Bradykinin Challenge Model in Humanized Bradykinin B2 receptor Transgenic Rat

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This presentation includes data for an investigational product not yet approved by regulatory authorities.

Conflicts of interest disclosures

J. Skarbaliene employee of Pharvaris, holds stocks/stock options in Pharvaris.

M. van Esdonk employee of Pharvaris, holds stocks/stock options in Pharvaris.

J. Bravo employee of Pharvaris, holds stocks/stock options in Pharvaris.

C. Gibson employee of AnalytiCon Discovery, holds stocks/stock options in Pharvaris.

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A. Lesage employee of GrayMatters Consulting and consultant to Pharvaris, holds stocks/stock options in Pharvaris; advisor to Kosa Pharma.

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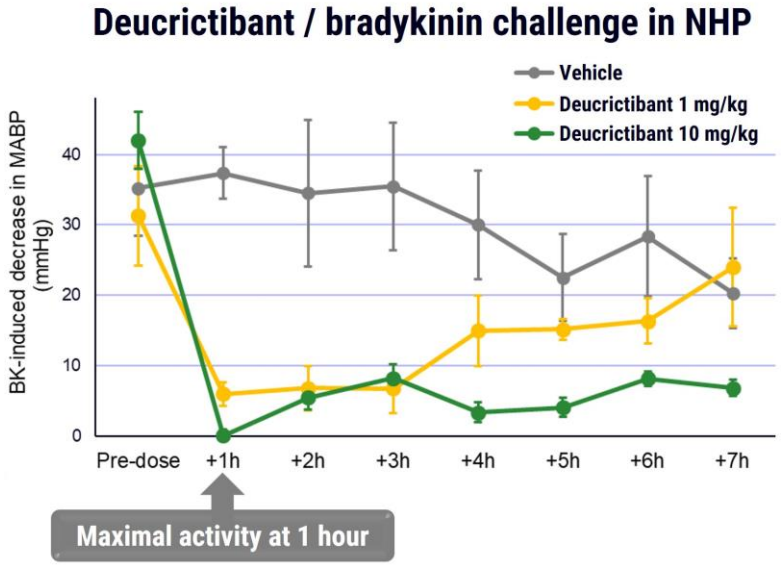
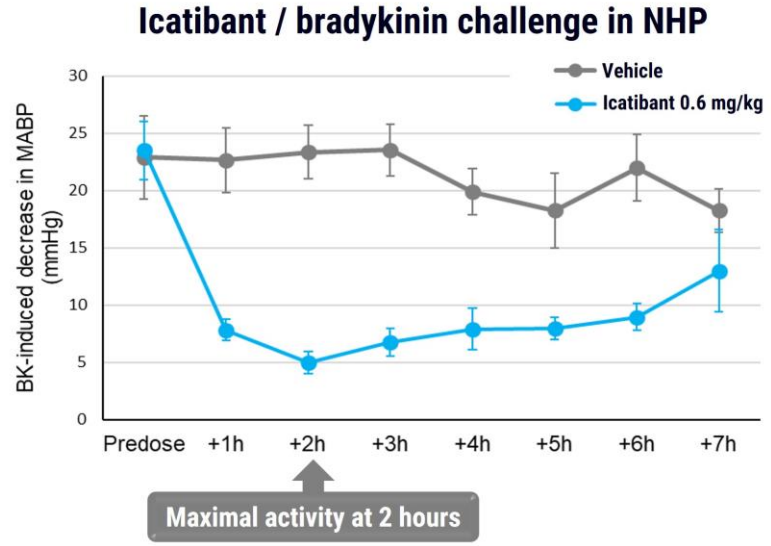
Why is a rat BK challenge model needed?

