Evaluation of the AERx Pulmonary Delivery System for Systemic Delivery of a Poorly Soluble Selective D-1 Agonist, ABT-431

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Purpose. ABT-431 is a chemically stable, poorly soluble prodrug that rapidly converts *in vivo* to A-86929, a selective dopamine D-1 receptor agonist. This study was designed to evaluate the ability of the AERx™ pulmonary delivery system to deliver ABT-431 to the systemic circulation via the lung.

Methods. A 60% ethanol formulation of 50 mg/mL ABT-431 was used to prepare unit dosage forms containing 40 μL of formulation. The AERx system was used to generate a fine aerosol bolus from each unit dose that was collected either onto a filter assembly to chemically assay for the emitted dose or in an Andersen cascade impactor for particle size analysis. Plasma samples were obtained for pharmacokinetic analysis after pulmonary delivery and IV dosing of ABT-431 to nine healthy male volunteers. Doses from the AERx system were delivered as a bolus inhalation(s) (1, 2, 4, and 8 mg) and intravenous infusions were given over 1hr (5 mg). Pharmacokinetic parameters of A-86929 were estimated using noncompartmental analysis.

Results. The emitted dose was 1.02 mg (%RSD = 11.0, n = 48). The mass median aerodynamic diameter of the aerosol was 2.9 ± 0.1 μm with a geometric standard deviation of 1.3 ± 0.1 (n = 15). T_{max} (mean ± SD) after inhalation ranged from 0.9 ± 0.6 to 11.5 ± 2.5. The mean absolute pulmonary bioavailibility (as A-86929) based on emitted dose ranged from 81.9% to 107.4%.

Conclusions. This study demonstrated that the AERx pulmonary delivery system is capable of reproducibly generating fine nearly monodisperse aerosols of a small organic molecule. Aerosol inhalation utilizing the AERx pulmonary delivery system may be an efficient means for systemic delivery of small organic molecules such as ABT-431.

KEY WORDS: AERx; dopamine D-1; ABT-431; DAS-431; pulmonary delivery; non-invasive delivery.

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INTRODUCTION

ABT-431 (all rights on ABT-431 have been licensed to Drug Abuse Sciences, Inc. Hayward, California, which will develop the drug under the designation DAS-431) is a diacetyl prodrug of A-86929, a potent and selective agonist to the dopamine receptor D1 and has demonstrated efficacy for treatment of Parkinson's disease (Fig. 1) (1–3). The conversion half-life of ABT-431 to A-86929 *in vivo* is estimated to be less than 2 min based on the appearance of A-86929 in serum. When administered to humans orally ABT-431 was extensively cleared by first pass metabolism leading to an oral bioavilibility of less than 4% (4). Pulmonary administration of small organic molecules such as ABT-431 has advantages over conventional delivery routes: rapid absorption into systemic circulation and significantly lower first pass metabolism (5). For these reasons ABT-431 was a prime candidate for systemic delivery via the AERx pulmonary delivery system (6). This delivery system, which provides the advantage of active breath control, has been shown to be well suited for precise delivery of systemically active drugs via pulmonary administration (7–10). This study was undertaken to provide information on the feasibility of formulating and delivering ethanolic solutions of poorly soluble drugs to the lung. The specific objectives of this study were: (i) to develop and evaluate a clinical formulation of ABT-431; (ii) to investigate the pharmacokinetics of ABT-431 after pulmonary and intravenous delivery to healthy male volunteers.

MATERIALS AND METHODS

Materials and Equipment

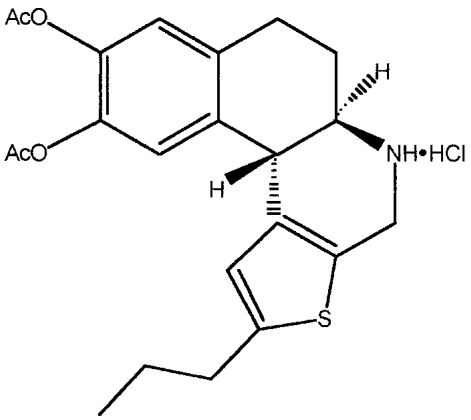
The following materials were used for this study: ABT-431 (Pharmaceutical Products Division, Abbott Laboratories, North Chicago, Illinois); Alcohol, Dehydrated USP, 200 Proof (Spectrum Chemical Co, Gardena, California); Sterile Water for Injection, USP (Fischer Scientific, Hanover Park, Illinois). All other chemicals and reagents were HPLC or reagent grade.

