Comparative pharmacokinetics of Zileuton's active pharmaceutical ingredient, nanocrystal-drug, and physical mixture in male and female Sprague Dawley rats

Chandra Mohan Reddy Muthumula[1], Bhagya Wickramaratne[1], Sangeeta Khare[1], Angela Lee[1], Sushanta Chakder[2], and Kuppan Gokulan[1]

[1]Division of Microbiology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, AR, 72079

[2]Center for Drug Evaluation Research, US Food and Drug Administration, White oak, Silver Spring, MD, 20993

Synopsis

We compared the oral pharmacokinetics of zileuton's nanocrystal-drug (NDZ) formulation with the micron-sized active pharmaceutical ingredient (API). Additionally, gender-dependent changes following administration of API, ND, and physical mixture was assessed in 10-week-old rats using gelatin capsules. This study demonstrated the differences between male and female rat's pharmacokinetic responses to zileuton's NDZ & API.

Abstract

The biopharmaceutical classification system (BCS) has been used as a prognostic tool to determine the bioavailability of an oral drug based on its solubility and permeability properties. Zileuton, a 5-lipoxygenase inhibitor used to treat chronic asthma, is classified as BCS class II drug due to its poor solubility and high permeability. To address solubility issues and improve bioavailability of a BCS class II drug, Zileuton was used as a model drug to develop a nanocrystal-drug formulation. In this study we evaluated the sex dependent changes in the pharmacokinetic properties of Zileuton in 10 weeks old male and female Sprague Dawley rats following oral administration of active pharmaceutical ingredient (API; 30 mg/kg bw), nanocrystal-drug formulation (NDZ; 30 mg contains 7.5 mg of API) and physical mixture (PM; 30 mg contains 7.5 mg of API) using gelatin capsules. A reproducible HPLC technique was developed to determine the concentration of Zileuton in the ileum (15 days post treatment), plasma (1, 2, 4, 6, and 24h post treatment), and urine (24h post treatment) samples. The results of this study showed that female rats had lower Zileuton concentrations in the ileum after oral administration of the API and nanodrug formulation than male rats. Following treatment with API, NDZ, and PM, plasma Zileuton concentrations were found to be higher in female rats than in male rats. The lower plasma Zileuton concentration in the male rats can be correlated to the higher expression of phase-1 and phase-II metabolic enzymes in the intestinal tissue. The Tmax of Zileuton in the male rats was found to be 2h after NDZ and PM treatment, compared to 1h after API treatment. In the female rats, the Tmax of Zileuton was 1h following administration of NDZ and PM, and 2h following administration of API. Following administration of NDZ and PM, male rats had a higher concentration of Zileuton in their urine at 24h than female rats. Overall, pharmacokinetic variations of Zileuton were observed between male and female rats following oral administration of API, NDZ, and PM. This study justifies further investigation of the NDZ in the translational model of asthma.

Introduction

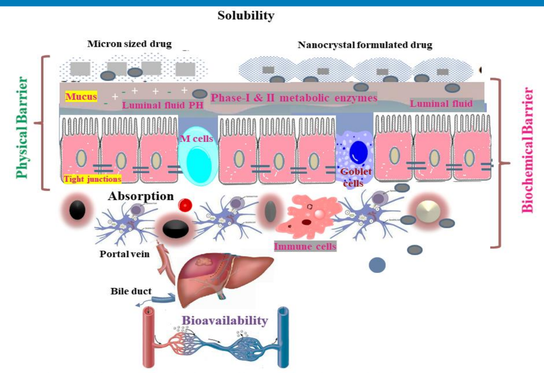


Figure 1: Physical and Biochemical barriers in the body affecting drug bioavailability.

Pharmacokinetics, the study of how drugs are absorbed, distributed, metabolized and eliminated through the body, plays a crucial role in determining their safety and efficacy. However, drug delivery to target tissues and organs is limited by several physical and biochemical barriers in the body as well as the properties of the drug molecules. Therefore, drug formulations such as nanocrystals have been developed to improve the solubility, bioavailability and drug delivery.

