Review

Drugs of the Kallikrein-Kinin System: An Overview

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Abstract: The kallikrein-kinin system consists of the two kininogen substrates, present in blood plasma, and of two serine proteases, the plasma and tissue kallikreins. The action of the latter on kininogens produce small peptides, the kinins, short lived but endowed by powerful pharmacologic actions on blood vessels and other tissues. Many recent and exciting therapeutic developments in the field are briefly summarized. Notably, various novel strategies are being clinically developed to inhibit the formation of bradykinin or block its receptors in the management of hereditary angioedema. The interventions include orally bioavailable drugs, biotechnological proteins, and gene therapy. These approaches are currently explored in a variety of other inflammatory and thrombotic disorders. Harnessing controlled kinin formation is also of potential therapeutic interest as shown by the clinical development of recombinant tissue kallikrein for ischemic stroke and renal disease. Biomarkers of a kinin-mediated disorders, frequently implicating edemas, include the consumption of kininogen(s), plasma kallikrein activity, and the detection of circulating kinin metabolites such as fragments BK_{1-5} and BK_{2-9}. Novel opportunities to clinically apply the underexploited drugs of the kallikrein-kinin system are briefly reviewed. This personal perspective is offered by an observer of, and a participant in drug characterization during the last 4 decades.

Keywords: kallikrein-kinin system; kininogens; bradykinin; B1 receptor; B2 receptor; hereditary angioedema

1. The kallikrein kinin systems: formation and clearance of kinins

Both protective and pathogenic effects are mediated by two largely separate kallikrein-kinin systems (KKS; abbreviations are defined in Table 1) via the formation of small and unstable peptides, the kinins (Fig. 1, schematic representation). Thus, vascular effects (vasodilation, increased microvascular permeability), inflammatory manifestations (edema, pain, increased local blood flow), smooth muscle contraction, and epithelial cell stimulation are potentially initiated by kinins [1]. The nonapeptide bradykinin (BK; H-Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9-OH) is the reference kinin sequence found in domain 4 of two circulating proteins, the high molecular weight and low molecular weight kininogens (HK, LK; about 20 and 80% molar proportions, respectively). The hepatic synthesis of both kininogen forms is based on the alternative splicing of a single gene product, *KNG1*.

HK (110 kDa), circulating in a complexed form with prekallikrein (85 had) and factor XI, is part of the contact system (Fig. 1) along with coagulation factor XII (FXII, Hageman factor, 80 kDa). When exposed to negatively charge surfaces, such as the basal membrane of denuded vascular endothelial cells, all these components assemble into a tetramolecular complex with ensuing proteolytic reactions: the mutual activation of FXII and prekallirein into their proteolytically active forms factor XIIa (FXIIa) and plasma kallikrein, respectively, the cleavage of HK releasing BK and the cleavage of factor XI that initiates the intrinsic coagulation pathway [2]. The contact system is tightly controlled by a circulating serpin inhibitor, C1-esterase inhibitor (C1INH, 105 kDa) that is also part of the complement cascade. FXIIa and plasma kallikrein are irreversibly inhibited by C1INH [3]. Blood clots are cleared by the fibrinolytic system which is connected to the contact system (Fig. 1): plasmin, the fibrinolytic enzyme, activates FXII into FXIIa to a certain extent, indirectly promoting BK

production via secondarily activated plasma kallikrein. C1INH is a secondary inhibitor of plasmin [3]. Whether HK is directly cleaved by additional proteases has been suggested, but not well established in whole blood where endogenous inhibitors are present: plasmin and the complement associated protease MASP-1 may directly release BK from HK [4,5]. There is no evidence of BK release when platelets or neutrophils are activated in human whole blood [6], casting a doubt about previously suggested activation pathways demonstrated using purified components of the contact system (e.g., that triggered by polyphosphate nanoparticles from platelets [7]).

Table 1. List of abbreviations.

Abbreviation	Standing for	Corresponding gene
ACE	angiotensin-I converting enzyme	*ACE*
	angiopoietin 1	*ANGPT1*
APN	aminopeptidase N	*ANPEP*
Arg-CP	arginine carboxypeptidase	
B1R	bradykinin B1 receptor	*BDKRB1*
B2R	bradykinin B2 receptor	*BDKRB2*
BK	bradykinin	
C1INH	C1-esterase inhibitor	*SERPING1*
D6	Domain 6 of HK	
FXII	coagulation factor XII	*F12*
FXIIa	activated factor XII	
HAE	hereditary angioedema	
HAE-C1INH	HAE caused by C1INH haplodeficiency	
	heparan sulfate-glucosamine 3-sulfotransferase 6	*HS3ST*
HK	high molecular weight kininogen	*KNG1*
KKS	kallikrein-kinin system	
KLK-1	tissue kallikrein	*KLK1*
LK	low molecular weight kininogen	*KNG1*
Lys-BK	kallidin	
mAb	therapeutic monoclonal antibody	
MASP-1	mannan-binding lectin associated serine protease 1	*MASP1*
	myoferlin	*MYOF*
NPA	non-peptide antagonist	
	plasminogen	*PLG*
tPA	tissue plasminogen activator	*PLAT*
uPA	urokinase-type plasminogen	*PLAU*

