Biomedicine & Pharmacotherapy 123 (2020) 109797

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Cyclooxygenase activity in bradykinin-induced dermal extravasation. A study in mice and humans

Ehsan Gholamreza-Fahimi[a], Marion Bisha[a], Janina Hahn[b], Ulrich Straßen[c], Michael Krybus[a], Farbod Khosravani[a,b,c], Thomas K. Hoffmann[b], Thomas Hohlfeld[a], Jens Greve[b], Murat Bas[c], Sören Twarock[a,1], Georg Kojda[a,1,*]

[a] Institute of Pharmacology andClinical Pharmacology, University Hospital, Heinrich Heine University, Düsseldorf, Germany
[b] Department of Oto-Rhino-Laryngology, Head and Neck Surgery, Ulm University Medical Center, Germany
[c] Otorhinolaryngology Department, University Hospital Rechts der Isar, Munich Technical University, Munich, Germany

ARTICLE INFO

Keywords:
Bradykinin
Endothelium permeability
Permeability
Endothelial barrier
Angioedema
Cyclooxygenase

ABSTRACT

Background: Non-allergic angioedema is largely driven by increased plasma levels of bradykinin and over-activation of bradykinin receptor type II (B2), but the specific downstream signalling pathways remain unclear. The aim of this study was to identify signal transduction events involved in bradykinin-induced dermal extravasation.

Methods: Quantification of dermal extravasation was accomplished following intradermal (i.d.) injection of bradykinin or the B2 agonist labradimil in mice with endothelial NO-synthase (eNOS) deficiency and in C57BL/6J mice pre-treated with vehicle, NO-synthase or cyclooxygenase (COX) inhibitors. In the multicentre clinical study ABRASE, 38 healthy volunteers received i.d. bradykinin injections into the ventral forearm before and after oral treatment with the COX inhibitor ibuprofen (600 mg). The primary endpoint of ABRASE was the mean time to complete resolution of wheals (TTCR) and the secondary endpoint was the change of maximal wheal size.

Results: Neither NOS inhibitors nor eNOS deficiency altered bradykinin-induced extravasation. In striking contrast, the COX inhibitors ibuprofen, diclofenac, SC560 and celecoxib significantly diminished this extravasation when given before injection. As for diclofenac, a similar but significantly lower effect was observed when given after i.d. injection of bradykinin. Similar results were obtained when bradykinin was replaced by labradimil. In volunteers, ibuprofen significantly reduced TTCR ($P < 0.001$) and maximal wheal size ($P = 0.0044$).

Conclusion: These data suggest that COX activity contributes to bradykinin-induced dermal extravasation in mice and humans. In addition, our findings may open new treatment options and point to a potential activity of drugs interfering with the release of the COX substrate arachidonic acid, e.g. glucocorticoids.

1. Introduction

Beside its well-known role as a mediator inducing inflammation and pain [1], bradykinin is involved in the regulation of vascular tone and capillary fluid turnover. Bradykinin-induced dermal extravasation occurs in a variety of conditions such as hereditary angioedema (HAE) or non-allergic angioedema as a side effect of cardiovascular drugs including angiotensin-converting enzyme inhibitors (ACEi) [2], angiotensin II receptor type 1 blockers (sartans) [2], Sacubitril [3] and tissue plasminogen activators [4]. In general, angioedema is a swelling of the mucosa and/or submucosa and the skin which is usually self-limiting, but is known to occur recurrently. It may impair breathing and

Abbreviations: AA, arachidonic acid; ABRASE, A Bradykinin in Skin Edema Trial; ACEi, angiotensin-converting enzyme inhibitor; B1, bradykinin receptor type I; B2, bradykinin receptor type II; c_{max}, maximal plasma concentration; COX, cyclooxygenase; DAG, diacylglycerol; EC, endothelial cells; eNOS, endothelial NO-synthase; $eNOS^{-/-}$, eNOS knock-out mouse strain; G, G-protein; HAE, hereditary angioedema; i.p., intraperitoneal; IP, PGI_2-receptor; EET, epoxyeicosatrienoic acid; IP_3, inositol 1,4,5-trisphosphate; LE, largest expansion; L-NAME, L-nitroarginine methyl-ester; L-NMMA, NG-monomethyl-L-arginine; PBS, phosphate buffer solution; PG, prostaglandin; PG-S, PG synthase; PKC, protein kinase C; PLA_2, phospholipase A_2; sartan, angiotensin II receptor type 1 blocker; SE, smallest expansion; t_{max}, time to reach maximal plasma concentration

