A new hypothesis to investigate bioequivalence of pharmaceutical inhalation products

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Abstract

Background This short communication reports a new hypothesis regarding bioequivalence of inhalation products which can potentially provide a reliable means to compare pharmaceutical aerosol formulations and inhalers.

Methods Available methods regarding the bioequivalence studies, inhaled drugs and advantages of exhaled breath condensate (EBC) samples were reviewed to develop this hypothesis.

Results It is postulated that two inhalation products providing the same drug concentrations in airway lining fluid (ALF) could be considered bioequivalent. The use of EBC tests which reflect ALF composition can be recommended as an alternative to current testing methods for consideration of bioequivalence.

Conclusion The methods based on EBC analysis can potentially be applied to bioequivalence study of inhalation products and could reflect drug concentration in ALF. However, experimental studies would be necessary to support or refute this hypothesis on the novel application of EBC to bioequivalence in the future.

Keywords Exhaled breath condensate · Bioequivalence · Inhaled drugs · Hypothesis

Introduction

It is generally accepted that inhalation therapy is the most effective way to treat patients with chronic obstructive pulmonary disease (COPD), asthma or other lung diseases. Indeed, inhalation of relatively small drug doses not only results in a high local concentration in the airways, but also minimizes the risk for side-effects by decreasing the amount of drug which the systemic circulation receives. In view of the increasing concern about potential systemic effects of inhaled drugs, an “ideal inhaler” should: 1) contain a drug which is not absorbable from the gastrointestinal tract nor from the airways, or be inactivated or cleared from the systemic circulation as quickly and as completely as possible; and 2) produce an aerosol characterized by maximal deposition in the intended parts of respiratory tract (e.g. conducting airways or alveoli) and minimal deposition at the oropharynx [1]. Two commonly used devices employed for drug delivery in the treatment of lung diseases are included pressurized metered dose inhalers (pMDIs) and dry powder inhalers (DPIs). Compared to pMDIs, DPIs do not require coordination between inhalation

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and device actuation [2]. Age, gender, duration of disease, correct inhalation technique or type of multiple inhalers used, education of the patient, and type of inhaler used may affect the effectiveness of drugs for inhalation [3–5]. Therefore, it would be difficult to correlate the in vitro performance of an inhalation product and the in vivo activity [6].

Nowadays cost of pharmacotherapy has increased due to the expensive R & D process for new pharmaceutical products. This cost can be decreased by replacing cheaper generic copies. Therapeutically, the generic (test) copy should be equivalent to the innovator (reference) drug product. So, bioequivalence studies are performed to obtain the required information about the quality of generic brands. According to the US Food and Drug Administration (FDA), bioequivalence can be considered as: the absence of a significant difference in the rate and extent of availability of two pharmaceutical products after administration in the same molar dose under similar conditions in an appropriately designed study [7]. The principal variables for bioequivalence comparison of two oral formulations include AUC, $C_{\max}$ (maximum drug concentrations found in the plasma) and $T_{\max}$ which are the parameters of the rate and level of drug absorption and are important criteria for bioequivalence required by the regulatory bodies [8, 9]. According to these parameters (i.e. AUC, $C_{\max}$ and $T_{\max}$), bioequivalence of oral formulations could be determined whether the desired average ratios (test/reference) were fully placed in the defined acceptance range of bioequivalence or not. For example, in bioequivalence study of generics, the AUC and the maximum obtained concentration ($C_{\max}$) must be no less than 80% or no more than 125% of the reference product [10].