Materials and Methods

Zileuton, a 5-lipoxygenase inhibitor used to treat chronic asthma, is classified as BCS class II drug due to its poor solubility and high permeability. To address solubility issues and improve bioavailability of a BCS class II drug, Zileuton was used as a model drug to develop a nanocrystal-drug (ND) formulation. In this study we aim to compare the pharmacokinetics of zileuton's active pharmaceutical ingredient (Micron-sized), nano-crystal drug formulation and physical mixture in male and female Sprague Dawley rats. This study will provide insights into the impact of these formulations on drug absorption, distribution, metabolism and elimination, and the influence of gender on zileuton's pharmacokinetic parameters thereby providing information related to potential effectiveness as treatments for asthma and other related conditions.

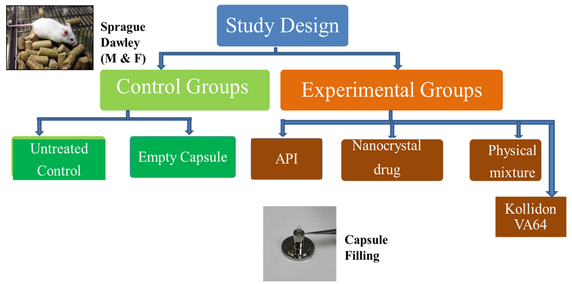


Figure 2: Schematic representation of the pharmacokinetic study design.

To evaluate the pharmacokinetics of the test agents, Sprague Dawley rats (10 weeks old male and female) were dosed with gelatin capsules containing the test agents, as shown in the schematic diagram, via oral gavage.

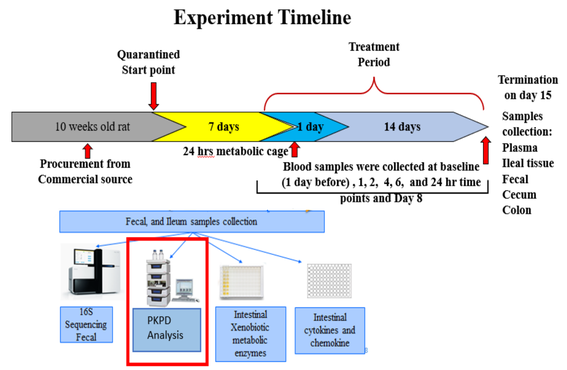


Figure 3: Experimental scheme representing treatment period, sample collection and analysis.

The rats were quarantined in a metabolic cage for the first 24 hours, at which time we collected blood plasma and urine samples from each group; after the 24 hours, we housed the rats in separate gender and experimental groups, and continued drug treatment for 15 days. On day 15 animals were sacrificed and samples (plasma, fecal, and colon) were collected for various biochemical, immunological, and PK/PD endpoint analysis.

Results and Discussion

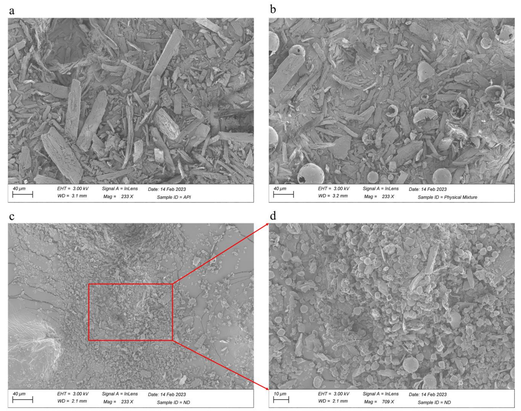


Figure 4: Scanning Electron Microscopic images of a) Micron sized Zileuton API, b) Physical mixture of zileuton c &d) Nano-crystal zileuton formulation.

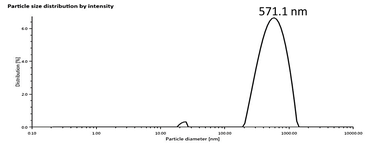


Figure 5: Particle size distribution analysis (using DLS) of ND formulation.

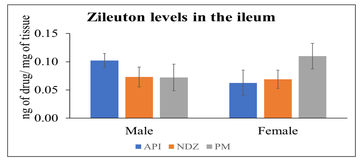


Figure 6: A comparison of zileuton levels in the ileum of male and female rats.

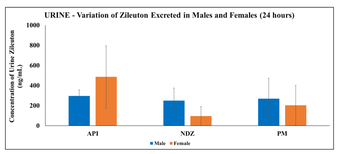


Figure 8: Zileuton levels in the urine of male and female rats 24 hours post oral dose of API, NDZ, and PM (30 mg/kg b.w.)

