Icatibant, the bradykinin B2 receptor antagonist with target to the interconnected kinin systems

Delphine Charignon, Peter Späth, Ludovic Martin & Christian Drouet†
†Université Joseph Fourier Grenoble 1, GREPI/AGIM CNRS-UJF FRE 3405 and Centre de Référence des Angioédèmes CREAK, Grenoble France

Introduction: HOE-140/Icatibant is a selective, competitive antagonist to bradykinin (BK) against its binding to the kinin B2 receptor. Substitution of five non-proteogeneic amino acid analogues makes icatibant resistant to degradation by metalloproteases of kinin catabolism. Icatibant has clinical applications in inflammatory and vascular leakage conditions caused by an acute (non-controlled) production of kinins and their accumulation at the endothelium B2 receptor. The clinical manifestation of vascular leakage, called angioedema (AE), is characterized by edematous attacks of subcutaneous and submucosal tissues, which can cause painful intestinal consequences, and life-threatening complications if affecting the larynx. Icatibant is registered for the treatment of acute attacks of the hereditary BK-mediated AE, i.e., AE due to C1 inhibitor deficiency.

Areas covered: This review discusses emerging knowledge on the kinin system: kinin pharmacological properties, biochemical characteristics of the contact phase and kinin catabolism proteases. It underlines the responsibility of the kinins in AE initiation and the potency of icatibant to inhibit AE formation by kinin-receptor interactions.

Expert opinion: Icatibant antagonist properties protect BK-mediated AE patients against severe attacks, and could be developed for use in inflammatory conditions. More studies are required to confirm whether or not prolonged and frequent applications of icatibant could result in the impairment of the cardioprotective effect of BK.

Keywords: bradykinin, des-Arg9-bradykinin, hereditary angioedema, HOE 140, icatibant, kinins, kinin B1 receptor, kinin B2 receptor, receptor antagonism

1. Kinin generation and its control

The term kinin, introduced by Abelous and Bardier in 1909 includes bradykinin (BK), kallidin (KD) and substance P and their biologically active degradation products. Below, the activating pathways, the control of BK, des-Arg⁸BK, KD and des-Arg¹⁰KD generation and their interaction with kinin receptors are reviewed.

1.1 The kinin-kallikrein system
1.1.1 BK and KD generation
1.1.1.1 BK generation
BK is released from the High Molecular Weight Kininogen (HK) upon plasma kallikrein (KK) cleavage. The plasmatic enzyme KK derives from the proenzyme prekallikrein (pKK) and is mainly complexed with HK (1). The majority of this complex is bound to the endothelial cell surface, where pKK can be activated by
factor XII protease (FXIIa). FXIIa is activated from factor XII (FXII) proenzyme, by two pathways: slow autoactivation process upon electronegative surface contact and efficient proteolytic activation by KK-like enzymes or plasmin [2]. Additional components might participate in the pKK to KK activation, e.g., prolylcarboxypeptidase [3] or heat shock protein 90 [4].

1.1.2 Catabolism of BK and KD
BK-producing enzymes circulate as proenzymes. Their activation process and enzyme activity are under the control of C1 inhibitor (C1inh), a Serine protease inhibitor (Serpin), acting as a suicide substrate [5].

1.1.3 KD generation
KD, or Lys-BK, is a decapeptide released from the Low Molecular Weight Kininogen (LK) by tissue kallikreins (TK). LK is an alternative splice product of the KNG1 gene encoding HK; it differs from HK by the length of its L chain [1]. TK constitutes a group of Serine proteases, encoded by three genes (hKLK1, hKLK2, hKLK3). The product of hKLK1 is the actual TK or hK1 which is expressed by epithelial or secretory cells of various organs [6]. hK1 is either secreted as an inactive (prokallikrein) or an active enzyme. Prokallikrein is activated by enzyme-like TK. The control of hK1 activity is achieved by kallistatin [7] or alpha antitrypsin [8].

1.1.4 KD conversion into BK
KD might be converted into BK by the membrane aminopeptidase M [9] (Figure 1, see Section 1.1.2.4).

1.1.2 Catabolism of BK and KD
Due to their rapid proteolytic cleavage by peptidases (Figure 1) [10], BK, KD and des-Arg10KD have a very short half-life (27 ± 10s, 0.32min, 32 ± 6s, for BK [11], KD [12] and des-Arg10KD [13], respectively) whereas des-Arg4BK displays a much longer half-life (643 ± 436s) [11].

1.1.2.1 Angiotensin-I-Converting Enzyme (ACE or kininase II) (EC 3.4.15.1)
ACE is the major enzyme responsible for the inactivation of BK by removing the dipeptide Phe8-Arg9 and then the Ser9-Pro10 to transform BK into the inactive BK1-5 [11]. ACE is the second enzyme in importance for the des-Arg9BK catabolism by removing the C-terminal tripeptide Ser8-Pro9-Phe8.

1.1.2.2 Aminopeptidase P (APP) (EC 3.4.11.9)
APP is the second enzyme in the catabolism of BK and the major enzyme involved in des-Arg9BK catabolism [14]. APP transforms BK and des-Arg9BK into inactive peptides [10]. The APP is encoded by the XPNPEP2 gene (X chromosome). Some polymorphisms are associated with a low APP activity [16,17]. APP does not directly metabolize KD and des-Arg10KD [18].