Although international guidelines for bioequivalence of inhalation drug products exist [11], they are not universal with major differences in the approaches between the FDA in the US and the EMA in Europe. Moreover, in vitro-in vivo correlation is not completely established for inhaled drugs [12]. Normally, bioequivalence experimental steps for two pharmaceutical products can be listed as: i) equality study of qualitative and quantitative of the active pharmaceutical ingredient and excipients, ii) in vitro study of dissolution and iii) in vivo pharmacokinetic (PK) studies. However, these steps are not suitable for inhaled drugs, due to investigation necessity of inhaled drug concentration in the lung as its action site(s). It should be noted that drug concentration in the systemic circulation does not show concentration of drug in the lung. Therefore, other sources of data and more steps are required for establishing of inhaled drug bioequivalency. These steps are: i) equality study of qualitative and quantitative of the active pharmaceutical ingredient and excipients, ii) similarity in device used by patients for obviation of the patient-device interface, iii) in vitro study of formulation performance including particle size profiling and emitted dose, iv) in vivo study of formulation performance including lung deposition and PK data and v) verification of equivalence in local efficacy [13]. PK studies for inhaled drugs

should be a single-dose study at the high level of the dosing range, and $C_{\max}$, $t_{\max}$, AUC, half-life, and elimination rate constant should be determined. Some criteria had been reported in performing the PK investigations for bioequivalence study of inhaled drugs including i) using the low dose and developing sensitive bioanalytical methods, ii) selection of healthy and non-smoking subjects for study, and iii) training of subjects for correct using of the device employed for drug delivery [14]. Moreover, pharmacodynamic (PD) investigations performed for study of clinical efficacy and safety of generic inhalational formulations should be performed in the healthy volunteers over patients to prevent probable converse influences of past or current steroid therapy, and variation in degree of the airway inflammation and obstructive impairment, which could decrease the sensitivity of the analysis [15].

Aerodynamic particle size distribution (APSD) of inhaled drugs is usually obtained by cascade impaction to predict lung deposition of different particle sizes of drugs [6]. However, the impactor systems which are widely employed do not sufficiently simulate the anatomy of the respiratory tract. Additionally, a constant flow rate used for aerosol drawn in an impactor is dissimilar to the complicate flow profiles against time during patients use of inhalers. Moreover, in contrast with the airways of the lungs, inlets of impactors have invariant diameters and lengths [16]. However, in the case of anatomical casts, inlets of impactor may improve in vitro-in vivo comparisons, but their standardization is still difficult. In comparison with a standard impactor inlet with smooth surfaces, the thoroughly elution of anatomical cast with solvent to retrieve deposited drug is considered as a problem. To resolve this problem, idealized mouth-throat geometries that closely mimic in vivo oropharyngeal deposition have been developed [17].

Cascade impaction determines the mass of drug along with specific size bands, so it is considered as a sizing method for inhalers. Laser scattering as an optical method is useful for nebulizers, in which the APSD is determined as the spray exists the nebulizer mouthpiece [18]. Furthermore, gamma scintigraphy or the charcoal block PK approach can be used to determine total lung drug deposition [19]. To obtain local lung deposition models, a three-dimensional (3D) imaging techniques such as single photon emission computed tomography (SPECT) [20] and positron emission tomography (PET) [21] may be used. Gamma scintigraphy and SPECT generally employ the radionuclide ^{99m}Tc to radiolabel particles or droplets, while in PET, it is possible to introduce a positron-emitting radionuclide such as ^{11}C into the structure of a drug molecule, so that deposition and clearance of drug can be identified [16]. No information about the depth of the deposition site within the lungs can be obtained by two-dimensional (2D) gamma scintigraphic images. Whereas, more precise information on deposition in specific lung regions can be obtained in 3D imaging methods such as SPECT [6]. However, the latter is more complex and more technically demanding

