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Clinical investigation of metabolic and renal clearance pathways contributing to the elimination of fevipiprant using probenecid as perpetrator

ONLINE DATA SUPPLEMENT

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Materials and methods

Inclusion criteria

Healthy volunteers eligible for inclusion in this study had to fulfill all of the following criteria:

1. Written informed consent was obtained before any assessment was performed.
2. Healthy male and/or female subjects 18 to 55 years of age, inclusive, and in good health as determined by past medical history, physical examination, vital signs, ECG, and laboratory tests at screening and/or first baseline visit as indicated.
3. At screening and first baseline visit, vital signs (systolic and diastolic blood pressure and pulse rate) were assessed in the sitting position after the subject had rested for at least 3 minutes, and again after 3 minutes in the standing position. Sitting vital signs were to be within the following ranges:
    - oral body temperature 35.0°C to 37.5°C;
    - systolic blood pressure, 90 to 139 mmHg;
    - diastolic blood pressure, 50 to 89 mmHg;
    - pulse rate, 40 to 90 bpm.

    If vital signs were out-of-range, the Investigator could obtain two additional readings so that a total of up to three consecutive assessments were made, with the subject seated quietly for approximately 5 minutes preceding each repeat assessment. At least the last reading was to be within the ranges provided above in order for the subject to qualify. Subjects were to be excluded if their standing vital signs (relative to sitting) showed findings which, in the opinion of the Investigator, were associated with clinical manifestation of postural hypotension (i.e. absence of any other cause). The Investigator was required to carefully consider enrolling subjects with either a > 20 mmHg decrease in systolic or a > 10 mmHg decrease in diastolic blood pressure, accompanied by a > 20 bpm increase in heart rate (comparing standing to sitting results).

4. Subjects were required to weigh between 50 and 90 kg (inclusive) to participate in the study, and were required to have a body mass index (BMI) within the range of 18 to 30 kg/m^2. BMI = Body weight (kg)/(height [m])^2.
5. Able to communicate well with the Investigator, to understand and comply with the requirements of the study.

Exclusion criteria

Healthy volunteers fulfilling any of the following criteria were not eligible for inclusion in this study:

1. Use of other investigational drugs at the time of enrollment, or within 5 half-lives of initial study drug administration, or within 30 days of initial study drug administration, whichever was longer; or longer if required by local regulations.
2. A history of clinically significant ECG abnormalities, or any of the following ECG abnormalities at screening or first baseline visit:
    - PR > 200 msec;
    - QRS complex > 120 msec;
    - Fridericia QT correction formula (QTcF) > 450 msec (males);
    - QTcF > 460 msec (females).
3. History or presence of long QT syndrome or other clinically significant ECG abnormalities, e.g., arrhythmia or tachycardia.
4. History or presence of malignancy of any organ system (other than localized basal cell carcinoma of the skin or in-situ cervical cancer), treated or untreated, within the past 5 years, regardless of whether there was evidence of local recurrence or metastases.
5. Pregnant or nursing (lactating) women, where pregnancy was defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin laboratory test.
6. Women of childbearing potential, defined as all women physiologically capable of becoming pregnant, unless they were using basic methods of contraception during study drug treatment. Basic contraception methods included:
    - Total abstinence from heterosexual intercourse (when this was in line with the preferred and usual lifestyle of the subject). Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal were not acceptable methods of contraception;
    - Female sterilization (had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before taking investigational drug. In case of oophorectomy alone, only when the reproductive status of the woman had been confirmed by follow-up hormone level assessment;
    - Male sterilization (at least 6 months prior to screening). For female subjects on the study, the vasectomized male partner was to be the sole partner for that subject;
    - Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps);
    - Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that had comparable efficacy (failure rate < 1%), for example hormone vaginal ring or transdermal hormone contraception or placement of an intra-uterine device or intra-uterine system.

      In case of use of oral contraception women were to be stable on the same pill for a minimum of 3 months before taking study drug. Women were

considered postmenopausal and not of childbearing potential if they had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g., age appropriate, history of vasomotor symptoms) or had surgical bilateral oophorectomy (with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before. In the case of oophorectomy alone, only when the reproductive status of the woman had been confirmed by follow-up hormone level assessment was she considered not of childbearing potential.

