al.* performed a meta-analysis with 24 randomized, double-blind, placebo-controlled clinical studies with DSCG in childhood asthma [9]. The authors came to the conclusion that there is insufficient evidence that DSCG has a beneficial effect as maintenance treatment in children with asthma compared with placebo. A similar conclusion by the same authors was taken by a second DSCG meta-analysis by van der Wouden *et al.* [10] based on partly new selected, 24 randomized, double-blind, placebo-controlled clinical DSCG studies in childhood asthma including day activity, night cough/wheeze, sleep disturbance and the use of bronchodilators. The pooled results of the trials using as primary outcome measure symptom-free days showed no difference between DSCG and placebo but did show significant differences in favor of DSCG for some secondary outcome measures. Following criticisms of this review regarding selection of studies and questionable statistical interpretation [11], the authors updated the review in 2009 and corrected some of the errors but not the conclusions [30].

The first meta-analysis by Tasche *et al.* [9] showed in fact a statistically significant difference in favor of DSCG for the end points wheeze and cough. In the second meta-analysis by van der Wouden *et al.* [10], a significant difference in favor of DSCG was found for the pooled parameters day wheeze score, day activity, night cough, night wheeze, overall symptom/severity score and bronchodilator use. No significant difference from placebo was found for symptom-free days, day cough and sleep disturbance.

The final conclusion of the authors was that there is insufficient evidence for DSCG being more effective than placebo. This yielded some debate in response letters to the editor, including the question regarding interpretability of the tolerance intervals and the selection criteria of the clinical trials included in Edwards and co-workers and Silverman [76-78]. An excellent review article by Stevens *et al.* describes in detail all pitfalls of the meta-analyses for DSCG in childhood asthma [11]. The meta-analysis by van der Wouden *et al.* [30] considered 23 studies with 1026 participants. DSCG was favorable versus placebo regarding daytime cough, day wheeze scores and bronchodilator use. Although the authors could not rule out the possibility that DSCG is of benefit in children above the age of 4 years, the negative rating of DSCG was not corrected [30] despite the fact that multiple papers showed therapeutic efficacy of DSCG, excellently summarized by Storms and Kaliner [7]. Although the method of administration was regarded to be a critical factor, nebulization was claimed to be less effective than an MDI or DPI treatment, which is in clear contradiction to the results discussed before [7,12,15,18,39,60,64,69,71]. Furthermore, it was not discussed whether the poor clinical outcome was possibly linked with an inadequate nebulizer performance, insufficient lung dose and/or formulation tolerability issues [12,13,15,16,69]. It appears that the conclusions drawn from the DSCG meta-analyses [9,10,30], which have served as the basis for the change of therapeutic guidelines and treatment recommendations in pediatric asthma, are questionable. In conclusion, the overall clinical efficacy assessment of DSCG is hampered by many factors, such as suboptimal study designs, unknown nebulizer performance and a lack of effective dose delivered to the lungs. Pharmacokinetic data were not considered to be a valuable tool to understand drug delivery aspects better [15].

9. New pharmacological properties of DSCG supporting its value in asthma

Hashimoto *et al.* [3] conducted a study in respiratory syncytial virus (RSV)-infected mice to investigate the effects of DSCG on RSV-induced illness using a well-characterized murine model of RSV-infected mice. Mice treated with DSCG were protected against RSV-induced weight loss. No differences in viral replication between mice treated with DSCG and those treated with saline were observed, but the level of inflammation observed in the lungs in RSV-infected mice treated with DSCG was less severe compared with mice treated with saline. The authors concluded from their results that DSCG may be an effective agent for the prevention of RSV-induced disease and the relief of symptoms caused by RSV infections.

A potential inhibition of influenza virus infection *in vivo* and *in vitro* by DSCG was investigated by Hidari *et al.* [79]. DSCG treatment of kidney cells during or after, but not before adsorption by the influenza virus A/PR/8/34 significantly inhibited influenza viral infection when DSCG was administered in a concentration of 10 mM, corresponding to ~ 5.12 mg/ml. *In vivo* experiments were carried out in 4-week-old BALB/c mice treated with 20 µl DSCG or water (control) intranasally 4 h post-viral infection with A/PR/8/34 for 1 – 4 days twice daily. The ED_{50} at 9 days post-infection was 15.5 mg/kg for DSCG, 16.2 mg/kg for ribavirin and 1.16 mg/kg for zanamivir. A weight gain was observed for mice at a dose of 32 mg DSCG/kg surviving to

day 9 post-infection. The viral protective effect of DSCG was comparable to ribavirin, but ~ 14 times less potent compared with zanamivir. The authors concluded that DSCG acts on events late in the viral infection cycle and exerts anti-influenza effects [79].