Tissue kallikrein (KLK-1; kallidinogenase) is a member of a family of 15 secreted proteases encoded on human chromosome locus 19q13.4 [8]. These serine proteases assume different, often uncertain physiological functions. Only KLK-1 is a relevant kininogenase in this family. This was verified with two KLKs normally found in the prostate: they release no kinins from purified HK (KLK-3), or very little (KLK-2 is 1000-fold less active than KLK-1 in this respect) [9]. KLK-1 releases the biologically active decapeptide Lys-BK (= kallidin) from both forms of kininogen, but mostly from the more abundant LK. KLK-1 is widely expressed and abundant in the kidney, pancreas, salivary glands, lungs, blood vessels and other tissues; its secretion and activation, via the removal of a N-terminal sequence, are not well understood. KLK-1 is regulated by its own irreversible inhibitor, kallistatin (*SERPINA4* gene product). The previously claimed direct agonist effect of KLK-1 on human BK B2 receptor (B2R) has been disproved using the pure recombinant enzyme in its active form [10].

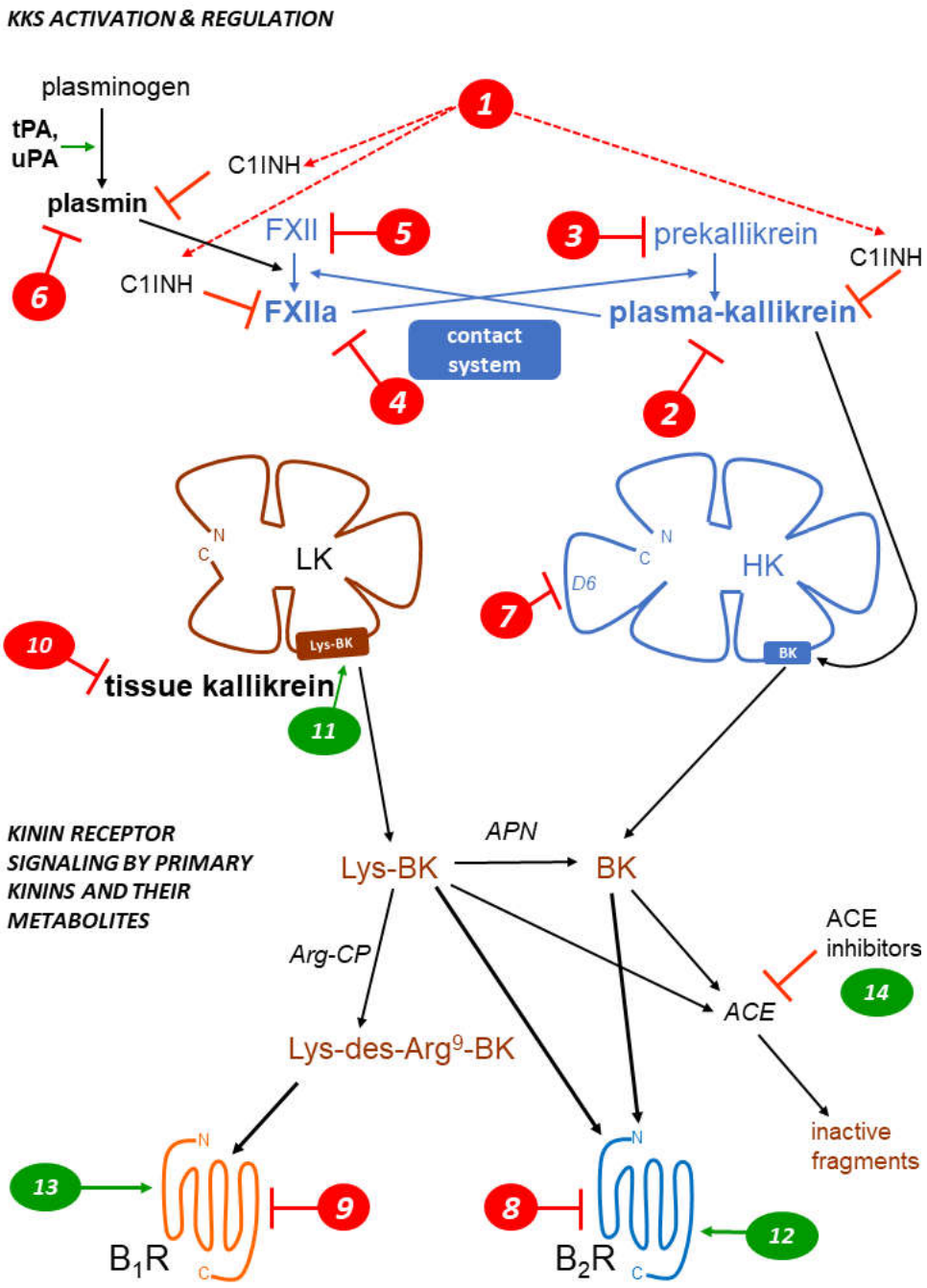

Figure 1. Schematic representation of the KKS, featuring the 2 validated pathways of kinin generation, that of plasma kallikrein (part of the contact system) releasing bradykinin (BK) from high molecular weight kininogen (HK) and that mediated by secreted tissue kallikrein (KLK-1), generating Lys-BK mainly from low molecular weight kininogen (LK). Two G protein coupled receptors (B1R, B2R) mediate the cellular effects of kinins. Three types of metallopeptidases that hydrolyze kinins are indicated (APN, Arg-CP, ACE). Numerical markers indicate the mode of action of inhibitory (red) or stimulatory drugs (green) of the KKS and are referred to in Tables 2-4 and main text. See Table 1 for abbreviations. Modified from [11, 12].

Kinins are inherently unstable, with a half-life well under 1 min [11], and metabolized by several metallopeptidases. An in vivo study showed that angiotensin converting enzyme (ACE, kininase II) is the dominant BK-inactivation pathway in rats, followed by aminopeptidase P [13]. Both peptidases inactivate BK, initially producing the fragments BK_{1-7} and BK_{2-9}, respectively. The fragment BK_{1-5} is the relatively stable product of a second cycle of BK_{1-7} cleavage by ACE. Lys-BK is also inactivated by

ACE. Aminopeptidase N (APN, CD13) can remove the N-terminal Lys residue from Lys-BK to produce BK [1]. The arginine carboxypeptidases (Arg-CPs), plasma carboxypeptidase N and glycosylphosphatidylinositol-linked carboxypeptidase M, remove the C-terminal Arg residue from BK and Lys-BK, producing des-Arg^9-BK (BK_{1-8}) and Lys-des-Arg^9-BK, respectively, also subsequently substrates of ACE [1]. Arg-CPs represent only a minor metabolic pathway if circulating kinins are considered [13,14], but may be important in inflammatory exudates. Crucially, Arg-CPs connect the KKS with the pharmacological profile of the kinin B1 receptor (B1R) selectively responsive to the des-Arg^9 metabolites of kinins (see below).