7. Smokers (use of tobacco products in the previous 3 months). Smokers were defined as any subject who reported tobacco use and/or who had a urine cotinine ≥ 200 ng/mL at screening, first baseline or second baseline.
8. Use of any prescription drugs (with the exception of oral or injectable contraceptives) and/or herbal supplements within 4 weeks prior to initial study drug administration, and/or over-the-counter (OTC) medication, dietary supplements (vitamins included) within 2 weeks prior to initial study drug administration. If the subject had an incidental and limited need for a medication (e.g., for a headache), refer to Appendix 16.1.1-Section 5.2 for additional considerations. Medications must have been documented in the Concomitant Medications/Significant Non-drug Therapies page of the electronic case report form (eCRF).
9. Donation or loss of 400 mL or more of blood within 8 weeks prior to initial study drug administration, or longer if required by local regulation.
10. Plasma donation within 4 weeks prior to initial study drug administration.
11. Hemoglobin levels outside of normal ranges of local laboratory for males and females, respectively at screening and first baseline visit. The laboratory assessment could be repeated once prior to initial treatment assignment.
12. Uric acid plasma concentrations above normal ranges of local laboratory at screening and first baseline.
13. Significant illness or infection which had not resolved within 2 weeks prior to initial study drug administration.
14. Recent (within the last 3 years) and/or recurrent history of autonomic dysfunction (e.g., recurrent episodes of fainting, palpitations, etc.).
15. History of multiple and recurring allergies or allergies/hypersensitivities or allergy/hypersensitivity to the investigational compounds/compound class being used in this study (e.g., probenecid or any of its ingredients and DP2 antagonists).
16. History of or current nephrolithiasis.
17. History of or current diagnosis of gout.
18. History of hemolytic anemia.
19. Known or suspected glucose-6-phosphate dehydrogenase (G6PD) deficiency.
20. History of any food allergies.
21. Any surgical or medical condition which could significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which could jeopardize the subject in case of participation in the study. The Investigator was to make this

determination in consideration of the subject's medical history and/or clinical or laboratory evidence of any of the following:

- Inflammatory bowel disease, peptic ulcers, gastrointestinal including rectal bleeding within 12 months prior to screening;
- History of major gastrointestinal tract surgery such as gastrectomy, gastroenterostomy, or bowel resection;
- Pancreatic injury or pancreatitis within 12 months prior to screening;
- Liver disease or liver injury as indicated by abnormal liver function tests. Alanine aminotransferase (ALT) (serum glutamic pyruvic transaminase), aspartate aminotransferase (AST) (serum glutamic oxaloacetic transaminase), gamma-glutamyltransferase (GGT), alkaline phosphatase and serum bilirubin were tested;
- Alanine aminotransferase or AST > 2.0 × the upper limit of normal (ULN) or total bilirubin > 1.3 × ULN at screening or at the first baseline visit;
- Gamma-glutamyltransferase or alkaline phosphatase > 1.5 × ULN at screening or at the first baseline visit;
- Any elevation above ULN of more than 1 parameter of ALT, AST, GGT, alkaline phosphatase or serum bilirubin at screening or at the first baseline visit excluded a subject from participation in the study;
- Glucose-6-phosphate dehydrogenase enzyme testing to rule out G6PD deficiencies. Glucose-6-phosphate dehydrogenase result had to be within normal range according to the reference range of the local laboratory. If necessary, laboratory testing could be repeated on 1 occasion (as soon as possible prior to enrollment, to rule out any laboratory error).
- History or presence of impaired renal function as indicated by elevated creatinine or blood urea nitrogen, and/or urea values above limits of local laboratory, or abnormal urinary constituents (e.g., albuminuria), or estimated glomerular filtration rate < 90 mL/min/1.73m^2
- History of urinary obstruction or difficulty in voiding at screening or at the first baseline visit.

22. History of immunodeficiency diseases or active disease, including a positive human immunodeficiency virus (HIV) (e.g., chemiluminescence assay and MultiSpot) test result.
23. A positive hepatitis B surface antigen or hepatitis C test result.
24. History of drug or alcohol abuse within the 12 months prior to study drug administration, or evidence of such abuse as indicated by the laboratory assays conducted during screening, first baseline and second baseline.

No additional exclusions were to be applied by the Investigator, in order to ensure that the study population was representative of all eligible subjects. In the case where a safety laboratory assessment at screening and/or initial baseline was outside of the range specified in the exclusion criteria, the assessment could be repeated once prior to enrollment. If the repeat value remained outside of the specified ranges, the subject was excluded from the study.

Pharmacokinetic analyses

In the frame of the validation of the bioanalytical method for fevipiprant and its AG metabolite in human urine, the stability of both fevipiprant and its AG was tested at different pH (physiological pH of human urine varies from 4 to about 8, whereas the average pH of urine is below 7). It could be demonstrated that AG is stable for at least 24h at pH 4.1, pH 6.8 and for 8.5h at pH 8 at +5°C ± 5°C (in this study, during the urine sampling interval, the urine was pooled either on ice or in a refrigerator at approximately 0-4°C. Afterwards an aliquot of 1ml urine was transferred into FC tubes and was frozen until analysis).