1.1.2.3 Dipeptidyl peptidase IV (DPPIV) (EC 3.4.14.5)
DPPIV degrades BK [2-9] into BK [4-9] [10]. DPPIV is inhibited by the so-called gliptin, a newly approved antidiabetic pharmaceutical class [19].

1.1.2.4 Aminopeptidase M (APM) (EC 3.4.11.2)
APM hydrolyzes KD into BK and des-Arg4KD into des-Arg9BK [9,20]. APM represents the main pathway for the KD degradation (Figures 1 and 2).

1.1.2.5 Neutral Endopeptidase (NEP) (EC 3.4.24.11)
NEP inactivates BK and KD by same methods as ACE to produce the inactive BK1-4 but contrary to ACE, its expression by vascular endothelial cells is low [15].

1.1.2.6 Carboxypeptidase M (CPM)
CPM a membrane bound enzyme (EC 3.4.17.12), and Carboxypeptidase N (CPN), a plasmatic enzyme synthesized in the liver (EC 3.4.17.3), are 41% identical. CPN and CPM, alternatively called Kininase I, cleave the C-terminal -Arg or -Lys residue of the B2 agonists and transform BK into des-Arg9BK and KD into des-Arg10KD, the B1 agonists (Figures 1 and 2) [21].

1.1.3 Receptors of kinins: the B1 and B2 receptors
Kinins activate two types of seven-span transmembrane receptors coupled to G proteins, the B1 (B1R) and B2 (B2R) receptors (Figure 2). B2R is activated by the binding of BK and KD, while the ligands of B1R are des-Arg9BK, des-Arg10KD and KD (Table 1). B2R is constitutively expressed whereas the B1R expression is induced upon inflammatory conditions and cytokine induction (IL-1β, TNF-α), bacterial endotoxin stimulation or anoxia. Upon agonist activation, B2R is rapidly desensitized by phosphorylation [22] accompanied by a redistribution in caveolae and is endocytosed [23], could be potentially recycled to the membrane. Conversely, B1R is not desensitized due to a different phosphorylation property [24]. In physiological situations, cells respond only to B2R activation, whereas in physiopathological conditions, in addition to B2R, B1R is fully expressed (heavy smoking, autoimmune disease, infections, others). Since B1R is not desensitized and des-Arg9BK exhibits a relative long half-life, B1R is of major importance.
as a B2R antagonist, is approved for treatment of adult cutaneous, abdominal and laryngeal attacks of HAE. These physiological effects of BK have been demonstrated when applying ACE inhibitors (ACEi) [27] and counteracted by application of BK antagonists [28].

1.2.2 Physiology of kinins: BK, des-Arg9BK, KD

des-Arg9BK, KD are mediators of inflammation and immunity

Kinin is potent inflammatory mediators, increasing blood flow, vascular leakage, edema and pain and can cause degranulation of mast cells [29]. Exogenous BK injected into human or animal tissues reproduces the four classic signs of inflammation: redness, local heat, swelling, and pain. The identity of the major target cell types for kinins explains this profile: the redness and local heat are determined by local endothelium-dependent vasodilation. The stimulation of endothelial cells also results in increased microvascular permeability, which contributes to the exudation of protein-rich fluid from the circulation (swelling). Furthermore, kinins are expected to play a role in adaptive immunity because monocytes, dendritic cells [30], and lymphocytes T express kinins receptor [31].

1.2.3 Pathophysiology of kinins: BK-related hyperalgesia

Because non-myelinated afferent neurons possess receptors, kinins stimulate nociceptive nerve and cause pain [32]. Kinins can sensitize other neurons to various stimuli [33].

1.2.4 Pathophysiology of kinins: the etiopathogenesis of BK-related angioedema

Angioedema (AE) is induced by a leakage of microvasculature of deep cutaneous tissues. There are various BK-related AE, depending on the trigger(s) and on individual etiopathogeneses. They result from uncontrolled activation of plasma protein cascade systems and more rarely by abnormal kinin catabolism which cause an acute accumulation of BK.

1.2.4.1 Symptoms of BK-mediated AE

BK-mediated angioedema is characterized by episodic and recurrent hard, pale and non-itching swellings involving subcutaneous (s.c.) or submucosal tissues: extremities, the gastrointestinal tract (GIT), face and lips including the upper respiratory tract. While swellings of the extremities, face and lips are disfiguring, GIT attacks induce violent pain [34] and obstruction of the upper airways is potentially life-threatening [35].

1.2.4.2 Classification of BK-mediated angioedema

BK-mediated angioedema are inherited (HAE), acquired (AAE), or iatrogenic (Table 2).

1.2.4.2.1 The hereditary forms of BK-mediated AE

HAE types I and II are associated with C1inh deficiency (HAE-C1Inh), either quantitative (Type I) or qualitative (Type II). The lack-of-function results from about 400 mutations in the SERPING1 gene ([36] and URL http://hae.enzim.hu/). Another HAE is associated with a gain-of-function of the plasma kinin formation capacity [37]; it is associated with normal or slight decrease in C1inh function. HAE type III is defined with missense mutation or deletion in the F12 gene encoding FXII [38,39]. In the majority of the gain-of-function cases, the genetic defect remains unexplained so far. In addition low kinin catabolism due to the defective BK/des-Arg9BK degrading enzymes represents an undefined situation of HAE, as demonstrated by the loss of CPN activity [40]. The defective APP and DPPIV activities are most often associated with iatrogenic AE [16,41] or with a risk factor for the development of severe form of HAE [42,43].