Biomarkers of kinin-mediated disorders include the consumption of kininogen(s) and the detection of circulating kinin metabolites such as fragments BK_{1-5} and BK_{2-9}, and the detection of plasma kallikrein activity, for instance using the synthetic substrate based on the C-terminal BK sequence HD-Pro-Phe-Arg-*pNA*. These assays are technically challenging, but one or more of them have been applied to hereditary angioedema (HAE), either during attacks or in remission [15,16], to other edematous conditions such as ascites secondary to liver cirrhosis [17] and chronic urticaria [18, 19] and to animal models of sepsis and sickle cell disease [20,21].

2. Kinin receptors

Before the era of molecular biology, the number and identity of kinin receptor subtypes in each mammalian species was uncertain. Historically, the first proposed kinin receptor subtype, the B1R, was discovered as the one mediating contraction in the isolated rabbit aorta in response to kinins based on classical pharmacologic criteria: a typical order of potency for agonists and antagonism by newly discovered peptide antagonists [22]. Native kinins (BK and Lys-BK) from which the Arg^9 residue has been removed by Arg-CPs (des-Arg^9-BK, Lys-des-Arg^9-BK, respectively) are the optimal agonists of the B1R, even if this kinin metabolic pathway is not prominent. Only Lys-des-Arg^9-BK, also called des-Arg^{10}-kallidin, has a subnanomolar affinity for the human (and rabbit) B1R [1]; this agonist is presumably generated from Lys-BK (kallidin), itself derived from the cleavage of kininogens by tissue kallikrein (Fig. 1), hence independently from the contact system. Early peptide antagonists, such as [Leu^8]des-Arg^9-BK, consolidated the pharmacological identity of the B1R; the other pharmacologic preparations, directly responsive to BK and Lys-BK but insensitive to the des-Arg^9 metabolites, were said to possess the still not fully defined B2R subtype. The first B2R antagonists were discovered in the early 1980's; they featured a constrained peptide backbone and were more or less protected from peptidases. Icatibant (Hoe 140; D-Arg-[Hyp^3, Thi^5, D-Tic^7, Oic^8]BK) is the success story among these early drugs [1,23] (Table 2). Selected modern, nonpeptide antagonists (NPAs) of both kinin receptor subtypes are presented in Fig. 2. It is very typical that BK receptor antagonists exhibit species-dependent alterations of affinity and competitive behavior for their pharmacological targets [1]; thus, clinically developed antagonists have gone through a structural optimization process for the human forms of the B1R or B2R [1,24].