* Corresponding author at: Institut für Pharmakologie und Klinische Pharmakologie, Heinrich-Heine-Universität, Universitätsstr. 1, 40225 Düsseldorf, Germany.
E-mail address: kojda@uni-duesseldorf.de (G. Kojda).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2019.109797
Received 26 November 2019; Received in revised form 12 December 2019; Accepted 15 December 2019

swellings of the head and neck region, particularly in the pharynx and the larynx, are potentially life-threatening. This condition often requires emergency treatment and several days of hospitalization [5]. Other tissue locations of angioedema manifestations occurring mainly in HAE are the gastrointestinal tract, the genital region or the extremities and are often painful [6]. Among non-allergic angioedema the most frequent cause appears to be treatment with ACEi [7]. Worldwide, many million patients are treated with ACEi and in view of the increasing need to treat elderly and chronically ill patients with cardiovascular and antidiabetic drugs, one can expect increasing numbers of drug-induced angioedema and thus increasing numbers of potentially life-threatening events. In fact, the mortality rates of HAE in the USA decreased between 1979 and 2010 from 0.28 to 0.06, while such rates for other forms of non-allergic angioedema increased from 0.24 to 0.34 per million [8]. It appears quite reasonable that this increased mortality of angioedema is at least in part associated with increased usage of ACEi [9]. While one randomized clinical trial using the B2 inhibitor icatibant provided evidence that over-activation of bradykinin receptor type II (B2) is crucial in ACEi induced angioedema [10], other studies with icatibant found no benefit [11,12] suggesting the involvement of other mediators, e.g. substance P. However, a later clinical study confirmed the involvement of bradykinin in ACEi induced angioedema and explained the obvious discrepancy between the randomized trials, e.g. with the later time of presentation of patients in their randomized trial [12]. In addition, consistent evidence from studies in transgenic mice strongly suggests as well that the increase of fluid extravasation induced by bradykinin is mediated by over-activation of B2 [2,13,14].

Studies in non-dermal tissues and cells have revealed that bradykinin signals via two distinct receptors, the bradykinin receptor type I (B1) and B2 [1,2,15]. Among many other tissues B2 is constitutively expressed in vascular endothelial and smooth muscle cells, while B1 expression is dependent on inflammatory mediators such as cytokines. Activation of endothelial $G\alpha_{q/11}$-coupled B2 results in activation of distinct signalling pathways, including activation of phospholipase C-ß and subsequent IP_3-dependent release of intracellular calcium; activation of endothelial NO-synthase (eNOS) and subsequent generation of NO; and activation of phospholipase A_2 (PLA_2) and D and subsequent generation of prostaglandins, leukotrienes and epoxyeicosatrienoic acids [2,16]. The aim of this study was to identify signal transduction events downstream of B2 activation in small dermal blood vessels which are involved in the pathophysiology of non-allergic angioedema.

2. Material and methods

2.1. Animal studies

2.1.1. Animals

C57BL/6 J mice were purchased from JANVIER LABS (Le Genest-Saint-Isle, France). Mice of an eNOS knock-out mouse strain ($eNOS^{-/-}$) [17,18] were bred and housed in the animal facility (ZETT, UKD Düsseldorf, Germany). All mice used for experiments were male and 3–4 months old (24 − 28 g). The animals (n = 3–5 per cage) received a standard mouse chow and acidified water (pH = 3–4) ad libitum. The experiments were performed according to the guidelines for the use of experimental animals, as given by the German 'Tierschutzgesetz' (approval references: 84-02.04.2012.A194, 84-02.04.2016.A114) and the 'Guide for the Care and Use of Laboratory Animals' of the US National Institutes of Health. Animal studies are reported in compliance with the ARRIVE guidelines [19,20].