1.2.4.2.2 The acquired and iatrogenic forms of BK-mediated AE

AAE are associated with loss of C1inh function and normal production of C1inh but with an excessive C1inh consumption associated with lymphoproliferation and anti-C1inh autoantibodies [44]. The distinction between AAE type I and type II seems artificial and is not appropriate for AE diagnostic and decision of icatibant (Box 1) application. Application of ACEi [45], angiotensin II receptor blocker [46] or gliptin [47] therapies can precipitate BK-associated AE. Patients under ACEi with a history of AE showed a prolonged half-life of des-Arg9BK [48], reduced plasma APP [41] or DPPIV [49] activities, as compared to patients with no AE history. Iatrogenic AE might develop in conditions with elevated inherited risk [16].
1.2.4.3 Registered and off-label therapeutic options of BK-mediated AE

Treatment modalities of BK-AE are short- or long-term prophylaxis and treatment of acute attacks.

For long-term prophylaxis of HAE and AAE, attenuated androgens and antifibrinolytics are already used for decades. Attenuated 17α-alkylated androgens such as danazol or stanozolol have been used in HAE while their use in AAE is controversial because of the mainly proliferative character of the underlying disease. Danazol increases twice both the expression of the SERPING1 gene [50] and C1inh function and concomitantly ten-fold the APP activity contributing to a more effective control of the BK degradation [42]. Unfortunately, androgens potentially develop significant side effects [51]. Antifibrinolytic agents such as tranexamic acid could be used off-label as long- or short-term prophylaxis or as treatment for moderate attacks. The target of antifibrinolytic agents in AE is likely dependent on the amplification of kinin formation, i.e., through tissue plasminogen activator (tPA)–plasminogen interaction [52] with subsequent activity of plasmin on kinin release [53].

C1inh replacement therapy is used for short- and long-term prophylaxis [54]. Because of its constrained application (i.e., administration, potential hypersensitivity reactions), it is rarely used in prophylaxis but more adapted to the treatment of attacks [55].

Treating HAE attacks needs an adequate medication with the self-administration. This will give patients the independence they expect with improved quality of life: B2R antagonist (icatibant) [56], inhibitor of KK (ecallantide, not approved for self-administration), C1inh concentrate, and recombinant C1inh.

2. Icatibant, a unique therapy option for BK-mediated AE: target, properties, trials

Today the armamentarium to treat BK-mediated AE is astonishingly rich. In its mode of action, icatibant strongly differs from the other drugs that have an application for AE treatment.

2.1 History and drug design development (Figure 1)

Icatibant was primarily developed by Hoechst with the first acronym HOE-140.

HOE-140/icatibant is a synthetic decapeptide of comparable structure to BK containing five non-proteinogenic amino acids. It is developed as a competitive antagonist specifically and selectively to the B2R.

A first generation of B2R antagonists was developed from BK by substitution of Pro⁷ by D-Phe⁷, the D-aromatic amino acid residue changes the steric conformation of the C-terminal dipeptide (Phe⁸-Arg⁹) of the BK analogue, whereby D-Phe⁷ is becoming a partial agonist of the B2R [57]. The D-amino acid at position 7 also protects the peptide from ACE activity. In the second generation of B2R antagonist, increased specificity and affinity for BK receptors is achieved.
Figure 2. Ligand–receptor interactions. A. Non-inflammatory situation, only B2R is expressed and is associated with the BK/KD-dependency on the endothelium permeability. B. Inflammatory situation. The B1R expression is induced by inflammatory circulating cytokines, e.g., TNF-α, IL-1β and IL6. Both B2R and B1R are involved in the permeability process induced by the kinins. Aminopeptidase M (APM); Carboxypeptidase N/M (CPN/M).
Table 1. Ligand affinities (Ki) evaluated on human cloned B1R and B2R transiently expressed in 293 cells (B1R) and in COS-7 cells (B2R) from binding competition with 0.1 to 5 nM $[^3H$Lys-des-Arg<sup>9</sup>BK] for B1R [87] and with 100 pM $[^3H]$BK for B2R [88].