Established BK challenge models in non-human primates (NHP) and humans were effectively used to determine pharmacokinetic and pharmacodynamic (PK/PD) relationships of bradykinin B2 receptor antagonists



Advantages of a rat BK challenge model

- Enables early-stage PK/PD characterization
- Enables the refinement of human dose predictions
- Practical and scalable for broad research applications
- More cost-effective alternative to existing models
- Can be applied to genetically modified animals to address specific research questions

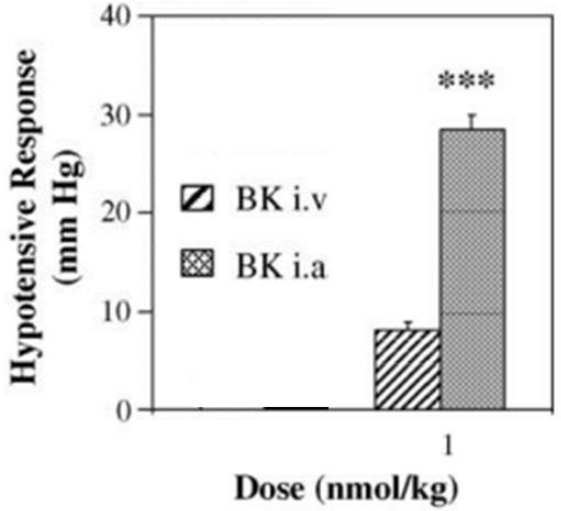


Lesage ASJ et al. AAAAI 2020.

Development of a BK challenge model in a humanized bradykinin B2 receptor transgenic rat line

Background

Bradykinin induces a transient hypotensive reaction following intra-arterial administration in rats, compared to intravenous administration.



Adapted from Blais et al. 2005. Blood pressure modulator effect of BK administered via intravenous (i.v.) and intra-arterial (i.a.) routes in rats n = 5–9.***p < 0.001 vs. BK i.v.

Aim of the study

To develop the BK challenge model using transgenic humanized bradykinin B2 receptor Sprague Dawley male rats for evaluation of species selective and non-selective bradykinin B2 receptor antagonists

1. Identify the effective dose of BK for inducing hemodynamic effects.
2. Assess desensitization of hemodynamic effects after repeated BK administration.
3. Validate the model using icatibant as reference bradykinin B2 receptor antagonist

A humanized bradykinin B2 receptor transgenic rat model was established and validated

- The recombinant humanized bradykinin B2 receptor was expressed in HEK293 cells and the effect of the mutation on antagonist potency of deucrictibant and icatibant was investigated.

Antagonist potency at recombinant bradykinin B2 receptors in HEK293

	Kb in nM (Ca^{2+} mobilisation)		
	WT rat B2	Humanized rat B2	Ratio K_b values WT vs humanized B2
Deucrictibant	61.0 ± 23.0	0.45 ± 0.10	136
Icatibant	0.59 ± 0.02	0.53 ± 0.10	1

- A transgenic humanized rat line was generated on a Sprague Dawley background using CRISPR/Cas9-mediated gene editing. This rat line showed no adverse phenotypes and appeared a healthy strain.

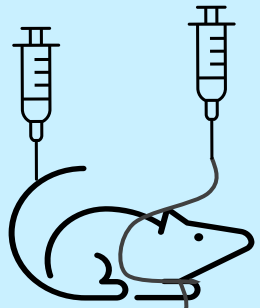
Antagonist affinity for endogenous bradykinin B2 receptor in uterus of WT or transgenic rat

	Ki in nM (^{3}H-BK)		
	WT rat uterus	Transgenic rat uterus	Ratio K_i values WT vs transgenic rat
Deucrictibant	14.9 ± 4.1	0.55 ± 0.14	27
Icatibant	0.34 ± 0.10	0.14 ± 0.05	2

- **Humanization of the rat bradykinin B2 receptor increases the antagonist potency and affinity of deucrictibant, a selective antagonist of the human bradykinin B2 receptor.**
- **Icatibant's antagonist potency and affinity for the recombinant bradykinin B2 receptor are not affected by humanization, confirming its equipotent antagonism of the human and rat bradykinin B2 receptors.**

Development and validation of a BK challenge model in a humanized bradykinin B2 receptor transgenic rat line – Study design