In the BA validation study, the pH of urine samples after transfer into FC tubes was 4.1. However, as the pH of untreated human urine varies from 4 to about 8, it cannot be concluded that for all urine samples the pH was 4.1. Nevertheless, taking the above mentioned stability data of AG at different pH, and the sample collection procedure into consideration, it has been concluded that there was no impact on stability of AG after collection.

Blood plasma is normally tightly regulated at approximately pH 7.4. Stability of the AG metabolite in human plasma was clearly demonstrated during the method validation for 0.5 mL to 1.5 mL plasma in FC tubes.

LC-MS/MS methods for probenecid

The method for probenecid consists of protein precipitation followed by reverse phase liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) in negative mode using ESI as the ionization technique (probenecid precursor ion m/z

284.2, product ion m/z 198.0, probenecid –d7 (internal standard) precursor ion m/z 291.3, product ion m/z 205.3). The method is suitable for the determination of probenecid in human EDTA blood over the range of 1.00 (LLOQ) to 400 µg/mL using 50 µL of human blood.

The selectivity of the method against interference of endogenous components of the matrix (blood) and against fevipiprant and its AG metabolite was tested. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the probenecid and its internal standard that would interfere with quantitation of study samples

Results

Table S1 Baseline demographics (safety analysis set)

Characteristic	Total N = 16
Age, years (median [min–max])	27.5 [19, 55]
Female, n (%)	14 (87.5)
Race n (%)	
White	11 (68.8)
Black or African American	4 (25.0)
Native Hawaiian or Other Pacific Islander	1 (6.3)
Height, cm	167.7 ± 8.59
Weight, kg	68.3 ± 10.37
BMI, kg/m^2	24.2 ± 2.44

Data presented as mean ± SD, unless otherwise specified

BMI = body mass index

Table S2 Mean concentration-time data for fevipiprant and its AG metabolite without and with probenecid

	Fevipiprant		AG metabolite	
Scheduled Sampling Time (h)	Without probenecid	With probenecid	Without probenecid	With probenecid
0	BLQ (0)	BLQ (0)	BLQ (0)	BLQ (0)
0.25	67.5 (82.5)	179 (169)	5.62 (7.44)	63.4 (75.0)
0.5	127 (97.9)	518 (463)	49.4 (55.6)	377 (326)
1	363 (302)	777 (637)	238 (185)	788 (553)
1.25	504 (416)	934 (744)	366 (261)	906 (589)
1.5	601 (429)	967 (720)	549 (388)	1010 (645)
2	675 (357)	975 (590)	823 (489)	1160 (584)
3	385 (194)	743 (370)	847 (433)	956 (395)
4	-	619 (438)	-	821 (583)
4.5	216 (122)	576 (473)	593 (332)	789 (711)
6	151 (113)	417 (317)	389 (292)	633 (649)
8	51.6 (27.5)	142 (64.3)	174 (120)	266 (258)
12	54.3 (14.9)	110 (65.1)	108 (40.8)	156 (122)
24	21.7 (6.18)	73.0 (54.7)	51.5 (14.2)	107 (83.1)
48	10.5 (5.64)	60.2 (40.2)	23.2 (10.4)	81.7 (68.0)
72	6.23 (4.66)	26.0 (16.2)	13.1 (8.56)	40.5 (30.2)
96	2.60 (2.25)	14.3 (11.3)	6.20 (5.02)	25.4 (20.7)

BLQ: Below the limit of quantification

Table S3 Adverse events by treatment (safety analysis set)

Adverse event	**Fevipiprant (N = 16)**	**Probenecid (N = 16)**	**Fevipiprant + probenecid (N = 16)**
Subjects with any adverse event, n (%)	*2 (12.5)*	*4 (25.0)*	*8 (50.0)*
Lower abdominal pain	0	0	1 (6.3)
Diarrhea	0	0	1 (6.3)
Flatulence	0	1 (6.3)	0
Nausea	0	1 (6.3)	1 (6.3)
Vomiting	0	1 (6.3)	1 (6.3)
Vessel puncture site pain	0	0	1 (6.3)
Gastroenteritis	0	0	1 (6.3)
Upper respiratory tract infection	0	0	1 (6.3)
Viral pharyngitis	0	1 (6.3)	0
Decreased appetite	0	0	1 (6.3)
Musculoskeletal chest pain	1 (6.3)	0	0
Postural dizziness	0	0	1 (6.3)
Headache	1 (6.3)	0	2 (12.5)
Anxiety	0	0	1 (6.3)