than 2D gamma scintigraphy. Validation testing of these methods includes a comparison of size distributions of drugs and radiolabel by cascade impaction. It should be demonstrated that addition of the radiolabel has not changed the nature of the drug product, and that the size distributions of drug and radiolabel are similar [22]. However, the obtained data using these methods may not be used for bioequivalence studies of inhalers and the FDA does not accept lung deposition measurements as a means of demonstrating bioequivalence. Hence, the use of principles of PKs and PDs assessment is of importance. PK studies are relatively sensitive in showing systemic exposure. In vitro assessments of emitted doses of inhalers are not necessarily predictive of PK and PD outcomes in clinical studies, PK data do not always correlate well with in vitro or clinical efficacy data, and PD data lack the precision of PK data when used to determine bioequivalence. On the other hand, plasma drug concentrations after inhalation are generally very low (often in the lower pg/mL range). Thus, for a number of drugs, PK profiles could not be generated during the drug approval process of the innovator product [16]. Although sufficient sensitive analytical techniques are available nowadays for monitoring plasma concentrations for most inhalation drugs after inhalation of therapeutically relevant doses, it remains a challenge to achieve straightforward methods for bioequivalence studies and describe the relationship between in vitro and in vivo observations.

Bioanalytical approaches employed for the quantitative measurement of drugs and their metabolite levels in biological samples play a crucial role in assessment of bioequivalence, bioavailability, and PK data. It is necessary to employ sensitive, selective and fully validated analytical methodology to obtain reliable results that can be rationally interpreted. Bioanalytical methods are continually being improved. The type of biological sample is a key determinant for the sensitivity and accuracy of a bioanalysis. Blood (serum, plasma) samples are commonly used in bioequivalence studies. The blood contents of drugs, or its related fluids, show the systemic analyte's amounts, and therefore they are widely admissible samples in the biomedical analysis. Exhaled breath (EB) or exhaled breath condensate (EBC) can be a good superseded sample type for determination of drug concentration and PK/PD studies of inhaled drugs. EBC contains tiny droplets of ALF or water vapor contained in breath which has condensed by employing a cooling collector (at sub-zero temperatures). Sampling of EBC is performed by chilling the exhaled air and collecting the resulting condensate. Three commercial available equipments for EBC sampling are i) ECoscreen (Erich Jaeger, Hoechberg, Germany), ii) RTube™ (Respiratory Research, Charlottesville, VA, USA), and iii) Turbo DECCS (ItalChil, Parma, Italy). In addition, different home-made devices have been developed in the literature [23]. The subjects breathe continuously into a mouthpiece connected to the condenser for a fixed time. Sampling of EBC is non-invasive,

easy and inexpensive; moreover, because the condensate consists primarily of water, very little sample preparation is required. EBC is a low-protein and highly diluted aqueous medium containing volatile compounds, as well as low-volatile (i.e. less volatile than water) and non-volatile compounds [24].

Studies of EBC collected via tracheostomy and through the mouth by developed EBC collection devices showed that most of the biomarkers/compounds are overplused to EBC samples from the lower airways [25]. The breath from deep within the lungs (alveolar gas) is in equilibrium with the blood; the blood is returned from the whole body tissues to the right side of heart where it is pumped to the alveolar capillaries of the lungs. During passage from the alveolar capillaries, the blood is in contact with alveolar air by a very thin layer of diffusion membrane. Volatile, low-volatile and non-volatile compounds present in blood can easily diffuse to alveolar gas or epithelial ALF. Volatile compounds exist in EBC as a result of partitioning between the alveolar gas and the blood. The mechanism of transferring of non-volatile compounds in EBC is aerosolization of the ALF due to air turbulence in the airways [26] or the sudden opening of jugated respiratory bronchioles and alveoli in the absence of bulk airflow, as supported by the bronchiole fluid film burst model proposed by Johnson and Morawska [27]. Therefore, the concentrations of molecules present in breath could be correlated with those found in the blood. Recently, attention has been drawn to EBC based analyses as a noninvasive approach for surveying the composition of ALF [20]. As EBC sampling is completely noninvasive, it has advantages in compared with sputum or lavage fluid for pharmacokinetics studies of respiratory drugs and is appropriate sample for longitudinal investigations and for monitoring the response to the pharmacological therapy [28]. The lung concentration of an inhaled drug may be many times higher than the blood concentration depending on the solubility, permeability and clearance of the drug as well as sampling time after inhalation. As a result, the EBC may reflect better the inhaled drug concentration in the lungs than in the blood. Numerous different methods for EBC collection and preconcentration and quantification of analytes have been reported including UV detection, fluorescence detection, mass spectrometry, electrochemical detection, antibody-based assays, gas chromatography in combination with capillary electrophoresis, mass spectrometry, and some new techniques. These techniques have been employed in the study of several different respiratory diseases and exposure studies such as exposure to air pollution [29]. Multivariate techniques such as nuclear magnetic resonance (NMR)-based metabolomic analyses are often employed to discriminate between patient subjects.