2.1.2. Induction of extravasation in mouse's skin

We used the Miles assay to quantify dermal extravasation [21]. Under anaesthesia (i.p. application of 100 mg/kg ketamine and 5 mg/kg xylazine) mice received an i.v. bolus injection of Evans blue (30 µmol/kg). Following depilation of the dorsal skin, i.d. injections (30 µl each) of vehicle (physiologic buffer solution, PBS), bradykinin, the specific B2 agonist labradimil [22] and histamine (2 nmol) were applied. C57BL/6 J mice received i.v. injections of either 16 mg/kg of the NOS-inhibitor L-nitroarginine methyl-ester (L-NAME), or 5 mg/kg diclofenac, 12 mg/kg ibuprofen, 5 mg/kg of the selective COX1-inhibitor SC-560 or 10 mg/kg of celecoxib prior to the injection of Evans blue. To study the effect of COX-inhibition on already developing bradykinin-induced extravasation 5 mg/kg Diclofenac was given i.p. 10 min after dorsal i.d. injections of 2 nmol bradykinin, labradimil, and histamine. Thirty minutes after the dorsal i.d. injections, the mice were sacrificed by cervical dislocation. The dye within the skin specimens was eluted in 1 mL *N,N*-dimethylformamide overnight at 55 °C and quantified at 620 nm using a spectrometer (Beckman Instruments GmbH, Munich, Germany). The concentrations obtained were set in relation to the wet weight of the specimens and expressed as µg dye/g tissue. The value obtained in vehicle-treated tissue was defined as reference and was set to 1, i.e., all other values from the same animal were related to this control.

2.2. ABRASE study design

The multicentre clinical study "A Bradykinin in Skin Edema Trial (ABRASE)" comprised 38 healthy human volunteers at three centres in Germany. One participant was excluded by ROUT test (calculated using Graph Pad Prism 6.07) and one participant was excluded after enrolment due to withdrawal of informed consent. A more detailed study protocol including inclusion and exclusion criteria and a table for some characteristics of the volunteers (Tab S1) is provided in the Supporting information available with the full text of this article. The effect of ibuprofen on bradykinin-induced extravasation was assessed in each volunteer. With each intradermal injection 18.9 nmol bradykinin dissolved in 20 µl of 0.9 % sterile isotonic saline solution (Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) corresponding to a concentration of 1 mg/mL was applied and compared to the effect of an intradermal injection of 20 µl of 0.9 % saline which served as internal control. The solutions were injected into the skin of the ventral forearm and sizes of the forming wheals were measured for 120 min at pre-defined time points. Wheal sizes were measured with a calliper gauge by recording the largest (LE) and smallest (SE) expansion of a wheal at various time points. Consecutively, 180 min after the initial intradermal injections, volunteers took orally 600 mg of IbuHEXAL® (Hexal AG, Holzkirchen, Germany). Another 60 min later, bradykinin was applied again and wheal size was measured for 120 min as described above. The primary endpoint of ABRASE was the time to complete resolution of wheals, as assessed at one of the pre-defined time-points of measurement. The secondary endpoint was a change of the mean maximal wheal size before and after the intervention. Ethical approval for this study was granted by the Ethics Commission of the Medical Faculty of the Heinrich-Heine University (Study number in the clinical trial register of the medical faculty of the university of Düsseldorf: 2015-11-4583, ethics committee vote number: 5339R). The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

2.3. Compounds

All chemicals were purchased from Sigma-Aldrich (Munich, Germany) or Merck (Darmstadt, Germany), except otherwise stated in the text.

2.4. Statistics

All animal data expressed as mean ± S.E.M. of n individual samples were analysed by the computer program GraphPad Prism PC software, version 6.07 (La Jolla, CA, USA). Statistical comparisons between more than two groups were performed by Tukey's multiple

comparison post-hoc test following One-way ANOVA.

The primary endpoint of ABRASE yielded a non-parametric data set. Data were analysed by Wilcoxon matched-pairs signed rank test. Changes of wheal size before and after intervention with COX-inhibitors (secondary endpoint) were calculated by paired two-tailed t-test. Change of wheal size over time were analysed by Two-way ANOVA and subsequent Sidak multiple comparison post-hoc test. Post-hoc tests were run only if F achieved $P < 0.05$ and there was no significant variance inhomogeneity. $P < 0.05$ was considered statistically significant.

A more detailed description of Methods and Materials is available in the supporting information.