Formulation and Manufacturing

A liquid formulation of ABT-431 was prepared by dissolving ABT-431 (50 mg/mL) in a 60:40 ethanol:water solution at room temperature. Each AERx unit-dosage form was filled with 40 μL of this liquid formulation and individually sealed. ABT-431 AERx unit-dosage forms containing 2.0 mg of ABT-431 were packaged and stored at 5 ± 3°C prior to use in the AERx device. Protocol requirements for this clinical study required AERx dosage forms not be used beyond 12 h after manufacture, therefore, 24 h of functional stability was sufficient to support the clinical formulation.

Determination of Emitted Dose and Emitted Dose Uniformity

The emitted dose (ED) and emitted dose uniformity (EDU) for each lot were determined as described by USP <601> (US Pharmacopoeia, 2000). Briefly, ten AERx dosage forms were individually loaded into the AERx device and aerosolized into the aerosol collection system. The aerosol collection system consisted of a Teflon filter holder (Savillex,

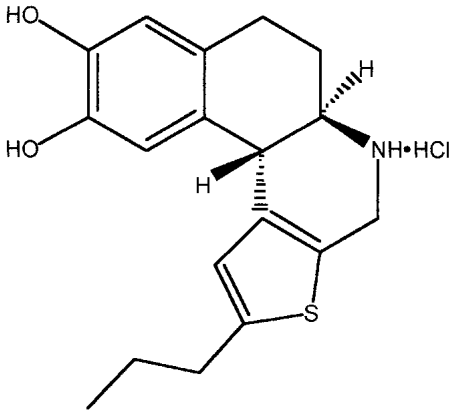


ABT-431

(-)-trans 9,10-acetoxy-2-propyl-4,5,5a,6,7,11

b-hexahydro-3-thia-5-azacyclopent-

1-ena[c]phenanthrene hydrochloride



A-86929

(-)-trans 9,10-hydroxy-2-propyl-4,5,5a,6,7,11b-hexahydro-3-

thia-5-azacyclopent-1-ena[c]phenanthrene hydrochloride

Fig. 1. Chemical structure of ABT-431 and A-86929.

catalogue no. 4750476) into which was secured a 47 mm glass fiber filter (Gelman A/E). The filter holder was then joined via a customized Delrin adapter to the male neck of a glass throat such as that used as the inlet port in the USP <601> Aerosols for Apparatus 2 (Single Stage Impactor). The entire aerosol collection apparatus was then positioned so that the emitted aerosol from the AERx device was directed into the center of the glass throat. Using a vacuum pump, air was drawn through the apparatus at an inlet flow equivalent to 70 L/min. This flow rate was programmed into the active breath control system and is required for efficient aerosol generation by this configuration of the AERx device. The AERx device was actuated and the pump turned off 10 s later (the aerosol cloud was generated over an approximate period of 1.4 s). The various parts of the AERx device and aerosol collection apparatus were then disassembled and quantitatively washed with 50:50 acetonitrile-water to remove any deposited ABT-431 aerosol. The amount of ABT-431 at each location was determined by HPLC. The emitted dose (ED) was calculated from the sum of ABT-431 retained in the glass throat and filter divided by the original amount contained in the AERx dosage-form. The emitted dose uniformity (EDU) was calculated using the following formula ($EDU = ED_{individual} \times ED_{mean} \times \%$).

Particle Size Distribution Analysis

Cascade impaction analysis was used to determine the aerodynamic particle size distribution of ABT-431 aerosols emitted from the AERx device. This test utilized a modified Andersen sampler (model no. 10-709) in which the pre-separator is fitted atop of stage 0 of the sampler as previously described by Schuster *et al.* (6). Briefly, for each test, one AERx dosage-form containing ABT-431 was aerosolized into the assembled cascade impactor while operating at 70 L/min. The AERx dosage form, AERx device, each collection stage, and the final filter (Whatman GF/F glass microfiber filters) were quantitatively rinsed with 50:50 acetonitrile-water prior to HPLC analysis. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosol collected in the cascade impactor was then estimated using least squares non-linear regression analysis for the plot of particle size vs. cumulative % undersize. The respirable dose was determined from the fraction of aerosolized ABT-431 collected on the stages of the Andersen impactor representing particles of aerodynamic diameter <5.7 μm and is represented as a percent of the measured emitted dose.

Analytical HPLC Assay for ABT-431

ABT-431 was analyzed using a reverse phase HPLC method, based on a C8 column (Zorbax, Rx-C8 column, 4.6 mm × 250 mm, 5 μm particle size). The mobile phase consisted of 0.2% perchloric acid mixed with acetonitrile (55:45 v/v). Elution of the HPLC column was performed at 1.0 mL/min with the perchloric acid:acetonitrile mobile phase. The column eluent was monitored by UV absorbance at 230 nm. The concentration of ABT-431 in analytical samples was calculated by peak area based on a standard curve (0.2–20 μg/mL ABT-431) prepared from a stock 1.0 mg/mL solution.