EBC analysis provides a facile, diagnostic and non-invasive method for investigation of ALF composition and lung inflammation, disease severity, exacerbations, and efficacy of treatment process. Recent studies presented importance of EBC analysis in

various clinical applications [30]. The possibility of employing selective sensors to determine specific inflammatory mediators in EBC is also under studying [31]. Furthermore, breath tests have been used to detect drugs of abuse including those commonly administered by inhalation such as cannabis (1(6aR, 10aR)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo [c] chromen-1-ol) [32]. After delivery of a nebulized drug in a sheep model, Esther et al. [28] demonstrated the feasibility of measuring the drug and its metabolites in EBC, thus allowing airway pharmacokinetic study of inhaled drugs. Importantly, the concentrations of the drug and its metabolites in EBC were 1 to 2 orders of magnitudes higher than that in the serum, indicating EBC enables quantification inhaled drug which would otherwise be difficult or not possible. However, to date, drug concentrations in EBC have not been used for bioequivalence studies. We hypothesize that if two inhalers of a drug provide similar EBC concentrations, they could be considered as bioequivalent. This simple, easy and low cost procedure can potentially provide an innovative approach to reliably determine the lung dose to achieve the goal. A major challenge in adopting this EBC-based approach is to establish a correlation and/or database to differentiate (i) between total and regional drug doses in the lungs and EBC, and (ii) between contributions of the deposited drug in the airways and the drug from the blood

compartment into the exhaled breath. While the former is yet to be established, the latter can be achieved by having the EBC collected soon after inhaled dosing when lung absorption has not occurred to a significant extent yet e.g. fluticasone propionate (with chemical name of S-(fluoromethyl) 6 α ,9-difluoro-11 β ,17-dihydroxy-16 α -methyl-3-oxoandrosta-1,4-diene-17 β -carbothioate, 17-propionate) administered by Diskus powder inhaler, mean T_{max} 79 min in patients with asthma [33]. For drugs that are rapidly absorbed (peaked within minutes) from the lungs e.g. glycopyrronium bromide (with chemical name of 3-[2-cyclopentyl(hydroxy)phenylacetoxy]-1,1-dimethylpyrrolidinium bromide) administered by metered-dose inhaler, mean T_{max} 4.8 min in COPD patients [34], the best timing to collect the EBC needs to be determined.

Significance and innovation

The common method for bioequivalence of oral formulations is analysing the serum concentration of the drug over time to obtain the PK parameters. In the case of inhalers, the lung is the main target of the inhaled drug and drug absorption into systemic circulation could be considered as a disadvantage. The main obstacle in pulmonary drug delivery is unsuitable particle size distribution of drugs resulting in either deposition

Fig. 1 Schematic representation of quality of formulations to efficient delivery of a drug into lung and its impact on profile of EBC concentration versus time

