Pharmacodynamic Effects of Deucrictibant on Carrageenan-Induced Edema in Humanized Bradykinin B2 Receptor Transgenic Sprague Dawley Rats

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Background

- Deucrictibant is an orally administered, highly potent, specific antagonist of the bradykinin B2 receptor under development for on-demand and prophylactic treatment of hereditary angioedema (HAE) attacks.
- Deucrictibant is a potent antagonist at the human bradykinin B2 receptor but is a weak antagonist at the rat ortholog (>100-fold lower potency), indicative of species selectivity.
- To address the challenge of deucrictibant's species selectivity in experimental models (eg, the paw edema model in rats), a humanized bradykinin B2 receptor transgenic (Tg) rat line was developed and validated.
- Following the validation, the Tg rat was used in the carrageenan-induced paw edema model to investigate the *in vivo* primary pharmacodynamic (PD) effects of deucrictibant.*

Methods

- A Tg rat line was generated on a Sprague-Dawley background using CRISPR/Cas9- mediated gene editing. This rat line, built on a Sprague-Dawley rat background showed no adverse phenotypes and appeared a healthy strain.
- The potency of deucrictibant and icatibant, an established bradykinin B2 receptor antagonist, to inhibit the bradykinin-induced intracellular Ca^{2+} mobilization was evaluated in HEK293 cells stably expressing the recombinant wild type (WT) or Tg rat bradykinin B2 receptor using a fluorimetric method. The half maximal inhibitory concentration (IC_{50}) and the equilibrium dissociation constant (K_b) values were calculated.
- Membrane preparations from WT and Tg rat uterus were used in radioligand binding inhibition experiments to determine the affinity of deucrictibant for the endogenously expressed B2 receptor. The assay was validated with icatibant. Deucrictibant was tested and the IC_{50} and inhibitor constant (K_i) values were calculated.
- The *in vivo* effects of deucrictibant and icatibant were examined on paw edema induced by unilateral intraplantar injection of carrageenan (0.75 mg in 0.05 mL/paw) in the hind paw of male Tg rats. Deucrictibant was administered orally 30 minutes before injection of carrageenan. Icatibant (1 mg/kg) was administered intravenously 2 minutes before injection of carrageenan. The positive control acetylsalicylic acid (512 mg/kg) was administered orally 60 minutes before injection of carrageenan.
- The volume of the paw was measured by hydroplethysmometry prior to carrageenan injection and at 2, 4, and 8 hours after carrageenan injection. Changes in paw volume relative to the measurement taken before carrageenan administration were calculated. Deucrictibant was evaluated at 5, 10, and 20 mg/kg.

Reference

1. Lesage A. et al. *Int Immunopharmacol.* 2022:105:108523.

Results

Potency of deucrictibant at recombinant WT and Tg rat bradykinin B2 receptors

- The potency of bradykinin measured as the half maximal effective concentration (EC_{50}) at the recombinant WT and Tg rat bradykinin B2 receptors was 105 and 113 pM, respectively.

Table 1: Potency of icatibant and deucrictibant at bradykinin B2 receptors from WT and Tg rats

	WT B2		Tg B2		Ratio
	IC_{50} (nM)	K_b (nM)	IC_{50} (nM)	K_b (nM)	K_b WT B2 vs K_b Tg B2
Icatibant	2.45 ± 0.12	0.59 ± 0.02	2.04 ± 0.48	0.53 ± 0.10	1.11
Deucrictibant	250.56 ± 75.70	61.26 ± 22.67	1.72 ± 0.45	0.45 ± 0.10	136.13

Values are mean ± SD; n=3 to 4 for icatibant and deucrictibant. IC_{50}, half maximal inhibitory concentration; K_b, equilibrium dissociation constant; Tg, transgenic; WT, wild type.

- Icatibant was equally potent at the WT and Tg rat bradykinin B2 receptors (**Table 1**). The antagonist potency of deucrictibant increased 136-fold at the heterologously expressed Tg receptor as compared to the WT rat receptor. The potency of deucrictibant 0.45 nM for the transgenic rat B2 receptor is similar to the potency for the human bradykinin B2 receptor (0.15 nM).
- Based on these data it was decided to create a Tg rat line expressing this humanized bradykinin B2 receptor.

Affinity of deucrictibant for the bradykinin B2 receptor in tissue of WT and Tg rats

- Saturation binding experiments with [^{3}H]BK showed a mean binding capacity (B_{max}) of 0.027 and 0.010 pmol/mg protein, and a mean dissociation constant (K_D) of 0.72 and 0.39 nM for WT and Tg rat uterus membranes, respectively (n=3).