The receptor classification was confirmed by the discovery of a kinin receptor locus: in human chromosome region 14q32, genes encoding the G protein coupled receptors B2R and B1R, respectively termed *BDKRB2* and *BDKRB1*, are found in tandem [25]; a similar organization is found in the genome of other mammals. While the expression of both genes is regulated, the B2R generally accounts for the in vivo effects of kinins in healthy laboratory animals: this receptor subtype is constitutively expressed in many cell types, including vascular endothelial cells, smooth muscle cells, some epithelia, sensory neurons, and other cell types [1]. The B1R, initially discovered in rabbit isolated blood vessels maintained in vitro for several hours, is not generally detectable in healthy animals. The paradox was resolved when the B1R was found to be expressed following tissue trauma, inflammation (such as the injection of bacterial lipopolysaccharide to animals) under the control of inflammatory cytokines (e.g., interleukin-1, tumor necrosis factor-α, interferon-γ) and signaling pathways (e.g., mitogen activated protein kinases, NF-κB, Jak/Stat) [25-28]. While B1R and B2R are structurally related, only the latter is phosphorylated, internalized, and recycled following agonist stimulation [1,25]. Thus, the B1R is potentially important in sustained inflammatory states and infectious disease. For instance, treatment with a B1R antagonist decreased mortality and mitigated cardiac inflammation and dysfunction in an experimental Chagas disease model in mice [29]. It does

not follow that B1R should be systematically blocked in tissue injury situations: for instance, the development of an adaptative collateral circulation is mediated by this receptor subtype following an arterial occlusion in a rodent model [30].

Both kinin receptor subtypes are coupled mainly to protein G_q and calcium signaling pathways. Those trigger smooth muscle contraction and vascular endothelial cell stimu-lation, including calcium-dependent prostanoid and nitric oxide release and plasma ex-travasation secondary the opening of endothelial junctions [1,25].

Fig. 1 is a schematic representation of the kinin-generating pathways and their re-ceptors; numerical markers indicate the mode of action of the numerous drugs of the KKS. Earlier achievements, such as the early peptide receptor antagonists, are reviewed else-where [31]. The present emphasis is on drugs that are currently in use, have reached clin-ical development (successfully or not), or are in preclinical development.

3. Pharmacological inhibition of the KKS: hereditary angioedema (HAE)

The therapeutic showcase of the KKS is presently hereditary angioedema (HAE), a rare disease most often caused by the haplodeficiency of C1INH: numerous mutations transmitted in an autosomal dominant manner are known in the corresponding gene SERPING1 [32]. HAE is characterized by recurrent episodes (attacks) of swelling due to fluid extravasation; limbs, the orofacial and genital areas, and the intestine can be affected. Attacks may be life-threatening (suffocation), painful and incapacitating. The physiopathology of HAE and its management have been recently reviewed [32-34]. While C1INH inhibits several proteases in the contact, fibrinolytic and complement systems, bradykinin is believed to be the ultimate mediator of HAE-C1INH attacks. Variants of six additional genes very rarely cause HAE independently from *SERPING1*: *PLG*, *F12*, *KNG1*, *HS3ST6*, *ANGPT1*, *MYOF*. The first three ones belong to the connected contact or fibrinolytic systems, also plausibly leading to the unregulated production of kinins, while the physiopathology of HAE caused by the exceedingly rare variants of the last 3 genes is essentially unknown. There are certainly other causal genes not currently identified in HAE patients with normal C1INH levels [35]. Age, sex, and hormonal status modulate the frequency and intensity of HAE attacks, the facilitatory effect of estrogens being particularly well documented.