3. Theory

Bradykinin signalling in small dermal blood vessels is mediated by bradykinin receptor type 2 and results in increased plasma fluid extravasation eventually causing non-allergic angioedema. Gq-protein coupling of this receptor is expected to enhance intracellular calcium concentration in endothelial and smooth muscle cells of this type of blood vessels. Among other important cellular proteins such as calmodulin, free intracellular calcium activates phospholipase A2 and/or endothelial NO synthase. However, the relative importance of these protein activities for dermal extravasation is not known.

4. Results

4.1. Animal studies

We adopted the Miles assay [21] to mice in order to study dermal extravasation in response to bradykinin in transgenic animals [23]. Intradermal injection of bradykinin induced extravasation as illustrated by the blue dots in Fig. 1A and the response was dose-dependent (Fig. 1B). This approach was used to investigate the importance of the two major signal transduction pathways of bradykinin primarily observed in larger blood vessels, i.e. activation of NOS and COX [2]. Histamine was used as a B2 independent control. As shown in Fig. 1C, the global loss of eNOS had no effect on dermal extravasation induced by bradykinin. A similar lack of effect was observed following treatment of C57BL/6 J with the NOS inhibitor L-NAME (Fig. 1D) suggesting that activation of NO signalling is rather unimportant in this setting.

To study the role of prostaglandins for the increase of permeability by bradykinin and labradimil in small dermal blood vessels, we pre-treated C57BL/6 J with COX-inhibitors of different specificity. This resulted in a significant reduction of bradykinin-induced dermal extravasation using the unspecific COX-inhibitor ibuprofen (Fig. 2A). A similar effect was evident in mice treated with diclofenac (Fig. 2B). These findings suggest a contribution of prostaglandins to the dermal extravasation in response to bradykinin in mice. We further pre-treated C57BL/6 J with compound SC560, a more specific COX1-inhibitor and dermal extravasation was significantly reduced (Fig. 2C), but the effect was smaller as compared with the effects of ibuprofen and diclofenac. Likewise, pre-treatment of C57BL/6 J with celecoxib, a more specific inhibitor of COX2, significantly reduced dermal extravasation (Fig. 2D), but again to a smaller extend. These data suggest that both COX1 as well as COX2 may be involved in the development of bradykinin-induced extravasation in small dermal blood vessels of mice.

In an additional approach we tested if this effect can be reproduced in the setting of bradykinin-induced extravasation developing already. Therefore, treatment of C57BL/6 J with diclofenac was delayed by 10 min (post-diclo) after the dorsal i.d. injections of bradykinin, labradimil and histamine. COX-inhibition did not change extravasation induced by histamine, but we observed a significant reduction of dermal extravasation by post-diclo compared to vehicle treatment for both B2 agonists (Fig. 3). However, the reduction of extravasation induced by both B2 agonists was significantly stronger if diclofenac was administered before i.d. injection of bradykinin (pre-diclo), while this difference reached no significance if labradimil was used as B2 agonist (Fig. 3). These results suggest that COX-dependent B2 signalling underlying extravasation in small dermal blood vessels of mice does not change during the development of extravasation and demonstrate the importance of early COX-inhibition on dermal extravasation.

4.2. ABRASE study

We sought to evaluate whether the results of the animal experiments would translate to humans. Bradykinin solution was applied by i.d. injection into the forearm and the development and resolution of wheals was monitored over time. A typical wheal developed to nearly maximal size is shown in Fig. 4A in comparison to injection of saline. The results according to the primary endpoint are illustrated in Fig. 4B. The mean time to complete resolution of wheal size as compared to no