Icatibant
iv injection
via the lateral
tail vein

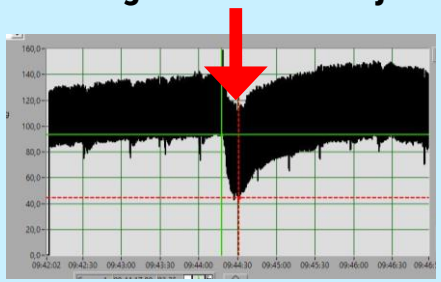


BK challenge
intra-arterial
injection of BK via
the left common
carotid artery

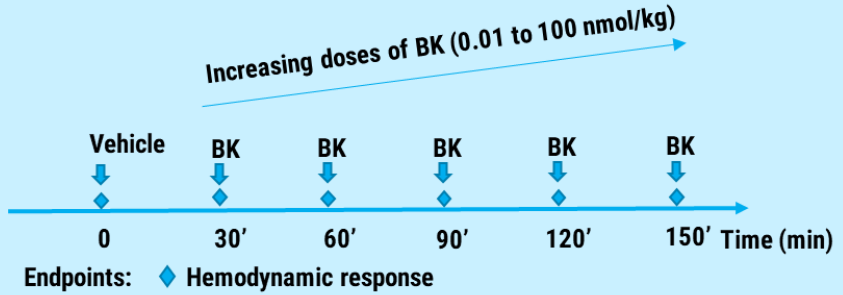
Anesthetized male rats

Blood pressure (BP)
measurement via the
right common carotid
artery and recorded
using a BP transducer

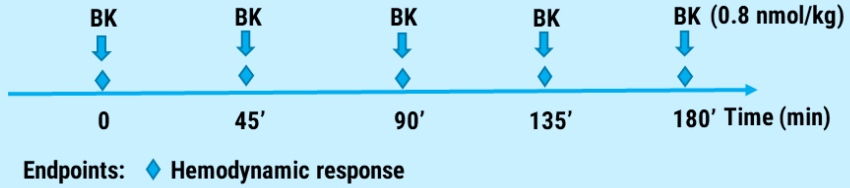
Representative tracings depicting hypotensive effects of BK following intra-arterial injection