<table>
<thead>
<tr>
<th>Ki values of kinin binding to human receptors</th>
<th>B1R [87]</th>
<th>B2R [88]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK</td>
<td>&gt; 10 000</td>
<td>0.54</td>
</tr>
<tr>
<td>KD</td>
<td>2.54</td>
<td>0.63</td>
</tr>
<tr>
<td>Des-Arg&lt;sup&gt;9&lt;/sup&gt;BK</td>
<td>1 930</td>
<td>8 100</td>
</tr>
<tr>
<td>Des-Arg&lt;sup&gt;9&lt;/sup&gt;KD</td>
<td>0.12</td>
<td>&gt; 30 000</td>
</tr>
<tr>
<td>HOE-140</td>
<td>437</td>
<td>0.41</td>
</tr>
<tr>
<td>Des-Arg&lt;sup&gt;9&lt;/sup&gt;HOE140</td>
<td>17.4</td>
<td>-</td>
</tr>
</tbody>
</table>

B1R: B1 receptor; B2R: B2 receptor; BK: bradykinin; KD, kallidin.

by the replacement of Phe<sup>5</sup> by a Thienylalaline, containing an aromatic group of higher aromatic properties than Phenylalanine, and by the hydrophobic nature of replaced amino acids residues D-tetrahydroisoquinoline carboxylic acid at position 7 (D-Tic<sup>7</sup>) and L-Octahydroindole-2-carbonyl at position 8 (Oic<sup>8</sup>) of BK [59] [60]. The C-terminal L-Arg is important but not required for the binding to B2R; its substitution by D-Arg maintains the binding capacity but inhibits its susceptibility to cleavage by APP. The substitution by Oic<sup>8</sup> provides resistance to cleavage by CPN. Resistance to APP and CPN increases peptide half-life in vivo. Substitution of Pro<sup>3</sup> by L-hydroxyproline (Hyp<sup>3</sup>) enhances the antagonist effect of HOE-140/icatibant.

2.2 Mechanism of action: receptor targeting
HOE-140/icatibant is a potent B2 antagonist with high affinity and specificity for the kinin receptors. It is more selective for the B2R relatively to the B1R (Table 1). Many questions of the antagonism mechanism still remain under debate. The competitiveness was demonstrated by Marceau et al. using human umbilical vein [61]. However, the log dose response obtained by Rhaleb et al. using rabbit jugular vein [62] and Cockcroft et al. using human forearm [63] suggests that HOE-140/icatibant is a non-competitive antagonist. This discrepancy is apparently due to the assay system and could be explained by distinct transduction processes [64], and different basal desensitization of the B2R [65]. A non-parallel change in receptor affinity observed from B2R mutation studies suggests that BK and HOE-140/ icatibant do not share the same binding site on B2R [66], strengthening a non-competitive antagonism hypothesis.

Besides, data are not consistent regarding the competitive model between icatibant and KD.

2.3 Pre-clinical studies
2.3.1 In vitro studies
2.3.1.1 Animal organ models
In isolated organs, HOE-140/icatibant inhibits the BK-mediated vasoconstriction or vasodilatation, prostaglandin E2 (PGE2), PG12, NO releases and decrease of the reflex fall in systemic blood pressure upon nociceptor stimulation [67,68].

HOE-140/icatibant is specific for BK pathway since it is ineffective on smooth muscle preparation regarding the effects of angiotensin II, substance P, neurokinin A, des-Arg<sup>9</sup>BK, noradrenaline or acetylcholine [62]. Selectiveness of HOE-140/icatibant for B2R is further supported because it is inactive in isolated rabbit aorta which expresses only the B1R [68,69]. Taken together these data demonstrate HOE-140/icatibant being a potent BK antagonist with high affinity, specificity and selectivity for the B2R.

The published results of the effects of HOE-140/icatibant need to be correctly interpreted with restriction to the tissue- and species-specificity. For example HOE-140/icatibant has agonist property regarding protein extravasation in rats (5µmol/kg), contraction of guinea pig ileum (10<sup>-4</sup>M) [68] or contraction of sheep femoral artery with or without endothelium (10<sup>-8</sup>M) [69], but has no effects on PGE2 release (2 × 10<sup>-5</sup>M) in rabbit perfused ear [67], on rat uterus contraction [68] or in rabbit jugular vein or aorta contraction (10<sup>-6</sup>M) [69].
Repeated s.c. dose studies have induced mild nephrotoxicity (10mg·kg⁻¹·day⁻¹ in rats), vomiting, swelling and erythema (10, 30 and 60mg·kg⁻¹·day⁻¹; dogs), diarrhea (60mg·kg⁻¹·day⁻¹; dogs) and genital toxicity (1 and 3.3 mg·kg⁻¹ three times a day on two days per week for 52 weeks). The NOAEL (No Observed Adverse Effect Level) was determined at 10mg·kg⁻¹·day⁻¹ s.c. in rats in a 13 week study [75], a very high level established in rodents compared to the NOAEL in the dog (400-fold less) [76].

In female rats and rabbits, HOE-140/icatibant (10mg·kg⁻¹·day⁻¹) increases rate of pre-implantation loss. However, no teratogenic effects or organ malformation and malfunction were observed. Mutagenic tests revealed no abnormal parameter.

### 2.4 Clinical trials

#### 2.4.1 Phase I trials

The effect of icatibant was studied on BK-induced vasodilatation in healthy human forearms. In a randomized, double-blind case-control study, icatibant (20, 50, or 100 µg·kg⁻¹ i.v.) was able to inhibit the BK (10, 30, 100, 300, 1 000, 3 000ng·min⁻¹ i.v.) effects in a dose-dependent manner [63].