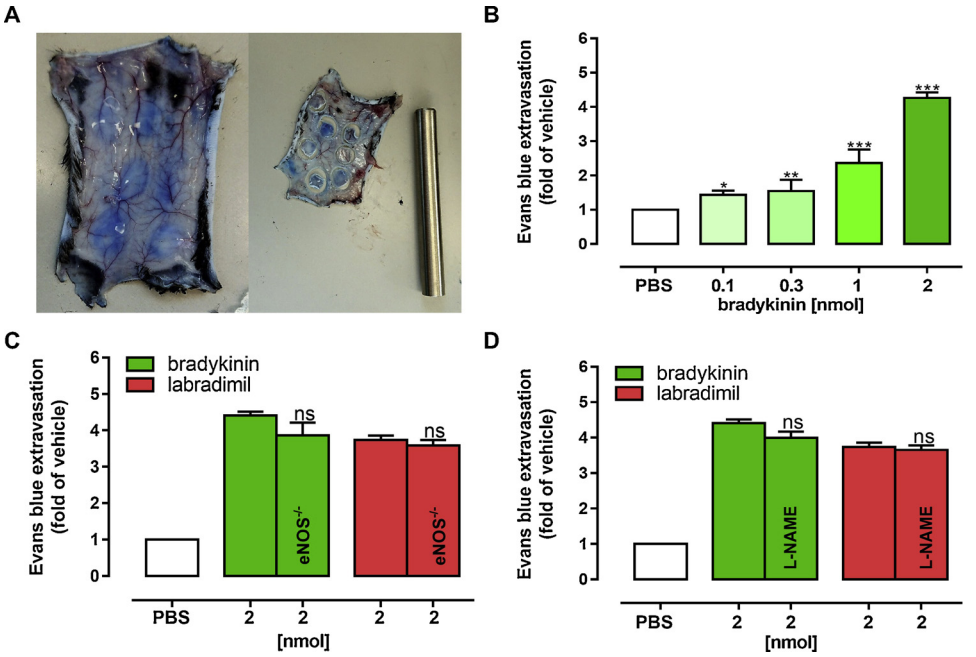

Fig. 1. Dermal extravasation in mice induced by intradermal (i.d.) injection of bradykinin as evaluated with the Miles assay. **A** Example of the dorsal skin of mice before and after excision of the skin specimens using a circular cutter shown on the right. **B** Dose-dependent extravasation induced by i.d. bradykinin ($n = 6$ each, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$ each vs. physiologic buffer solution (PBS), Tukey's multiple comparison test following One-Way ANOVA). **C** Extravasation in C57BL/6 J (left column) and eNOS$^{-/-}$ mice ($n = 6$ each) demonstrating that the global loss of endothelial NO-synthase doesn't significantly change (ns) skin extravasation induced by bradykinin and labradimil. **D** Extravasation in C57BL/6 J without (left column) and with i.v. L-nitroarginine methyl-ester (L-NAME) pre-treatment ($n = 6$ each) demonstrating that pharmacologic inhibition of NOS also doesn't significantly change (ns) skin extravasation induced by bradykinin and labradimil.