Table 1 List of some drugs analysed in EB/EBC samples

Drug	Chemical name	Breath collection device	Applied method	Detection range	Ref.
Alprazolam	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine	Sampling device holding a polymer filter	LC-MS/MS	4–53 pg/sample	[32]
Amphetamine	1-Phenylpropan-2-amine	Sampling device holding a polymer filter	LC-MS/MS	20–4700 pg/sample	[32]
Amphetamine	1-Phenylpropan-2-amine	SPEC DAS cartridge	LC-MS/MS	0.2–102 pg min ⁻¹	[36]
Amphetamine	1-Phenylpropan-2-amine	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	90–3280 pg min ⁻¹	[37]
Buprenorphine	(1S,2S,6R,14R,15R,16R)-5-(Cyclopropylmethyl)-16-[[2S)-2-hydroxy-3,3-dimethylbutan-2-yl]-15-methoxy-13-oxa-5-azahexacyclo[3.2.2.1 ⁰ .2.2.1 ⁰ .1 ⁰ .1 ⁰]-11-ol	Sampling device holding a polymer filter	LC-MS/MS	114–567 pg/sample	[32]
Buprenorphine	(1S,2S,6R,14R,15R,16R)-5-(Cyclopropylmethyl)-16-[[2S)-2-hydroxy-3,3-dimethylbutan-2-yl]-15-methoxy-13-oxa-5-azahexacyclo[3.2.2.1 ⁰ .2.2.1 ⁰ .1 ⁰ .1 ⁰]-11-ol	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	1.8–41 pg min ⁻¹	[37]
Cocaine	Methyl (1R,2R,3S,5S)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate	Sampling device holding a polymer filter	LC-MS/MS	29–13,000 pg/sample	[32]
Cocaine	Methyl (1R,2R,3S,5S)-3-benzoyloxy-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	80 pg min ⁻¹	[37]
Diazepam	7-Chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2-one	Sampling device holding a polymer filter	LC-MS/MS	2–145 pg/sample	[32]
Hexafluoroisopropanol	1,1,1,3,3,3-Hexafluoropropan-2-ol	Disposable bags wrapped and tightened around a PTFE tube connected to a stopcock, a one-way valve, and a mouthpiece.	GC/MS	0.001–0.267 ppmv	[38]
Metadone	6-(Dimethylamino)-4,4-diphenylheptan-3-one	EcoScreen condenser	LC-MS/MS	23.6–275 pg min ⁻¹	[39]
Metadone	6-(Dimethylamino)-4,4-diphenylheptan-3-one	Sampling device holding a polymer filter	LC-MS/MS	58–2421 pg/sample	[32]
Metadone	6-(Dimethylamino)-4,4-diphenylheptan-3-one	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	4.8–29,800 pg min ⁻¹	[37]
Metadone	6-(Dimethylamino)-4,4-diphenylheptan-3-one	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	41–7188 pg min ⁻¹	[40]
Metadone	6-(Dimethylamino)-4,4-diphenylheptan-3-one	Home-made cooling trap system	HPLC-UV	0.34–1.31 µg L ⁻¹	[41]
Methamphetamine	(2S)-N-Methyl-1-phenylpropan-2-amine	Sampling device holding a polymer filter	LC-MS/MS	30–790 pg/sample	[32]
Methamphetamine	(2S)-N-Methyl-1-phenylpropan-2-amine	SPEC DAS cartridge	LC-MS/MS	0.3–139 pg min ⁻¹	[36]
Methamphetamine	(2S)-N-Methyl-1-phenylpropan-2-amine	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	11.6–3780 pg min ⁻¹	[37]
Morphine	(4R,4aR,7S,7aR,12bS)-3-Methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7,9-diol	Sampling device holding a polymer filter	LC-MS/MS	25–4650 pg/sample	[32]
Morphine	(4R,4aR,7S,7aR,12bS)-3-Methyl-2,4,4a,7,7a,13-hexahydro-1H-4,12-methanobenzofuro[3,2-e]isoquinoline-7,9-diol	Sampling device holding a 47-mm Empore C ₁₈ filter	LC-MS-MS	15.1 pg min ⁻¹	[37]
O-desmethyltramadol	3-[(1R,2R)-2-[(Dimethylamino)methyl]-1-hydroxycyclohexyl]-phenol	Polymer filter inside Plastic bag	LC-MS/MS	30 pg/filter	[42]
Oxazepam	7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one	Sampling device holding a polymer filter	LC-MS/MS	60–84 pg/sample	[32]
Propofol	2,6-Di(isopropyl)phenol	Home-made setup (Face mask attached to gas-tight syringe and a glass collector placed in ice)	HS-SPME-GC-MS	0.04–0.53 nmol L ⁻¹	[43]
Sevoflurane	1,1,1,3,3,3-Hexafluoro-2-(fluoromethoxy)propane		GC/MS	0.4–74.7 ppmv	[38]