#### 2.4.2 Phase II and III clinical trials

In a first proof-of-concept, a Phase II study with 20 attacks in 15 HAE-C1inh patients were either treated i.v. with 0.4 or 0.8mg·kg⁻¹ and s.c. 30 or 45 mg. Several outcome evaluations were used to demonstrate efficacy, including the reduction of symptom intensity in the 10-cm visual analog scale (VAS) 4h after the beginning of the treatment. The results showed that efficacy was similar for 0.8mg·kg⁻¹ i.v. and 30mg s.c. [74], with retaining the 30mg s.c. as dosage for the Phase III studies [77-79].

Icatibant demonstrated clinical efficacy and safety to treat cutaneous, abdominal and laryngeal attacks in HAE-I/-II adults in the three pivotal FAST (For Angioedema Subcutaneous Treatment) studies. Simultaneous FAST-1 and -2 (56 and 74 patients, respectively) multicenter trials were double-blind and randomized studies. FAST-1 was placebo-controlled, and in FAST-2, the effect of icatibant was compared to that of tranexamic acid. The primary endpoint was the median time to significant relief of symptoms (>30%) and based on VAS of one of the three main symptoms (abdominal pain, cutaneous swelling or pain). Laryngeal attacks were evaluated separately in an open-label part of the studies.

FAST-1 failed to demonstrate a significant difference between icatibant and placebo with regards to the time to clinically significant relief of the index symptom (2.5h with icatibant and 4.6h with placebo). The FAST-2 trial demonstrated a significant higher efficacy of icatibant compared to tranexamic acid: the median time to clinically significant relief of the index symptom was significantly shorter in the icatibant group (2h) compared to the tranexamic acid arm (12h) (p < 0.001). Attention must be paid to the dose of tranexamic acid used (3g daily for 2 days), which is inferior to the dosage commonly used and stated in the recommendation of tranexamic acid treatment (4-6g, daily) [80].

In 2008, icatibant got marketing authorization in Europe (trade name Firazyr™), while Food and Drug Administration (FDA) required an additional randomized, double-blinded placebo-controlled study, the FAST-3 trial. In this study icatibant demonstrated a significantly faster symptom relief than placebo (2.0 and 19.8h for the median time to 50% reduction in symptom severity, respectively).
In summary, significant efficacy was demonstrated vs tranexamic acid in FAST-2 and vs placebo in FAST-3 in the treatment of acute moderate-to-severe attacks. Several post hoc analyses were undertaken to understand the differences between both studies. The assessment of the only index symptom in FAST-1 as a primary endpoint was questioned, and major concerns on the validity of VAS in both trials were raised. The discrepancy between FAST-1 and FAST-3 results remains puzzling but may be due to the use of suboptimal outcome criteria. The FDA stated that there was "consistent evidence that median time to onset of symptom relief was about 2 hours", in accordance with the clinical experience of most experts in the field.\textsuperscript{[81]}

2.5 Indications, pharmacology, and pharmacokinetics

2.5.1 Indications

2.5.1.1 Authorized applications

Icatibant is approved for the treatment of adult cutaneous, abdominal or laryngeal attacks of HAE, with country-specific applications (Table 3).

2.5.1.2 Reported off-label applications

Icatibant is not indicated for prophylaxis\textsuperscript{[56]}; however, icatibant has been documented being used in short-term prophylaxis\textsuperscript{[82,83]}. Furthermore, it has been successfully developed in HAE type III\textsuperscript{[84]}, in some ACEi-induced iatrogenic angioedema\textsuperscript{[85]}, in prevention of anaphylactic reactions to ACEi during LDL-apheresis\textsuperscript{[86]} and in AAE attacks\textsuperscript{[87]}.

2.5.2 Pharmacology

2.5.2.1 Dose\textsuperscript{[88]}

The Phase I clinical studies identified 0.4mg·kg\textsuperscript{-1} as the minimum dose with an effective concentration during 6 - 8h and 1.6mg·kg\textsuperscript{-1} as the maximum tolerated dose. At 0.4mg·kg\textsuperscript{-1}, C\textsubscript{max} (maximum concentration) is rapidly attained, representing 50- to 100-fold the EC\textsubscript{50} (half maximal effective concentration) values necessary for inhibition of BK-induced effects (Table 4).\textsuperscript{[81]} In healthy individuals, single i.v. application of 0.4mg·kg\textsuperscript{-1} was established to be equivalent to a single 30 mg s.c. dose\textsuperscript{[89]}, the retained dosage to treat HAE attacks by the agencies. In most cases a single injection is sufficient, but in cases of insufficient relief or recurrence of symptoms, a second injection can be administered 6h later. The prescribing information mentions no more than 8 injections per month.

2.5.2.2 Formulation

Icatibant is fully synthesized by a solid phase peptide synthesis process and does not contain any material or proteins of human or animal origin.

2.5.2.3 Administration

Both i.v. and s.c. administrations have been studied\textsuperscript{[88,90]} at equivalent exposure and with similar efficacy. The s.c. application route was pursued later because the C\textsubscript{max} is inferior to C\textsubscript{max} when i.v. applied and this reduces the risk of overdosing.