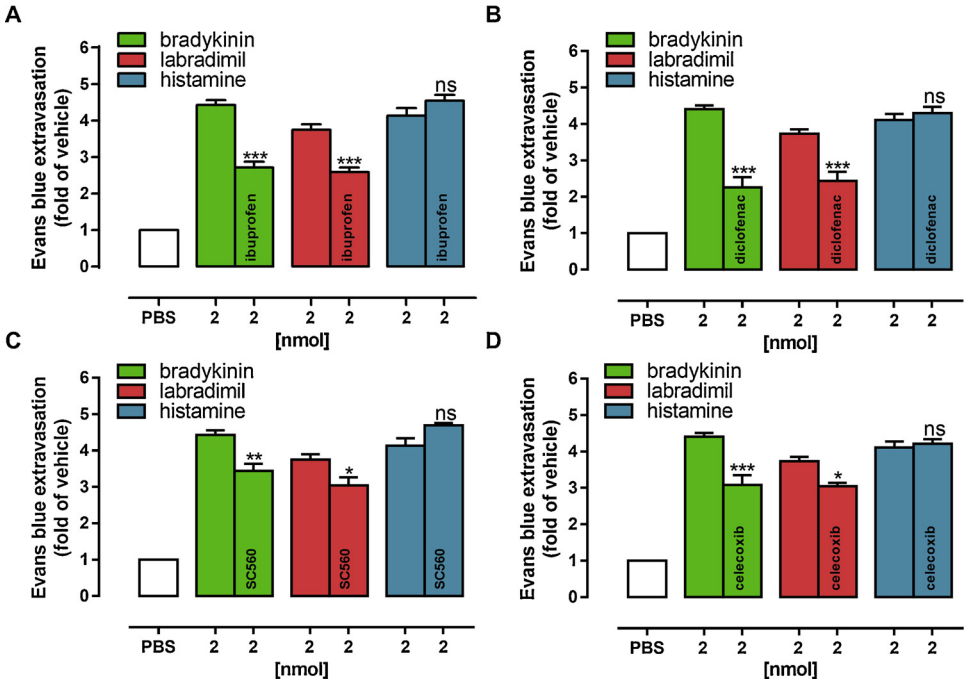

Fig. 2. Miles assay results in C57BL/6 J illustrating the effects of intraperitoneal injected COX-inhibitors on dermal extravasation induced by intradermal injection of bradykinin, labradimil, and histamine as related to intradermal injection of physiologic buffer solution (PBS). **A** Extravasation following pretreatment with vehicle (left column) and the unspecific COX-inhibitor ibuprofen showed that unspecific COX1/COX2-inhibition significantly reduces skin extravasation induced by bradykinin and labradimil (n = 5 each, ***P < 0.0001 vs. vehicle treatment). Of note, there was no effect on extravasation induced by histamine. Similar effects were observed after **B** COX-inhibition by diclofenac (n = 6 each, ***P < 0.0001 vs. vehicle treatment), **C** more COX1 specific inhibition with compound SC560 (n = 5 each, **P < 0.01, *P < 0.05 each vs. vehicle treatment), and **D** more COX2 specific inhibition with celecoxib (n = 6, ***P < 0.0001, *P < 0.05 each vs. vehicle treatment). The statistical significance was calculated using Tukey's multiple comparison test following One-Way ANOVA.

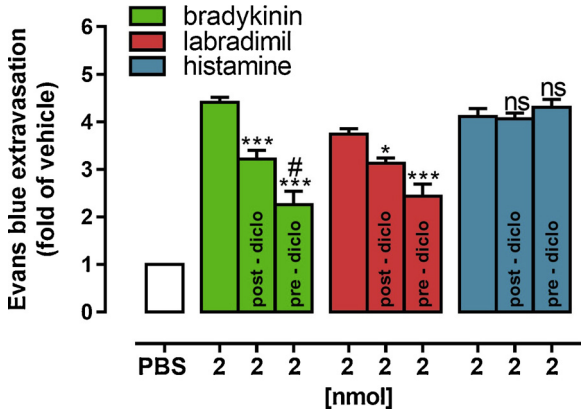

Fig. 3. Miles assay results showing the effects of unspecific COX-inhibition by intraperitoneal injection of diclofenac delayed for 10 min following intradermal injection of bradykinin or labradimil (post-diclo) in comparison to treatment before induced extravasation (pre-diclo) in the dorsal skin of C57BL/6 J. Extravasation is normalized to the effect induced by buffer solution (PBS). In comparison to the vehicle treatment group (left column) extravasation induced by bradykinin and labradimil was significantly reduced in both post-diclo (middle column) and pre-diclo (right column) treatment groups (n = 6, ***P < 0.0001, *P < 0.05 each vs. vehicle treatment). In addition, there was a significant difference between the two time points of administration in case of bradykinin but not labradimil (#p < 0.01 vs post-diclo). There was no effect on extravasation induced by histamine. These data reveal the importance of early COX-inhibition on dermal extravasation (statistical significance was calculated using Tukey's multiple comparison test following One-Way ANOVA).

treatment (n = 38, 99.5 min, 95 % confidence interval [CI], 92.2–106.8) was significantly lower after treatment with ibuprofen (82.6 min, 95 % CI, 74.3–90.9, ***P < 0.0001, Fig. 4B).

The secondary endpoint of ABRASE was a change of the mean maximal wheal size before and after the intervention with the COX-inhibitor ibuprofen. As shown in Fig. 5, there was a great variation of maximal wheal size among volunteers, both before and after intervention. Statistical analysis revealed a significant reduction of mean maximal wheal size before (n = 38, 468.9 mm^2, 95 % CI, 378.8–559.0) and after ibuprofen (385.5 mm^2, 95 % CI, 295.8–475.2, **P = 0.0044).

A separation of the results obtained in each study centre and the time course of the relative and absolute wheal sizes before and after ingestion of ibuprofen is provided in the supporting information (illustrated in S2).

In view of the marked variation of maximal wheal size we calculated whether the wheal size itself had a direct influence on the primary endpoint of the study, i.e., that a greater wheal size is associated with a prolongation of the time to complete resolution of wheals or vice versa. To investigate this particular point, we performed a direct comparison of maximal wheal size at the predefined measurement times for wheals sizes. As illustrated in Fig. 6, no significant difference in the variation of maximal wheals size between the different time points was observed suggesting that in the ABRASE study maximal wheal size had no influence on the time to complete resolution of wheals. This implies that the variation of wheal sizes did not interact with the primary endpoint of the ABRASE study.