Clinical Studies

A double blind placebo controlled single center sequential dose study was conducted with 12 healthy adult male subjects. Subjects were randomized to receive AERx ABT-431 ($n = 10$) or placebo ($n = 2$) on five consecutive days. The study consisted of a single period dose escalation study where drug was administered by oral inhalation for 4 days followed by 60 min intravenous infusion (IV) on day 6. Each dose was administered on the morning of each of the 5 study days. The following doses were given by oral inhalation (AERx programmed to deliver dose at inspiratory flow rate of 70 ± 5 L/min): One inhalation (1.02 mg) on day 1, two inhalations (2.04 mg) on day 2, four inhalations (4.08 mg) on day 3, eight inhalations (8.16 mg) on day 4, respectively. Blood samples were collected in EDTA containing tubes at specific time intervals following drug administration for determination of plasma A-86929 concentrations.

Table I. Emitted Dose Emitted Dose Uniformity, and Particle Size Distribution, Mean (RSD)

Batch	ED[a] (%)	EDU[b] (%)	MMAD[c] (μm)	GSD[d] (h)
Lot#AB970228	52.2 (9.4)	85.2–113.6	2.9	1.3
Lot#AB970303	50.5 (8.9)	85.0–110.7	3.0	1.3
Lot#AB970305	49.4 (14.8)	71.1–116.0	2.9	1.3

[a] Represented as a percent of the nominal loaded dose (NLD) of A-86929 (100% NLD = 2.0 mg A-86929).
[b] Reported as range of individual values expressed as the percentage of the lot mean.
[c] Mass median aerodynamic diameter (n = 5).
[d] Geometric standard deviation.

Blood samples were stored on ice prior to plasma harvest by centrifugation. Plasma A-86929 concentrations were determined using an HPLC method with electrochemical detection as previously described (4). The pharmacokinetic parameters of A-86929 after single oral inhalation and intravenous dose of ABT-431 were estimated using standard noncompartmental methods. The peak plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were taken directly from the plasma concentration-time data. The terminal rate constant (β) was determined by linear regression of the natural log-transformed plasma concentration in the terminal phases. The AUC from time zero to the last concentration measurement (AUC_t) was calculated by the linear trapezoid rule using the measured concentration data. The extrapolated AUC from t to infinity ($AUC_{t-\infty}$) was estimated by dividing the last measurable plasma concentration (C_{lsat}) by β. The AUC from time zero to infinity (AUC_∞) was estimated by adding $AUC_{t-\infty}$ to AUC_t. This research adhered to the tenets of the Declaration of Helsinki promulgated in 1964 and was approved by the institutional human experimentation committee, and subjects informed consent was obtained.

RESULTS AND DISCUSSION

Emitted Dose and Emitted Dose Uniformity

Results for three lots of AERx ABT-431 did not show any significant differences in ED at the initial testing point (ANOVA; $P = 0.5421$) Table I. The global mean ED at the initial testing point was 50.70%, (RSD = 11%). This indicates consistent ED performance across lots of AERx ABT-431. The dosage forms were also stored for 24 h at 5 ± 3°C and tested for ED and EDU. The global mean (±RSD) ED was 51% (9%). Furthermore the global EDU for all three lots was 71.1–116.0%. These experiments demonstrated satisfactory *in vitro* ED performance over the period tested.

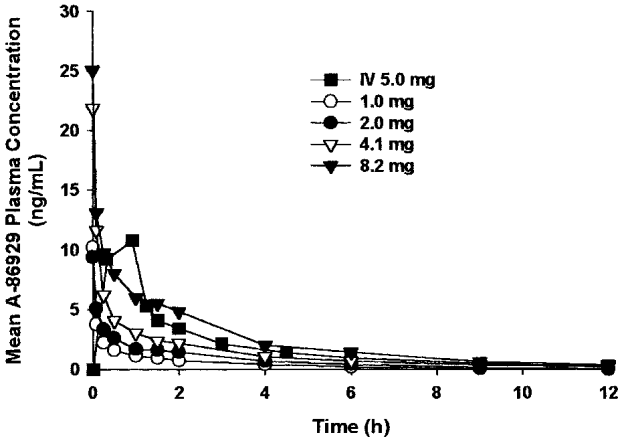


Fig. 2. Mean A-86929 plasma concentration-time profiles following oral inhalations and intravenous infusion of ABT-431.