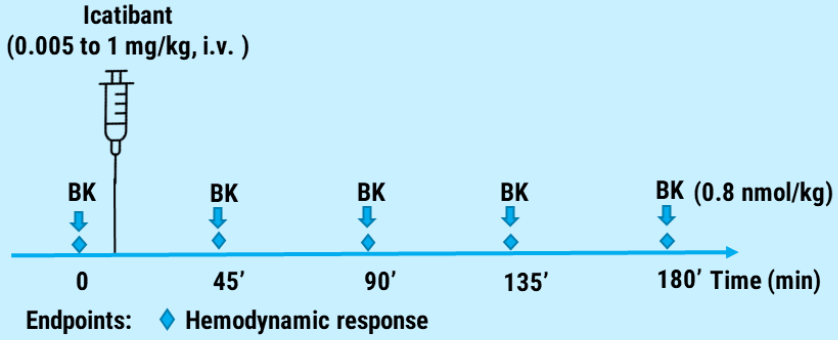
Dose-response of BK



Desensitization testing

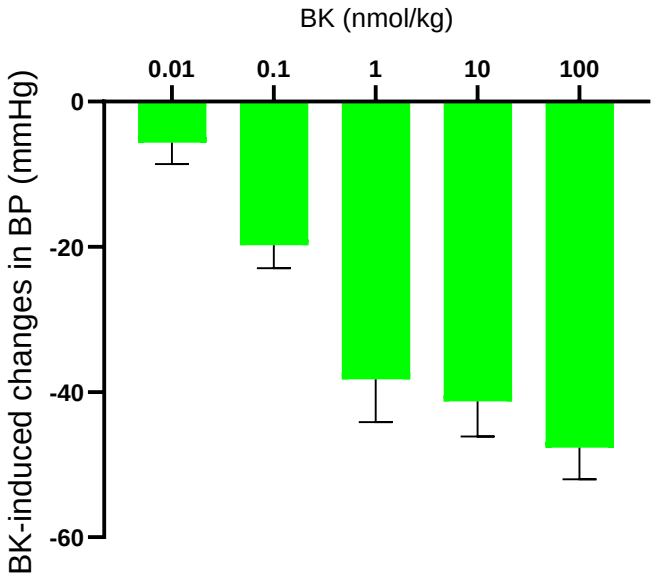


Icatibant testing

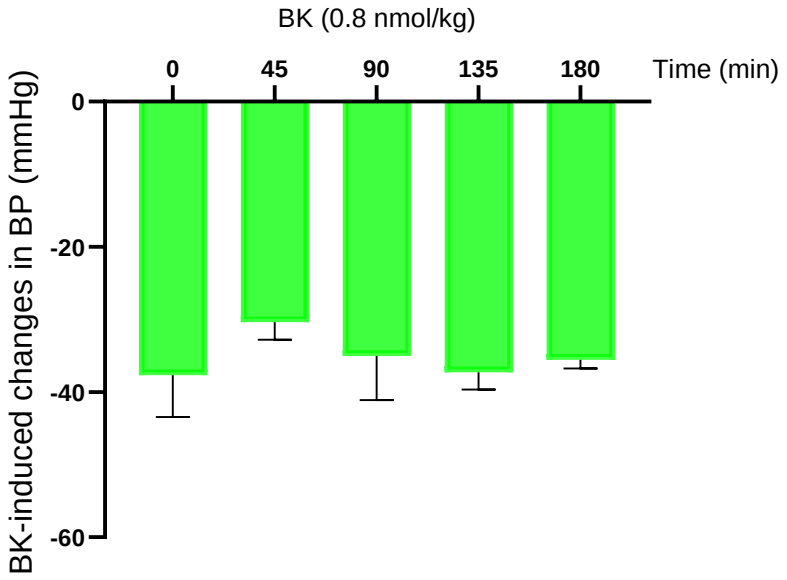


Development and validation of a BK challenge model in a humanized bradykinin B2 receptor transgenic rat line – Study results

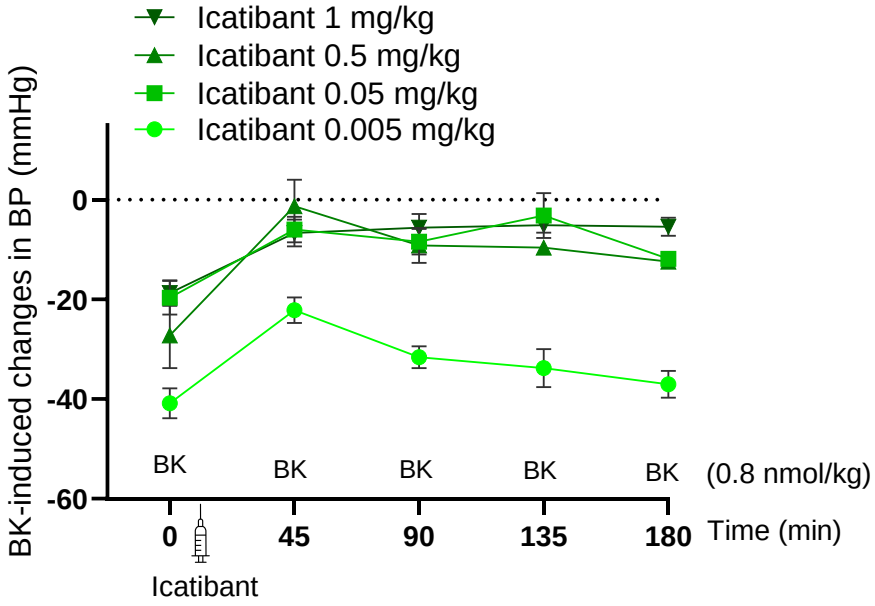
1. Evaluation of bradykinin-dose response (incremental BK doses every 30 min)