HAE attacks have a lot of consequences on patient’s quality of life because they are unpredictable in their location and their severity\textsuperscript{[91]}. Like all other drugs, self-administration at home considerably improves patient quality of life\textsuperscript{[56,92]}. The study EASSI (Evaluation of the Safety of Self-Administration) included 56 patients and proved safety and convenience of the self-administration of icatibant in acute HAE attacks. Prior entering the study all patients received comprehensive training on the self-injection technique. The EASSI trial showed self-administration of icatibant in acute HAE attacks being well tolerated and resulted in symptom relief for 96.2% of self-treated attacks. A majority of patients (94.6%) preferred the self-administration procedure and most patients (87.5%) found the self-administration technique easy and comfortable\textsuperscript{[93]}. S.c. administration has been authorized in European member states (Table 3).

Other experimental routes of application such as intranasal\textsuperscript{[94]}, inhaled\textsuperscript{[95]} and intra-articular\textsuperscript{[96]} have been evaluated.

---

**Table 3. Icatibant application authorizations and indications around the world.**

<table>
<thead>
<tr>
<th>Treatment of acute attacks of hereditary angioedema in adults with C1-esterase-inhibitor deficiency</th>
<th>USA</th>
<th>European Union, Lichtenstein, Norway, Iceland</th>
<th>Argentina, Australia, Israel, Brazil, Mexico, Russia, Switzerland.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of acute attacks of hereditary angioedema in adults</td>
<td>Approved</td>
<td>Approved</td>
<td>Documented but non-approved</td>
</tr>
<tr>
<td>Self-administration</td>
<td>Approved</td>
<td>Documented but non-approved</td>
<td>Approved</td>
</tr>
<tr>
<td>Treatment of acute attacks of acquired angioedema</td>
<td>Documented but non-approved</td>
<td>Documented but non-approved</td>
<td>Documented but non-approved</td>
</tr>
<tr>
<td>Treatment of acute attacks of iatrogenic angioedema</td>
<td>Documented but non-approved</td>
<td>Documented but non-approved</td>
<td>Documented but non-approved</td>
</tr>
</tbody>
</table>

For personal use only.
2.5.2.4 Precautions for use
Because BK develops cardioprotective effects in myocardial ischemia [26], its antagonist icatibant must be used with caution in patients suffering from acute ischemic heart disease, unstable angina pectoris or after stroke. Icatibant in its present formulation is not authorized in pediatric patients (ongoing current clinical trials NCT01386658), and during pregnancy and lactation.

2.5.2.5 Drug interaction
Icatibant metabolism is independent from cytochrome P450 (CYP450) activity, without any inhibitor or inducer property toward CYP450. Accordingly, no such drug–drug interaction has been observed.

The beneficial antihypertensive effects of ACEi therapy are depending on the well-dosed inhibition of BK degradation. Since icatibant develops BK antagonism capacity, it decreases the mean arterial pressure in response to ACEi, in rat [28], dog [97] and human [98]. This subsequently diminishes the efficacy of ACEi therapy.

2.5.2.6 Adverse effects
The 30mg s.c. icatibant administration was well tolerated with a consistent safety profile across all clinical studies.

The most common adverse event reported in clinical trials was the recurrence within 48h of the first attack or the worsening of an ongoing attack [79,81]. In uncontrolled pilot study from eight patients submitted to s.c. icatibant, two individuals (25%) suffer from a recurrent attack 20h after treatment [90]. In the FAST-1 and -3 trials, the rates of recurrence are 13% (13 events/103 attacks) [77] and 10.9% (5/46) [79], respectively, for up to 48h after administration. These percentages are confirmed in routine use of icatibant, e.g., the recurrence rate of 23.2% (13/56) is observed from routinely self-treated patients in a recent report [99]. Almost all patients in clinical studies receiving icatibant s.c. experienced localized injection site reaction including itching, erythema, swelling and burning, with its etiopathology developed above. These reactions were described being mostly mild or moderate in severity and were spontaneously resolved within 20min up to few hours [64,75,100]. Other adverse events include diarrhea, nausea, vomiting, dyspepsia, headache, asthenia, elevated alanine aminotransferase and respiratory affection [75]. It affected a part of the patients: 14% for FAST-1 and -2 and 41% for FAST-3.

Generalized and hypotension reactions because of the partial agonist effect of icatibant represent a theoretical risk, nevertheless the s.c. application of 30mg does not increase blood pressure and has no effect on heart rate [78]. But severe acute ischemia and angina pectoris foreseeingly contraindicate icatibant application. BK is cardioprotective. As yet no cardiac complication of frequent use of icatibant has been reported. However, the observation period might not be long enough for an appropriate evaluation.

Hypersensitivity to icatibant is also a potential risk even if icatibant has been recognized as weak sensitizer. From immunogenicity testing in FAST-1 and -2, three patients developed anti-icatibant IgG but its efficacy is retained in all patients. No hypersensitivity or anaphylactic reaction was reported from the Phase III studies [81].

Loss of efficacy after sequential repeated administration of icatibant has not been observed even in patients treated for more than 100 attacks [101].

2.5.3 Pharmacokinetics (Table 5)
Pharmacokinetic parameters of both s.c. and i.v. injections were comparable, from the AUCC values (area under the plasma concentration–time curve from time zero to signal extinction) of 3 114h-ng·mL⁻¹ and 3 208h-ng·mL⁻¹, respectively [102].