Let us limit ourselves here to a few remarks concerning the therapy of HAE, an admittedly crowded market for such a rare disease. Drugs and biotechnological treatments are used or proposed for attack prevention (prophylaxis), to abort attacks ("on demand" treatments), or both. Several HAE therapies that affect the KKS are approved or under development (Table 2). The parenteral administration of C1INH, or gene therapy to increase the hepatic biosynthesis of normal C1INH, is physiologically sound for HAE-C1INH. This approach is supported by multiple clinical trials for C1INH concentrates. Of note, this intervention also works in many HAE patients with normal C1INH levels, possibly because supraphysiological concentrations of this serpin suppress FXIIa and/or plasma kallikrein activity to a more complete level. The heart of the contact system is also targeted in HAE (Fig. 1, Table 2): plasma kallikrein or its proenzyme prekallikrein, FXIIa or its proenzyme FXII can be suppressed or pharmacologically inhibited by several pharmacological or biotechnological interventions. The proof of concept for a further level of intervention on the contact system has been recently reported in a preclinical study: the mAb 3E8 targets domain 6 (D6) of HK, thus inhibiting the assembly of the trimolecular complex HK-prekallikrein-factor XI (mode of action 7 in Fig. 1). In transgenic mice that express human HK, mAb 3E8 inhibits dextran sulfate-induced BK formation and FXII activation [36].

Table 2. Inhibiting the KKS for treating or preventing attacks of hereditary angioedema.

Type of agent *mode of action marker in Fig. 1*	Drug or intervention	Development status	Ref.
Parenteral replacement of C1INH *1*	various C1INH concentrates, natural or recombinant	approved, widely used	37
Gene therapy to increase the endogenous synthesis of C1INH *1*	BMN 311 HAE	clinical trials	38
	OTL-105 HAE	preclinical	39
Kunitz-domain based peptide inhibitor of plasma kallikrein *2*	ecallantide	approved	40
Small molecule inhibitors of plasma kallikrein *2*	berotralstat (BCX7353)	approved	41
	sebetralstat (KVD-900)	clinical trials	42
	ATN-249, ATN-111	clinical trials	43
Anti-plasma kallikrein mAb *2*	lanadelumab	approved	44
	STAR-0215	clinical trials	45
Transfer of a gene encoding an anti-plasma kallikrein mAb *2*	RegenxBio undisclosed	preclinical	46
Antisense suppressor of hepatic plasma prekallikrein production *3*	donidalorsen (PKK-L Rx)	clinical trials	47
Gene therapy to disrupt hepatic plasma prekallikrein production *3*	NTLA-2002	clinical trials	48
Small molecule inhibitor of factor XIIa *4*	KV998086	preclinical	49
Anti-factor XII mAb *4*	garadacimab (CSL312)	clinical trials	50
Small interfering RNA targeting factor XII mRNA *5*	ALN-F12	preclinical, halted?	51
	ARC-F12	preclinical, halted?	52
Plasmin/tPA inhibitor *6*	tranexamic acid	approved, 2nd line prophylactic agent	53
Bradykinin B2R antagonists *8*	peptide icatibant	approved	54
	NPA deucrictibant (PHA-022121, PHA-121)	clinical trials	24,55