Penttinen *et al.* [80] reported that DSCG can inhibit in high doses virus-induced cytopathic effects *in vitro*. The authors concluded from their findings that high DSCG concentrations in the airways' fluid may protect the mucosa from tissue destruction and diminish local inflammation induced by viruses.

Fukasawa *et al.* [81] reported an inhibition on the adherence of non-typeable *Haemophilus influenzae* (NTHi) onto RSV-infected A549 airway epithelial cells by DSCG, but not by dexamethasone and fluticasone propionate. Furthermore, DSCG suppressed the expression of ICAM-1, which is one of the NTHi receptors, explaining an inhibitory effect of DSCG on RSV infections.

Miyatake *et al.* [82] reported that DSCG inhalation decreased respiratory infections in adults with asthma. The design was retrospective, cross-sectional and questionnaire-based and supported by supplementary lung function and laboratory data. Over 200 patients were changed from ICSs to an ICS/DSCG combination. After DSCG was added to the ICS therapy, 56% of patients rated that asthma symptoms were alleviated and 66% that respiratory infections lessened.

Reijonen *et al.* [83] reported that infants suffering from viral-induced bronchiolitis [4] had significantly fewer wheezing episodes when treated with DSCG (19%) and budesonide (16%) compared with placebo. An early therapy with nebulized DSCG, particularly of children with atopy, reduces the number of wheezing episodes and hospital admissions after bronchiolitis.

Korppi reported in a letter to the editor [8] that 92 children aged < 24 months hospitalized for wheezing were treated for 4 months with DSCG, or ICS, or no maintenance therapy. About 6 – 7 years later these children were re-examined and those treated in infancy with DSCG had significantly less asthma (21%) than those with no treatment in infancy (54%) or with ICS (46%).

Yamazaki *et al.* [84] investigated the inhibitory effect of DSCG on the growth of *Chlamydia pneumoniae* (CP) *in vitro* by assessing the minimum inhibitory concentration (MIC) and pre-inoculation minimal cidal concentration (MCC). The MCC could be reduced by ~ 100% during a 60 – 90 min exposure with a dose of 20 mg DSCG/ml, whereas lower doses were less effective. The MIC was reduced to 80 ± 7% and 31 ± 10% for DSCG in concentrations of 10 and 20 mg/ml, respectively. The authors discussed that clinical anti-asthmatic effects of DSCG may be a result of the inhibition of *C. pneumonia*, known to be associated with respiratory illness, such as asthma, chronic obstructive pulmonary disease, bronchitis and pneumonia.

10. Conclusions

Although early pharmacokinetic investigations demonstrated a dose response [18,60], flow rate-dependent highly variable plasma concentrations [17,21] and therapeutic effects [60], drug delivery aspects resulting from the hygroscopic nature and particle growth of DSCG [13,14,28] were not considered adequately in publications [7,11,75-78] and meta-analyses [9,10,30]. Despite positive clinical results obtained with MDIs and DPIs, it can be concluded from the physicochemical properties of DSCG that MDIs and DPIs are less suitable for a reliable lung delivery [13,14,17,18,20]. In addition, patients have problems handling and using these systems properly [17,18,21]. Despite intensive instructions, patients still exhale into the DPI causing moisture ingression, forget exhalation before inhalation, cannot generate the requested inspiratory flow rate and do not hold their breath for 10 s [17,18,21]. Coordination problems are most frequent for MDIs, requiring, contrary to the DPI, a slow and deep inhalation maneuver. Furthermore, MDIs are most probably underdosed [15,16], and the drop of the fine particle dose and increase of the MMAD over time due to water ingression into the MDI canister are serious drawbacks.