Particle Size Distribution

Results from the particle size analysis were highly reproducible for the three lots of AERx ABT-431 at all testing times. The global mean MMAD was 2.9 μm (RSD = 4.2%; range, 2.5–3.1 μm) and the global mean GSD was 1.3 (RSD = 2.5%, range 1.2–1.4) (Table I). Based on these results 98% of the AERx ABT-431 ED contained respirable particles (defined as particles ≤5.7 μm). Excellent correlation has been shown between *in vitro* particle size analysis and *in vivo* deep lung deposition for aerosols generated by the AERx pulmonary delivery system therefore this value (~50% of nominal dose) was used to predict the lung dose subjects would receive per inhalation (11,12).

Table II. Noncompartmental Pharmacokinetic Parameters and Bioavailability (F) for A-86929 in Humans (mean ± SD)

Route	Dose[a] (mg)	n[b]	T_{max} (min)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng*h/mL)	$T_{1/2}$[c] (h)	F[d] (%)
1 Inhalation	1.02	9	0.90 ± 0.60	10.21 ± 6.16	5.69 ± 3.23	2.1 ± 0.3	107.4
2 Inhalation	2.04	9	2.88 ± 2.16	9.89 ± 8.20	9.84 ± 3.40	2.5 ± 0.3	92.8
4 Inhalation	4.08	9	4.56 ± 0.72	21.89 ± 8.30	17.49 ± 5.01	3.0 ± 0.4	82.5
8 Inhalation	8.16	7	11.58 ± 2.46	25.07 ± 16.67	33.31 ± 17.30	2.9 ± 0.5	81.9
IV	5.00	9	51.00 ± 11.40	12.05 ± 5.20	25.98 ± 5.20	3.0 ± 0.4	

[a] Delivered (emitted) dose with the nominal loaded dose in parentheses.
[b] Includes all subjects receiving the full dose within each inhalation treatment and the intravenous dose.
[c] Harmonic mean and pseudo-standard deviation.
[d] Calculated as ratio of dose-normalized means (AUC inhalation/AUC IV), based on delivered (emitted) dose.

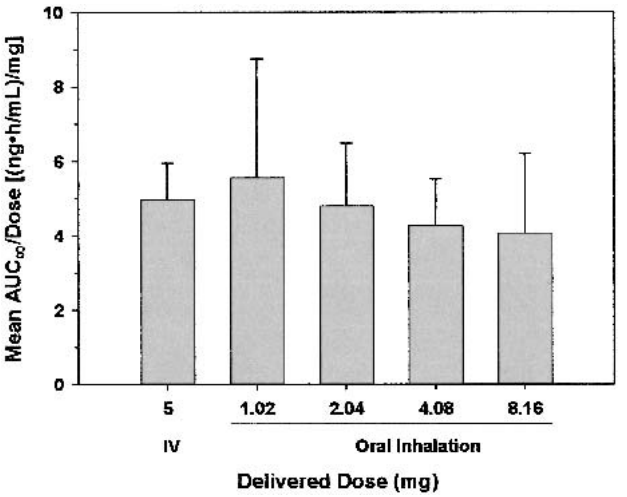


Fig. 3. Mean (SD) A-86929 dose-normalized AUCs following oral inhalations and intravenous infusion of ABT-431.

In Vivo Pharmacokinetics of AERx ABT-431

Plasma concentration time profiles for ABT-431 following oral inhalation of rising doses and IV administration in humans are shown in Fig. 2 with the corresponding pharmacokinetic parameters tabulated in Table II. All data shown are for subjects receiving the full dose within each inhalation treatment and the intravenous dose. Time to reach peak plasma A-86929 concentrations is rather short (0.90–11.58 min) for all inhaled doses. This early T_{max} indicates that absorption of ABT-431 from the lung was rapid and complete. The increasing trend observed in T_{max} after multiple inhalations, when compared to one inhalation, is likely due to the increased amount of time required (~45 s per inhalation) to deliver 8 inhalations (8.16 mg dose) vs. 1 inhalation (1.02 mg dose). The mean plasma AUC values increase with increasing dose from 5.69 ± 3.23 ng*h/mL for one inhalation to 33.31 ± 17.30 ng*h/mL for eight inhalations. An approximately linear dose response relationship (based on AUCs) was observed with inhaled ABT-431 for the dose range of 1.02–8.16 mg. There was a slight reduction of the bioavailability as the number of inhalations increased from one to eight (Fig. 3). This decrease in bioavialability could be due to a decrease in the soluble fraction of ABT-431 present at the epithelial surface after inhalation of higher doses of this moderately soluble compound. A similar decrease in bioavailibility was observed for higher doses of ABT-431 delivered by a propellant-driven metered dose inhaler (4).