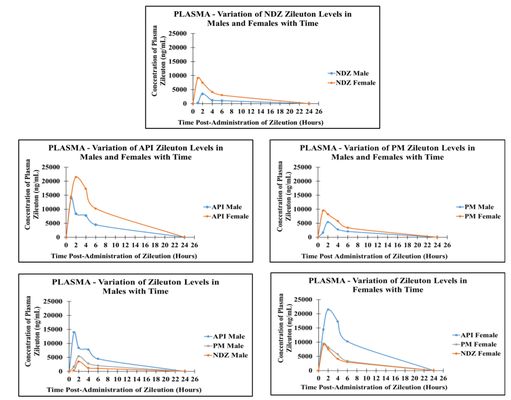


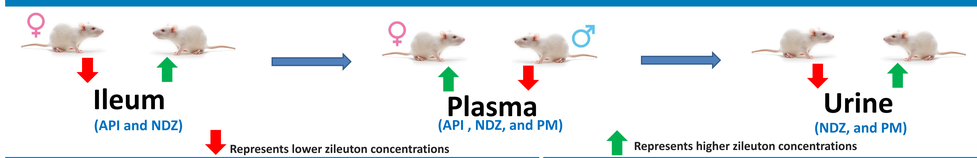
Figure 7: Mean plasma concentrations (ng/ml) *vs* time for zileuton following oral administration of API, NDZ, and PM at a dose of 30 mg/kg b.w. (n=3)

Table 1: Pharmacokinetic parameters for zileuton following oral administration of API, NDZ, and PM.

Parameter	Male			Female		
Formulation	API	NDZ	PM	API	NDZ	PM
Cmax (ng/ml) ± SD	13955 ± 176	3510 ± 37	5395 + 27	21467 ± 189	9092 ± 103	9466 + 67
Tmax (h)	1	2	2	2	1	1
K_a (h^{-1})	9.55 ± 0.0126	4.08 ± 0.0053	4.30 ± 0.0025	4.99 ± 0.0044	9.12 ± 0.114	9.16 ± 0.0071
K_e (h^{-1})	0.42 ± 0.0008	0.37 ± 0.0004	0.40 ± 0.0002	0.48 ± 0.0005	0.41 ± 0.0005	0.41 ± 0.0004
$T_{1/2}$(h)	1.64 ± 0.003	1.88 ± 0.0022	1.74 ± 0.0010	1.46 ± 0.0016	1.70 ± 0.0022	1.68 ± 0.0016
C_0(ng/ml)	30406.53 ± 558.92	7118.39 ± 73.632	15178.91 ± 89.0545	100394.90 ± 1346.57	19567.59 ± 244.96	22990.94 ± 255.27
Vd (ml)	986.85 ± 18.33	4214.74 ± 43.59	1976.47 ± 11.59	298.86 ± 4.0039	1533.31 ± 19.19	1304.97 ± 14.58
Clearance (ml/h)	417.92 ± 6.99	1553.11 ± 14.22	787.75 ± 4.19	141.99 ± 1.74	623.41 ± 7.016	538.13 ± 5.48
AUC $_{(0\text{-}24)}$	86926.32 ± 1133.29	18563.26 ± 150.18	36095.11 ± 489.51	183729.52 ± 1908.75	58626.39 ± 627.00	67261.39 ± 745.06

The NDZ showed uniform surface morphology compared to the API. Female rats had higher plasma levels of zileuton than in male rats following oral administration of API, NDZ, and PM. However, male rats showed higher levels of zileuton in the ileum and urine following treatment with NDZ. These results suggest that gender differences should be considered in the drug pharmacokinetics during preclinical studies to optimize formulations and dosing strategies.

Conclusion



FDA Significance

- **This research will support FDA to facilitate evaluation of in vivo models for safety assessment including PK of the nanocrystal-drug formulation.**
- **The outcomes of this study will help define guidelines for the safety and risk assessment associated with nanocrystal formulated drugs.**

Acknowledgements

Discussions with Dr. Banu Zolnik from CDER for the selection of zileuton as the study's model drug. University of Connecticut scientists Dr. Rajan Jog and Dr. Diane Burgess developed nano-crystal formulations. SEM analysis was carried out by Dr. Angel Paredes from the nanocore facility at NCTR-US FDA.

Projects: CDER #E0759001 | NCTR #E0759011 | CORES #E0759021