For a single s.c. injection of 10mg·mL⁻¹ icatibant, 96.1% of the dose is absorbed, resulting in maximal blood concentrations of 1 429ng·mL⁻¹ which was reached approximately within 0.5h. This fast absorption favors a rapid relief of symptoms. Plasma clearance after s.c. administration was 245 ± 58mL·min⁻¹ with a mean elimination half-life (t1/2) of 1.4 ± 0.4h [89]. There is no evidence of accumulation of icatibant after three doses administrated each 6h. Inter-individual variation in absorption rate might vary up to 25% [75].

The duration of the therapeutic effect of icatibant is quite independent on the administrated dose. This invariance is the reason why it is preferable to administer a second dose instead of elevating the dose of a single injection [75].

2.5.3.1 Distribution
The binding to blood plasma proteins is low (44% in human) [89], with no competition with drug carried by plasma proteins during the treatment. The distribution volume of 20 – 25L corresponds to an extended tissue distribution [102]. Animal studies using [³H]icatibant indicate that it is poorly distributed into adipose tissue and does not cross the blood–brain barrier. Icatibant crosses over the rat’s placenta and it is secreted into rat milk [75].
Icatibant parameters of strategic importance in human application

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-inf} (h·ng·mL⁻¹)</td>
<td>3 114</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>96.1</td>
</tr>
<tr>
<td>C_max (ng·mL⁻¹)</td>
<td>1 429</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>0.5</td>
</tr>
<tr>
<td>T_{1/2} (h)</td>
<td>1.4</td>
</tr>
<tr>
<td>Volume of distribution (L)</td>
<td>20 - 25</td>
</tr>
<tr>
<td>% binding to plasma proteins</td>
<td>44</td>
</tr>
</tbody>
</table>

AUC_{0-inf}: Area under the plasma concentration-time curve from time 0 to infinity; C_max: Maximum icatibant concentration; T_{1/2}: Half life; T_max: Times of maximum concentration.

2.5.3.2 Metabolism/elimination

Icatibant metabolism is independent on CYP450. Even if it is resistant to the three major endothelial proteolytic enzymes involved in BK degradation (ACE, APP and CPN), it remains a substrate for some unidentified proteases [76]. Icatibant is mainly eliminated after metabolism under two inactive metabolites: icatibant[1-5] (M1) and icatibant[7-10] (M2) in the rat, dog, and human. The unchanged drug is eliminated in urine with less than 10% of the dose [102]. Studies indicated that age, but not weight, gender, renal or hepatic insufficiency affected icatibant pharmacokinetics [75].

3. Conclusion

Icatibant is a decapeptide resistant to the BK-degrading proteases, resulting in a longer half-life than BK which it antagonizes. Icatibant exhibits efficacy in BK-mediated angioedema attacks, and induces rapid symptom relief. However, its short half-life is not pertinent for its application in the prophylaxis of HAE.

4. Expert opinion

Icatibant can be considered as a promising drug with non-understandable adverse effects. The total BK production (=1µmol) from the nearly complete HK cleavage is considerably lower than the dose of the antagonist in its current application (23µmol), as calculated in [43] (Table 4) in a molar excess around 21.3 for icatibant. This excess might contribute to the competition toward other B2R peptide ligands likely to participate in angioedema, e.g., KD. No investigation has firmly established the impact of KD in angioedema, for the in vivo or in vitro production capacity and the competition with icatibant.

As a consequence of icatibant application, BK is displaced from the nearly complete HK cleavage is considerely lower than the dose of the antagonist in its current application (23µmol), as calculated in [43] (Table 4) in a molar excess around 21.3 for icatibant. This excess might contribute to the competition toward other B2R peptide ligands likely to participate in angioedema, e.g., KD. No investigation has firmly established the impact of KD in angioedema, for the in vivo or in vitro production capacity and the competition with icatibant.

As a consequence of icatibant application, BK is displaced into the soluble compartment and transformed by CPN/CPM into a B1 ligand. This occurrence may explain some of the recurrent attacks post icatibant with the prerequisite that the B1 receptor is expressed (chronic inflammation, cigarette smoker, chronic viral infections, mast cell activation, and other not defined conditions).

Icatibant is a selective B2R antagonist of BK. Kinin system involves other biologically active components such as KD, des-Arg¹^10-KD, des-Arg¹ BK and B1R as showed in vitro by F Bossi (University of Trieste) with the requirement of a B1R antagonist in addition to icatibant for the total inhibition of BK-induced permeability [25]. The current reported clinical observations with icatibant might indicate the involvement of the B1R pathway at occasion of some of the recurrences of attacks post treatment. It is hypothesized that some of the recurrence might occur in the context of (subclinical) inflammation and accumulation of B1R agonists. Such events might be prevented by supplementation by a B1R antagonist as recommended by Bossi et al. [25].

Icatibant is registered in the USA and in European countries for the treatment of acute attacks of HAE and for self-administration at home. There is a rationale to extend registration to HAE with normal C1inh expression and AAE, all suggested to be mediated by acute increase of BK production, but as stated above, taking precaution in its application during the inflammatory processes.