CONCLUSIONS

This study indicates that a high concentration ethanolic formulation of ABT-431 can be used in conjunction with the AERx pulmonary delivery system to reproducibly deliver respirable aerosols where ~50% of the nominal (loaded) dose reached systemic circulation. Aerosol inhalation utilizing the AERx pulmonary delivery system may be an efficient means for systemic delivery of poorly soluble molecules.

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REFERENCES

1. O. Rascol, O. Blin, C. Thalamas, S. Descombes, C. Soubrouillard, P. Azulay, N. Fabre, F. Viallet, K. Lafnitzegger, S. Wright. J. H. Carter, and J. G. Nutt. ABT-431, a D1 receptor agonist prodrug, has efficacy in Parkinson's disease. *Ann. Neuro.* **45**:736–741 (1999).
2. O. Rascol, J. G. Nutt, O. Blin, C. G. Goetz, J. M. Trugman, C. Soubrouillard, J. H. Carter, L. J. Currie, N. Fabre, C. Thalamas, W. J. Giardina, and S. Wright. Induction by dopamine D-1 receptor agonist ABT-431 of dyskinesia similar to levodopa in patients with Parkinson disease. *Archiv. Neurol.* **58**:249–254 (2001).
3. K. Shiosaki, P. Jenner, K. E. Asin, D. R. Britton, C. W. Lin, M. Michaelides, L. Smith, B. Bianchi, S. Didomenico, L. Hodges, Y. F. Hong, L. Mahan, J. Mikusa, T. Miller, A. Nikkel, M. Stashko, D. Witte, and M. Williams. ABT-431-the diacetyl prodrug of A-86929, a potent selective dopamine D1 receptor agonist: in vitro characterisation and effects in animal models of Parkinson's disease. *J.Pharm. Exp.Ther.* **276**:150–160 (1996).
4. Y. Zheng, K. C. Marsh, R. J. Bertz, T. El-Shourbagy, and A. L. Adjei. Pulmonary delivery of a dopamine D-1 agonist, ABT-431, in dogs and humans. *Int. J. Pharm.* **191**:131–140 (1999).
5. I. Gonda. The ascent of pulmonary drug delivery. *J. Pharm. Sci.* **89**:940–945 (2000).
6. J. A. Schuster, R. Rubsamen, P. Lloyd, and J. Lloyd. The AERx aerosol delivery system. *Pharm. Res.* **14**:354–357 (1997).
7. M. E. Ward, A. Woodhouse, L. E. Mather, S. J. Farr, J. K. Okikawa, P. Lloyd, J. A. Schuster, and R. M. Rubsamen. Morphine pharmacokinetics after pulmonary administration from a novel aerosol delivery system. *Clin. Pharm. Ther.* **62**:596–609 (1997).
8. S. J. Farr, S. J. Warren, P. Lloyd, J. K. Okikawa, J. A. Schuster, A. M. Rowe, R. M. Rubsamen, and G. Taylor. Comparison of in vitro and in vivo efficiencies of a novel unit-dose liquid aerosol generator and a pressurized metered dose inhaler. *Int. J. Pharm.* **198**:63–70 (2000).
9. M. Dershwitz, J. L. Walsh, R. J. Morishige, P. M. Conners, R. M. Rubsamen, S. L. Shafer, and C. E. Rosow. Pharmacokinetics and pharmacodynamics of inhaled vs. intravenous morphine in healthy volunteers. *Anesthesiology* **93**:619–628 (2000).
10. G. A. Brunner, B. Balent, M. Ellmerer , L. Schaupp, A. Siebenhofer, J. H. Jendle, J. K. Okikawa, and T. R. Pieber. Dose-response relation of liquid aerosol inhaled insulin in type I diabetic patients. *Diabetologia* **44**:305–308 (2001).
11. I. Gonda, J. A. Schuster, R. Rubsamen, P. Lloyd, D. Cipolla, and S. J. Farr. Inhalation delivery systems with compliance and disease management capabilities. *J. Control. Release* **53**:269–274 (1998).
12. A. V. Annapragada. Biophysics of inhaled drug particles. In A. L. Adjei and P. K. Gupta (eds), *Inhalation Delivery of Therapeutic Peptides and Proteins* Vol. 107, *Lung Biology in Health and Disease* Marcel Dekker, Inc., New York, 1997 pp. 1–22.