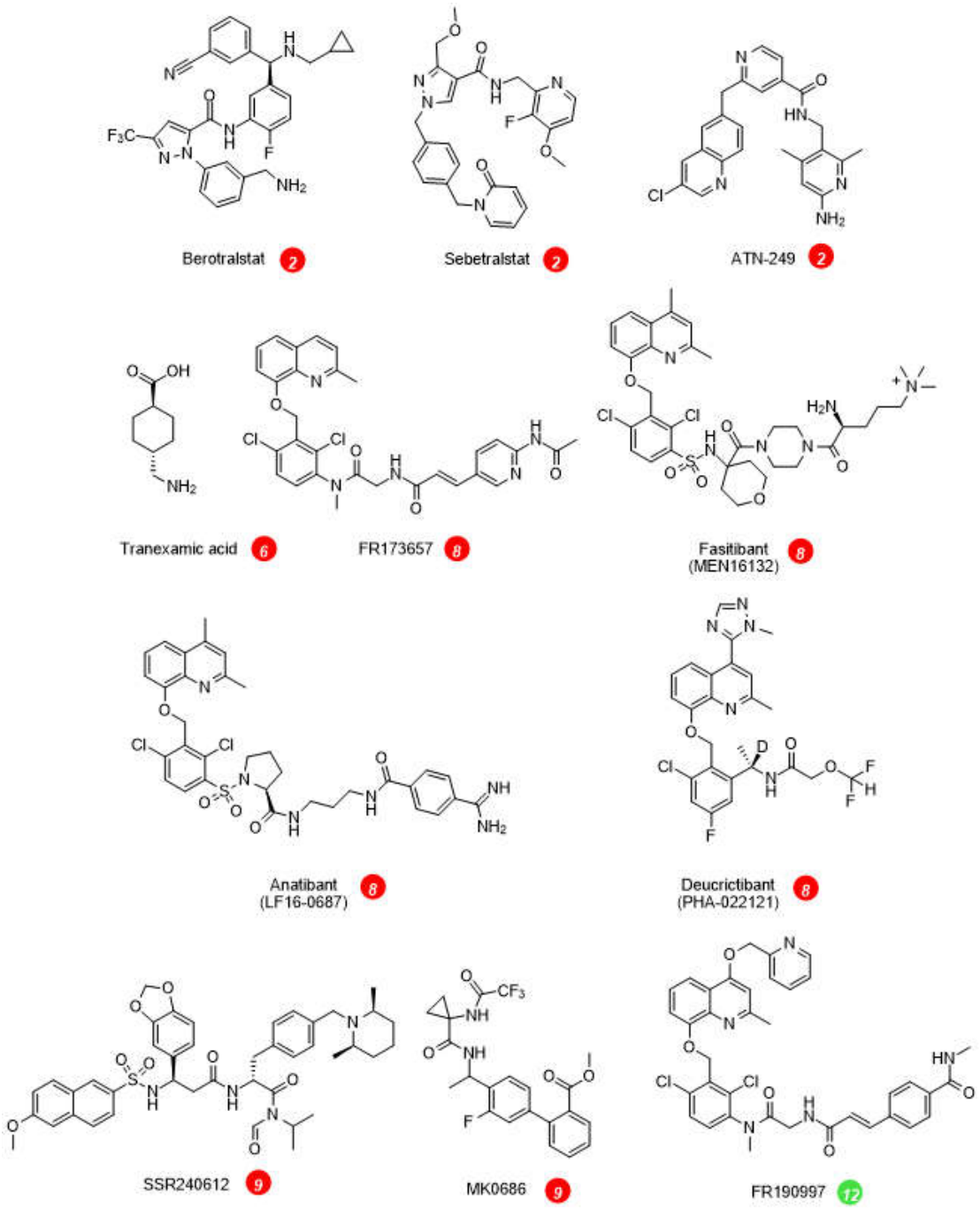

Figure 2. Structure of the small molecule drugs cited in tables 2-4 (except for KV998086 and BI1026706, currently undisclosed). The *numerical markers* indicate the mode of action as in Fig. 1. The structure of the antagonists of kinin receptors is optimized for the human forms of these receptors. Note the structural similarities of the B2R antagonists (marker 8); only deucrictibant is developed as an orally administered drug in this class.

On the effector side, the BK B2R antagonists inhibit the vascular manifestations of HAE (Table 2, Fig. 1). The injectable and rapidly cleared peptide antagonist icatibant is widely used to abort HAE attacks. The nonpeptide B2R antagonist deucrictibant [24] (Fig. 2) is orally bioavailable, more potent, and longer lived than icatibant in vivo; it is currently developed for on demand treatment of HAE attacks (a potentially convenient substitute to subcutaneous icatibant, Table 2). Chronically administered deucrictibant will also be tested for prophylaxis. Both icatibant and deucrictibant are competitive and reversible antagonists at the human B2R [24]; the affinity and/or competitive behavior of these drugs varies for B2Rs from other mammalian species. Kinin B1R antagonists have

not been tested for HAE, and the rationale for them may be weak, considering that the human form of the B1R is not effectively coupled to the contact system (see above).

There is clear evidence of fibrinolytic system activation during HAE attacks: the D-dimers that are fibrin degradation products are elevated in blood during attacks, but without thrombotic risks [56]. Whether fibrinolysis initiates the attacks is a complex question that has been reviewed [57]. Let us add two remarks to the debate. Indicating a particular vulnerability, adding recombinant tPA (10 nM) to human whole blood or citrated plasma from HAE-C1INH patients selectively increased the early BK production in vitro (measured by enzyme immunoassay and corroborated with bioassay) [6,58]. In addition, in a survey of patients concerning the circumstances preceding HAE attacks, most identified triggering factors could be related to limited coagulation followed by fibrinolysis: physical exertion, mechanical trauma, infection, menstruation, dental procedures, etc. [59]. Mental stress is one of the top patient-identified triggering factor of HAE attacks [59]. In well controlled clinical psychology studies in healthy volunteers, mental stress activates tPA secretion and the turnover of fibrin (detected by D-dimer formation) [60], further supporting the case for fibrinolysis-induced attacks.

Oral tranexamic acid, an inhibitor of plasmin and tissue plasminogen activator, has been approved as a second line prophylactic treatment of HAE. However, it lacks validation through extensive clinical trials for this indication, does not normalize the patient lives as well as more recent therapies, and does not work well in some patients [53]. Modern medicinal chemistry approaches are warranted to produce more effective inhibitors of fibrinolysis [61].