Icatibant has been proposed to treat inflammatory situations in non-HAE patients [103], e.g., BK-mediated formation of exudates where the kallikrein-kinin system can be massively activated [104]. In animals, icatibant has been investigated in pancreatitis treatment [105], pain [106], liver cirrhosis [107], central nervous system injury [108], ulcerative colitis [109], allergic inflammation associated with airway hyper-reactivity [110] and to prevent diabetic nephropathy [111], suggesting a deleterious role of the kallikrein-kinin system in these murine disease models. In humans intranasal, icatibant was tested to treat allergic rhinitis [94] and asthma with the inhaled formulation.

A third generation of B2R antagonists of BK is under development [60,112,113], because the second generation like icatibant is limited by its injectable form of application and its rapid elimination. New non-peptide B2R antagonists under development are already tested in vitro and in animal studies [61]. Their capacity to antagonize BK has been demonstrated, extending the data obtained with icatibant. This new drugs have potential for further kinin antagonism.

Acknowledgements

The authors are indebted to Prof Bernd Rosenkranz (University of Stellenbosch, Cape Town South Africa) for advice, Dr MV Chuong Nguyen (Université Joseph Fourier Grenoble, France) for critical reading of the manuscript and to I Andresen (Shire HGT, Switzerland) for comments. Shire Human Genetic Therapies was given the opportunity to review this manuscript for scientific accuracy, but the opinions and conclusions remain those of the authors and do not necessarily reflect the views of Shire HGT.
Declaration of interest

This paper was supported by the University Joseph Fourier Grenoble, the UNAM University, France, the University of Bern Switzerland and EU Erare 2007, grant acronym HAEIII. D Charignon and C Drouet declare no conflict of interest. P Spaeth participates by virtue of his previous employment in the employee share plan of CSL Ltd. L Martin has received honoraria from CSL Behring and Shire HGT.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (★★) to readers.


• This paper reports the global view of both BK and des-Arg9-BK metabolic pathways in human and determines the impact of ACEi on their half lives.


• The first description of XPNPEP2 polymorphism with low plasma APP activity associated with angioedema induced by ACEi.


• Describes impact of Aminopeptidase M on B2R agonist conversion into B1R agonist in vessels.


D. Charignon et al.


- This article demonstrates the impact of plasma B2 and B1 ligands on the endothelial permeability, with the interest of B1R in angioedema.


- Provides an overview of the role of bradykinin in cardiovascular disorders.


32. Rang HP, Bevan S, Dray A. Chemical


- Identification of 52 mutations on SERPING1 from 127 individuals suffering of hereditary angioedema.


- The first demonstration of the association between missense mutation in F12 gene and the increased amidase activity in a new hereditary angioedema context.


- First description of CPN1 mutation associated with CPN enzyme activity defect and angioedema symptoms.


- Describes the first relationship between ACEi induced angioedema and low APP activity.


- Describes the impact of the low dipeptidyl peptidase IV activity in ACEI induced angioedema.


Icatibant


- Summarizes market authorization obtained for Firazyr™ (icatibant) by the European Commission.


- Describes the evolution of kinin receptor antagonists.


- Describes the evolution of B2R antagonists.


- First trials of icatibant in human with inclusion of eight healthy normotensive men.


- Characterization of icatibant properties in human in vitro models.


- Characterization of icatibant properties in animal in vivo models.


- Characterization of icatibant properties in animals in vivo models.


- This report describes the comprehensive data of FAST-1 and FAST-2 clinical trials.


- This report describes the data of the FAST-3 trial.


81. Advisory Committee Meeting Briefing Package. Firazyr® (icatibant) NDA 22.150. 2011


85. Bas M, Greve J, Stelzer K, et al. Therapeutic efficacy of icatibant in angioedema induced by...
D. Charignon et al.


91. Toscani M, Riedl M. Meeting the challenges and burdens associated with hereditary angioedema. Manag Care 2011;20:44-51


95. Akbari AM, Wirth KJ, Scholkmens BA. Efficacy and tolerability of icatibant (Hoe 140) in patients with moderately severe chronic bronchial asthma. Immunopharmacology 1996;33:238-42


100. Maurer M, Church MK. Inflammatory skin responses induced by icatibant injection are mast cell mediated and attenuated by H1-antihistamines. Exp Dermatol 2012;21:134-5


102. Deeks ED. Icatibant. Drugs 2010;70:73-81

• Compilation of pharmacological and pharmacokinetic data on icatibant.


** This review reports progress on kinins and their receptors with the history and the updated knowledge of the kinins.

Affiliation
Delphine Charignon¹ MSc, Peter Späth² PhD, Ludovic Martin³ MD PhD & Christian Drouet⁴ PhD
¹Author for correspondence
¹Université Joseph Fourier Grenoble 1, GREPI/AGIM CNRS-UJF FRE 3405 and Centre de Référence des Angioédèmes CREAK, CHU Grenoble POBox 217, F-38043 Grenoble France
²Institut für Pharmakologie, Universität Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland
³Université d’Angers, Dermatologie, Centre de Référence des Angioédèmes CREAK, CHU Angers, F-49933 Angers, France
⁴Professor, Université Joseph Fourier Grenoble 1, GREPI/AGIM CNRS-UJF FRE 3405 and Centre de Référence des Angioédèmes CREAK, CHU Grenoble POBox 217, F-38043 Grenoble France
Tel: +33 476 767 201; Fax: +33 476 766 251; E-mail: christian.drouet@ujf-grenoble.fr