2. Evaluation of repeated administration of 0.8 nmol/kg BK (every 45 min)



3. Evaluation of icatibant effects on response to 0.8 nmol/kg BK (every 45 min)



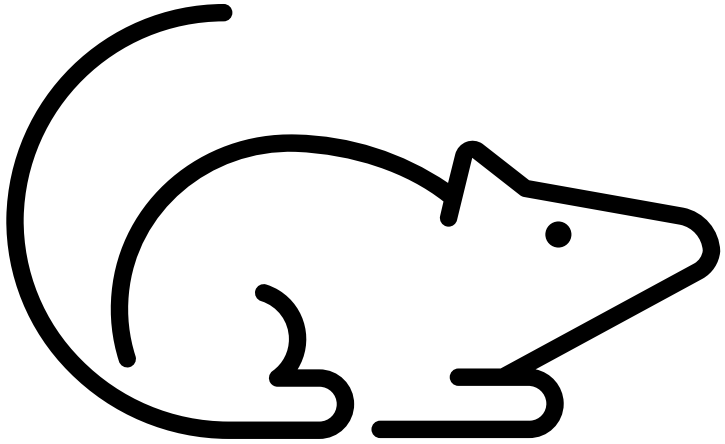
Mean ±SEM, N=3-8

- ✓ Dose-dependent lowering of blood pressure demonstrated with BK (ED_{80} was estimated at approximately 1 nmol/kg)
- ✓ No apparent BK-mediated desensitization
- ✓ Icatibant at all tested doses (0.005 to 1 mg/kg, i.v.) inhibited the BK-induced hemodynamic response

Summary

- We successfully developed a BK challenge model in humanized bradykinin B2 receptor transgenic rats that are pharmacologically responsive to bradykinin B2 receptor antagonists.

- The BK challenge model in humanized bradykinin B2 receptor transgenic rats offers a valuable, easy to manage, and cost-effective tool for efficacy studies compared to those involving non-human primates.



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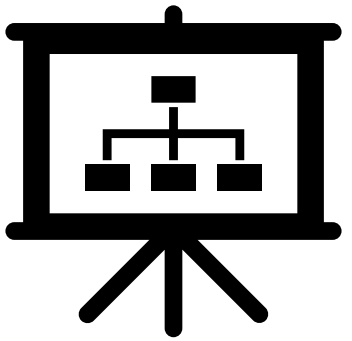

Oral Presentation. Session VII Hereditary angioedema on **Friday, 6th September**, from 15:05 to 15:15

- Prophylactic Treatment With Deucrictibant Improves HAE Disease Control and HRQoL (Markus Magerl et al.)

Posters Poster session on **Friday, 6th September,** from 15:35 to 16:15

- **The bradykinin challenge model translates across rat, monkey and human (Juan Bravo et al.)**
- Deucrictibant inhibits carrageenan-induced edema in bradykinin B2 receptor transgenic rat (Anne Lesage et al.)
- Cardiovascular safety of repeated oral administration of the B2-receptor antagonist deucrictibant (Nieves Crespo et al.)
- A novel kinin biomarker assay for characterisation of bradykinin-mediated disorders (Evangelia Pardali et al.)
- A HMWK capillary immunoblotting assay to characterise bradykinin-mediated disorders (Evangelia Pardali et al.)
- Clinical trials conformity with AURORA COS: a systematic literature review (Remy S. Petersen et al.)
- Deucrictibant vs. Standard of Care in HAE: Propensity Score-Matched Analysis (Marc A. Riedl et al.)
- Treatment of HAE Attacks With Oral Deucrictibant: RAPIDe-2 Extension Results (Emel Aygören-Pürsün et al.)
- Long-Term Safety and Efficacy of Oral Deucrictibant for HAE Prophylaxis (Marc A. Riedl et al.)
- Prophylaxis of Hereditary Angioedema Attacks With Oral Deucrictibant: CHAPTER-1 Results (Emel Aygören-Pürsün et al.)