Table 2: Affinity of icatibant and deucrictibant at bradykinin B2 receptors from WT and Tg rats

	WT B2		Tg B2		Ratio
	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)	K_i WT B2 vs K_i Tg B2
Icatibant	0.58 ± 0.18	0.34 ± 0.10	0.32 ± 0.10	0.14 ± 0.05	2.43
Deucrictibant	25.20 ± 6.90	14.90 ± 4.10	1.24 ± 0.33	0.55 ± 0.14	27.09

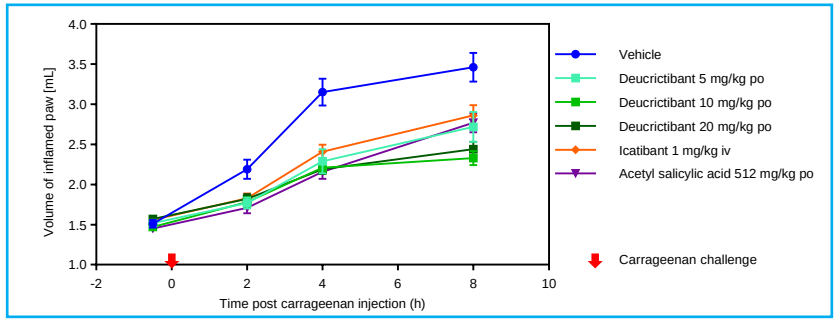
Values are mean ± SD; n=5 for icatibant and n=3 for deucrictibant. IC_{50}, half maximal inhibitory concentration; K_i, inhibitor constant; Tg, transgenic; WT, wild type.

- The affinity of icatibant for the bradykinin B2 receptor in transgenic rat uterus membranes was in the same order of magnitude as the affinity for the bradykinin B2 receptors in WT rat uterus membranes (**Table 2**).
- Humanization of the bradykinin B2 receptor resulted in a 27-fold increase in affinity of deucrictibant for the bradykinin B2 receptor in membrane preparations from Tg rat uterus compared to WT rat uterus. The K_i at the bradykinin B2 receptor in uterus membranes from Tg rats is in the same range as the reported K_i for the recombinant human bradykinin B2 receptor (0.55 nM *versus* 0.47 nM)[1].

Effect of deucrictibant on carrageenan-induced paw edema in Tg rats

- Intraplantar injection of carrageenan in the hind paw of Tg rats induced a marked increase in paw volume at 2, 4, and 8 hours postdose, indicative of gradual development of edema (**Figure 1**).

Figure 1: Carrageenan-induced paw edema in male Tg rats (mean ± SEM)



iv, administered intravenously; po, administered orally, SEM, standard error of the mean; Tg, transgenic.

- Deucrictibant, icatibant and the positive control acetylsalicylic acid partially prevented carrageenan-induced development of paw edema at 2, 4, and 8 hours after carrageenan injection (**Figure 1**, **Table 3**).

Table 3: Inhibition of carrageenan-induced paw edema

	% Inhibition of carrageenan-induced paw edema		
Time post-carrageenan injection	**2 hours**	**4 hours**	**8 hours**
Deucrictibant 5 mg/kg po	63.9 ± 8.5	53.0 ± 8.3	38.7 ± 9.9
Deucrictibant 10 mg/kg po	55.4 ± 6.8	55.2 ± 3.3	55.7 ± 4.1
Deucrictibant 20 mg/kg po	63.4 ± 10.3	62.4 ± 4.0	55.7 ± 4.9
Icatibant 1 mg/kg iv	59.5 ± 10.4	48.0 ± 6.0	33.2 ± 7.1
Acetyl salicylic acid 512 mg/kg po	62.1 ± 11.2	56.9 ± 5.3	32.5 ± 5.8

Values are mean ± SEM for n=11. iv, intravenous; po, administered orally.

- Oral doses of 10 and 20 mg/kg deucrictibant retained their efficacy up to 8 hours post-carrageenan injection (56% inhibition) differently to iv injection of icatibant, which showed 33% inhibition at this time point **(Table 3).**

Conclusions

- **These results show that the genetically engineered Tg humanized bradykinin B2 receptor rat model is a viable tool to address the challenge of species selectivity of deucrictibant.**
- **The rat line is pharmacologically responsive to bradykinin B2 receptor antagonists and can be used to study the pharmacodynamic properties of deucrictibant *in vivo*.**
- **Oral deucrictibant was effective at inhibiting carrageenan-induced edema in Tg bradykinin B2 receptor rats and demonstrated a long duration of action.**

COI: A.L.: employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma; **B.L:** consultant to Pharvaris, holds stocks/stock options in Pharvaris; **G.V.:** former employee of Pharvaris; **E.P.:** employee of Pharvaris, holds stock in Pharvaris; **C.G.:** employee of AnalytiCon Discovery, holds stocks/stock options in Pharvaris; **J.K.:** employee of JCK Consult and consultant to Pharvaris, holds stocks/stock options in Pharvaris.

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