Table 1 (continued)

Drug	Chemical name	Breath collection device	Applied method	Detection range	Ref.
Δ9-Tetrahydrocannabinol	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[c]chromen-1-ol	Disposable bags wrapped and tightened around a PTFE tube connected to a stopcock, a one-way valve, and a mouthpiece	LC-MS/MS	8–332 pg/sample	[32]
Δ9-Tetrahydrocannabinol	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[c]chromen-1-ol	Sampling device holding a polymer filter	LC-MS-MS	2.5–77.3 pg min ⁻¹	[44]
Δ9-Tetrahydrocannabinol	(6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydrobenzo[c]chromen-1-ol	Sampling device holding a 47-mm Empore C ₁₈ filter	GC/MS	0.21–5.7 ng L ⁻¹	[45]
Tramadol	(1R,2R)-2-[(Dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol	Tenax-GC 60/80 mesh with Linde molecular sieve and thermal desorption Polymer filter inside plastic bag	LC-MS/MS	675 pg/filter	[42]

HPLC, High-performance liquid chromatography; *CE-DAD*, Capillary electrophoresis with diode array detector; *LC-MS/MS*, Liquid chromatography tandem mass chromatography; *GC/MS*, Gas chromatography–mass spectrometry; *HS-SPME-GC-MS*, Headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry; *NPs*, Nanoparticles; *PTFE*, Polytetrafluoroethylene

of drug particles in upper region of the airways (for large particles) or exhalation of very fine particles. In general, particles with aerodynamic size 1–5 μm are considered suitable for delivery to the lungs, although the specific particle size will also depend on the inspiratory air flow rate and breath-holding maneuver. Figure 1 illustrated a schematic representation of the main idea of using EBC concentration in evaluating bioequivalence of inhaled formulations. After deposition of a drug in the respiratory tract, it could be eliminated via different mechanisms: 1) distribution into the systemic blood, 2) metabolism in the lung tissue and 3) mucociliary clearance and 4) macrophage uptake. In addition, exhalation can aerosolize very small droplets of drug-containing lung lining fluids due to re-opening of the collapsed alveoli.

In addition to the commercially available devices, home-made setups could be developed and validated to trap these aerosolized particles [35] which have been used to monitor the concentration of a number of drugs administered via oral and/or lung and the obtained results are promising (Table 1) [32, 36–45]. Research on EBC has been rapidly expanding over the past 40 years. EBC tests have been developed for detection and monitoring of diseases [46]. More recent research has branched into a new horizon of measuring drugs (including inhaled drugs) and their metabolites in the ALF [28, 32]. Since the use of EBC for inhaler bioequivalence study is novel, we believe it has sufficient merits to be tested in practice to provide the required evidences for moving this innovative approach forward.

Conclusion

The authors proposed that if two inhaled formulations of a given drug provide similar EBC concentrations, they could be considered as bioequivalent. Hence, the methods based on EBC analysis can potentially be applied to bioequivalence study of inhalation products, and allow rapid, non-invasive and reliable determination of drug concentrations in the lungs with a higher throughput than current methods. The proposed novel method of testing bioequivalence would require future studies to validate.

Compliance with ethical standards

Competing interest Authors declare that they have no financial conflict of interest.

Patent: A Jouyban, M Khoubnasabjafari, K Ansarin and V Jouyban-Gharamaleki. Breath sampling setup. Iranian Patent, 81,363, 2013.

This work was approved by the Ethics Committee of Tabriz University of Medical Sciences (approved No. IR TBZMED.REC.1397.695).

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