4. Other application of KKS inhibitors

Table 3 presents a selection of ongoing or terminated therapeutic projects that exploited inhibitors of the KKS. Some comments are offered here concerning specific indications. Pain is one of the cardinal signs of inflammation; despite good preclinical evidence, the clinical development of sophisticated and orally bioavailable B1R antagonists has failed due to their lack of efficacy in phase 2 trials (Table 3, Fig. 2) [62]. Fasitibant, a B2R antagonist injected in an intraarticular manner, has also failed to relieve pain associated with knee osteoarthritis (Fig. 2) [63]. These trials were a major disappointment, but preclinical research continues for a specific form of pain associated with breast cancer and its chemotherapy; the blockade of individual or both kinin receptor subtypes exert favorable effects on the associated mechanical and cold allodynia [64]. The unsuccessful clinical research concerning the B1R as a druggable target could benefit from the repurposing of potent and specific antagonists that have passed successfully clinical phase 1 development (Fig. 2), for instance for the prevention of COVID-19 complications [65].

Gliomas, including the highly malignant glioblastoma multiforme, overexpress the B2R in a manner correlated to clinical aggressivity [66]. In this clinical study, the B2R was a biomarker coupled to several pro-tumoral signaling systems. Blocking B2R may be of therapeutic value in this condition, based on a preclinical study that also shows the difficulty of reaching the tumor through the blood-brain barrier with the presently available B2R antagonists: intracerebroventricular icatibant was used in animals [67]. Interestingly, the B1R is also often present in glioma, but in this case the pharmacological blockade or genetic ablation of B1R expression had detrimental effects, enhancing tumor growth [67].

Table 3. Selected additional indications for the inhibition of the KKS.

Indication	Drug *mode of action marker in Fig. 1*	Status	Ref.
ACE-inhibitor induced acquired angioedema	C1INH concentrate *1*	ineffective in a small clinical trial	68
Idiopathic pulmonary fibrosis, interstitial lung disease	mAb garadacimab (CSL312) *4*	clinical trials	69
Thrombosis prevention	ALN-F12 *5*	preclinical	70
Intradyalytic hypotension	peptide B2R antagonist icatibant *8*	effective in a small clinical trial	71
COVID-19 pneumonia	icatibant *8*	ineffective in a clinical trial	72
Cerebral edema/head trauma	B2R NPA anatibant (LF16-0687) *8*	ineffective in an interrupted clinical trial	73
Osteoarthritis pain	B2R NPA fasitibant (MEN16132) *8*	largely ineffective in a clinical trial	63
Diabetic macula edema	B1R NPA BI1026706 *8*	ineffective in a clinical trial	74
Glioma	intracerebroventricular icatibant *8*	preclinical	67
ACE-inhibitor induced acquired angioedema	icatibant *8*	ineffective in a clinical trial	75
Diarrheal states induced by dextran sulfate	oral icatibant, oral FR173657 *8*	preclinical	76,77
Pancreatitis	icatibant, FR173657 *8*	preclinical	78,79
Circulatory complications of burns	icatibant *8*	preclinical	80,81
Inflammatory edema of various causes	FR173657, icatibant *8*	preclinical	82,83
Chagas disease myocarditis	B1R antagonist R-954 *9*	preclinical	29
Breast cancer	B1R NPA SSR240612, etc. *9*	preclinical, in vitro	84
Inflammatory pain	B1R NPA MK-0686, SS240612 *9*	ineffective in clinical trials	62
Inflammatory bowel disease	B1R NPA SSR240612 *9*	preclinical	85
Airway disease	mAb DX-2300 *10*	preclinical	86
Aortic aneurysm expansion	mAb DX-2300, etc. *10*	preclinical	87

ACE inhibitors (enalapril, ramipril, many others) constitute an important class of antihypertensive drugs with benefits on several organs. ACE is a carboxydipeptidase that activates the formation of the pressor hormone angiotensin II from its precursor angiotensin I, but also mediates the most important inactivation pathway for kinins such as BK and Lys-des-Arg^{9}-BK. A certain fraction of the beneficial effects of ACE inhibitors in human patients seems to derive from the potentiation of the vasodilator effect of kinins (mode of action *14* in Fig. 1) [88]. Many drugs are associated with acquired angioedema, an unpredictable and potentially life-threatening side effect. Several investigators attributed the angioedema induced by ACE inhibitors to the action of endogenous BK [89]; for instance, increased blood concentration of the BK metabolite BK_{1-5} was measured during such episodes [90]. However, such attacks did not respond to administration of icatibant [68] or of a C1INH concentrate [75] in clinical settings that are comparable to HAE attacks for which these interventions are therapeutic. Possible explanations include the lack of involvement of the contact system in kinin generation, a prominent role for the kinin B1R or, simply, the lack of developing fluid extravasation when the therapies were initiated relatively late during such unexpected episodes. Of note, thrombolytic therapy for ischemic stroke based on a plasminogen activator such as tPA is sometimes associated with brain edema, angioedema, and hypotension: these are serious side effects presumably mediated by excessive BK formation, due to the connection

between fibrinolysis and the contact system [4]. A beneficial effect of icatibant has been reported in such episodes [91,92].