Asthma is primarily a deep-lung disease and chymase-positive mast cells located there play a crucial role [1,2,49,50], making DSCG delivery to this site of inflammation necessary. Although pharmacokinetic, pharmacodynamic and lung deposition aspects have been published widely [12,13,15,16,18,21,24,25,37-42,45,60,61], these aspects have not been considered adequately as selection criteria applied to meta-analyses [9,10,30]. The conclusions taken are in contradiction to many other studies, which have demonstrated that DSCG is therapeutically effective and worth being reappraised [7,8,64-69,71,76-78,82,83].

In vitro aerosol characterization studies of DSCG delivery systems [13,14,27] are a valuable tool to understand better drug delivery aspects and to select more suitable delivery systems [13,14,26,27]. The RD can be increased and is less susceptible to humidity when new and highly efficient nebulizers are used in comparison with MDIs and DPIs [13,14,27], and *in vitro* study data are supported by a clinical study in asthmatic children [71]. However larger, randomized, double-blind, ideally placebo-controlled clinical studies will be required to improve the poor image of DSCG as a valuable asthma controller medication in order to reappraise it again as a first-line therapy in asthma and guidelines.

11. Expert opinion: prospects for optimizing DSCG drug delivery and therapeutic efficacy

Pharmacological investigations on the anti-inflammatory properties of cromons [5] and newer clinical studies have revealed that DSCG is a potent drug [54,55,71] that has a broader pharmacological effect when higher doses reach the deep lung, which is the site where inflammatory processes

occur [12,50-52]. Owing to its use for > 4 decades and outstanding safety profile [5-7,75-78], DSCG is an ideal drug for the treatment of pediatric and adult asthma, particularly when potential antiviral properties could be clinically demonstrated [3,4,8,79-83]. Although pharmacokinetic data direct towards a three to four times daily administration [15,21], there is also evidence from animal [62,63] and human studies [65-67] that the pharmacological effect may not be directly linked to the plasma concentration only [15,21]. Bronchoprotective effects were observed, for example, in animals up to 72 h after allergen challenge [53-55] and in man for several hours when higher nebulized doses were administered [16,60]. Hence, a twice daily treatment in doses of 2 × 40 mg or even a once daily treatment of a single 80 mg dose with an efficient nebulizer [26,44,46,47] may be therapeutically beneficial owing to potential drug trafficking and absorption into the lung tissue and better interference with viruses and bacteria [6,7,75-84]. A nominal dose of ~ 40 mg delivered twice daily by highly efficient nebulizers, as for instance the investigational eFlow electronic nebulizer generating small droplets (MMAD ~ 3.2 µm, GSD ~ 1.6) should facilitate a uniform lung deposition of 30 – 40% with a central-to-peripheral deposition ratio of ~ 1:1 applying normal tidal breathing [26,44,46,47]. Such a lung distribution pattern may be of importance regarding the phenotype distribution of tryptase- and chymase-positive mast cells within the lungs [1,2]. Inhalation of a 40 mg dose by means of an investigational eFlow 30S may result in a lung dose of ~ 12 mg and urine excretion of ~ 5 – 6 mg/24 h, which, however, has to be proven in a pK study. A well-tolerable isotonic DSCG formulation [12,69] with a higher drug concentration (4 – 6%) would make possible a treatment in < 3 min [26,46]. A higher drug concentration at the site of inflammation could allow a better interaction with cells, mediators, biomarkers, viruses and bacteria distributed throughout the airways, and requiring higher doses for an improved efficacy [7,8,75-84]. As viruses involved in asthma [3,4] are located in the upper and lower airways oral inhalation and exhalation through the nose may be advantageous because there is a close relationship between asthma and allergic rhinitis [85]. Alternatively, inhalation via a nose mask may be beneficial for young asthmatic children and those suffering from viral bronchiolitis [83]. Hence, clinical studies are warranted to investigate the above aspects in more detail. It is in the interest of patients and especially children that a pharmacologically active and very safe drug should have a chance to be reappraised [7,8,11,75-78,83].

Declaration of interest

M Keller is the inventor of the US Patent application US 2007/0193577 A1 claiming invention of the delivery of small droplets with highly efficient nebulizers. He is also a co-inventor of the granted US patent 6,475,467 claiming invention of the use of DSCG as a drying agent to maintain the dispersion properties of drugs in MDIs.

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