An efficient monoclonal antibody that blocks the enzymatic action of tissue kallikrein, DX-2300, has been developed and shown of potential interest in preclinical research (Table 3). Whether tissue kallikrein. a physiological regulator of circulation and renal function, could be inhibited in vivo without major side effects remains to be seen.

5. Therapeutic value of KKS stimulation?

Kinin generation via the contact system can be chronically blocked without apparent harm, as implied above when discussing the therapy of hereditary angioedema. The alternate KKS, mediated by tissue kallikrein, appears to be adaptative in various ways. Tissue kallikrein promotes reparative neovascularization following experimental ischemia and protects the heart in animal models of pathologies [93,94]. This enzyme, produced in a regulated manner in the kidney, is released in urine and protects from sodium overload and salt-sensitive hypertension [95]. Tissue kallikrein also participates to flow-dependent vasodilation, a local circulatory adaptative mechanism [96]. So, why not consider the parenteral administration of tissue kallikrein in therapeutics? In China, active KLK-1 purified from human urine has reached clinical use for acute ischemic stroke (Table 4). When added to standard thrombolytic therapy, parenteral tissue kallikrein improved the neurological recovery in a significant manner [97]. A pharmaceutically refined recombinant tissue kallikrein, DM199, is being clinically developed for cerebrovascular and renal dysfunctions [98,99].

Is intravenous infusion of kinins feasible in intensive care situations where the stimulation of vascular B2Rs has been proposed to have therapeutic value, such as myocardial infarction and ischemic stroke? A preclinical project addressed this possibility by characterizing peptides that are both prodrugs and "soft drugs" [100-102] (Table 4). For instance, BK-Arg (BK sequence prolonged with an Arg residue) has virtually no affinity for the B2R, but releases BK in vivo following the action of Arg-CPs in the circulation, with typical vasodilator responses [101]; thus BK-Arg is a prodrug. This peptide is also a soft drug, because its active form, BK, is rapidly cleared with minimal extra-vascular effects. Other activating peptidases and agonist designs have been explored.

6. Conclusions

The medicinal chemistry related to the KKS has reached maturity, with the development of modern drugs, injectable biotechnological proteins, and advanced gene therapy projects. In addition to C1INH replacement therapy, HAE has been the focus of intense drug development efforts based on a limited number of validated targets (plasma kallikrein, FXIIa and their respective zymogens, the B2R). The recent transition to oral therapies is also noted. Although drug targeting of KKS in animal models provided promising therapeutic leads, disappointing clinical outcomes followed, as in other therapeutic areas. The existence of orally bioavailable drugs that have at least passed clinical phase 1 development (B1R and B2R antagonists, plasma kallikrein inhibitors) could facilitate their repurposing for additional therapeutic indications. Opportunities, but also side effects in various neurological disorders should be considered. For instance, there is evidence for both neuroprotective and detrimental effects of kinins, mediated by either B1R or B2R, in various neurological disorders [103,104]. Let us hope that the underexploited pharmacopeia overviewed in the present paper finds novel clinical applications.

Table 4. Therapeutic stimulation of KKS stimulation.

Type of agent *mode of action marker in Fig. 1*	Drug	status	Ref.
Activated tissue kallikrein injection for cerebrovascular or renal indications *11*	purified from human urine: Kailikang	approved in China, stroke	97
	recombinant: DM199	clinical trials	98,99
B2R agonist to open the blood-brain barrier: adjuvant to brain tumor chemotherapy *12*	labradimil, other peptides	clinical trials of labradimil failed	105,106
Combined B1R and B2R agonists to open the blood-brain barrier: adjuvant to brain tumor chemotherapy *12, 13*	co-administered peptide agonists or kinin heterodimer	preclinical	107
Peptide pro-drugs that release bradykinin via the action of peptidases *12*	bradykinin-Arg, D-Arg-bradykinin-Arg-Arg, others	preclinical	100-102
B2R NPA for breast cancer *12*	FR-190997 and analogs	preclinical, in vitro	108
Attenuation of Alzheimer's disease development, B2R agonist *12*	[Hyp^3,Thi^5,NChg^7,Thi^8]-BK	preclinical, animal model	103
ACE inhibition *14*	enalapril, ramipril, many others	a fraction of therapeutic effects mediated by kinins	88

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