5. Discussion

The aim of this study was to get a more detailed insight into the mechanism of action of bradykinin-induced dermal extravasation occurring in a variety of conditions such as HAE or non-allergic angioedema as a side effect of cardiovascular drugs. Surprisingly, activation of eNOS does not seem to play a role in mice, while COX dependent generation of prostaglandins appears to be critically involved. Likewise, inhibition of prostaglandin synthesis decreased bradykinin-induced dermal extravasation in volunteers. These data suggest that the development of non-allergic angioedema depends on bradykinin-induced activation of COX. In addition, our findings may point to a potential activity of drugs interfering with the release of the COX substrate arachidonic acid, e.g. glucocorticoids, in drug induced non-allergic angioedema.

5.1. Studies in mice

In contrast to the lack of effect of NO, we observed a pronounced inhibition on dermal extravasation in response to i.d. bradykinin or i.d. labradimil in C57BL/6 by the two unspecific COX inhibitors ibuprofen and diclofenac, which block the enzymatic activity of both isoforms of COX. Similar results were obtained using the more COX1-specific

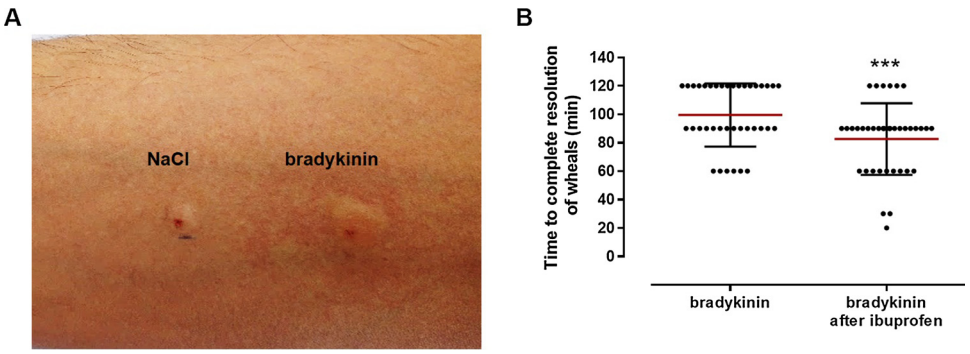

Fig. 4. Main results of the ABRASE study. **A** Example of wheals following intradermal injection of 18.9 nmol bradykinin and 0.9 % saline (NaCl) into the upper ventral forearm of a volunteer. Both wheals and flares were observed, but only palpable wheals counted in the course of the experiments. **B** The primary endpoint of the study, that is the mean time to complete resolution of wheals, was significantly lower after ingestion of 600 mg ibuprofen suggesting that activation of cyclooxygenase by bradykinin in small dermal vessels of the human skin contributes to the development of wheals (n = 38, ***P < 0.0001, calculated using Wilcoxon matched-pairs signed rank test).

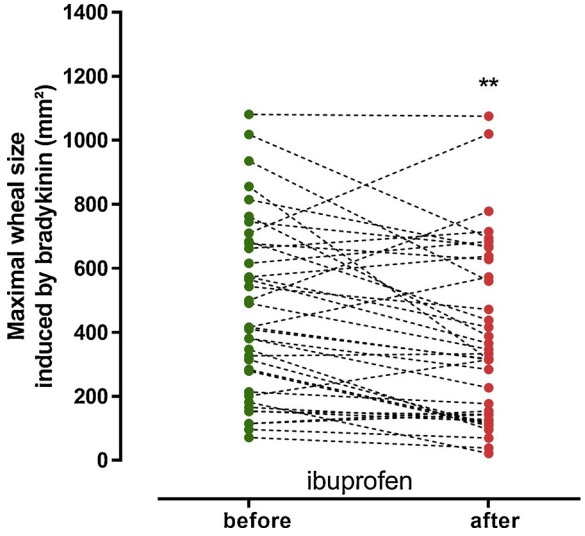

Fig. 5. Illustration of the variation of maximal wheal size among volunteers before and after ingestion of 600 mg ibuprofen. Such changes were defined as the secondary endpoint of the ABRASE study. Although there was a great variation of maximal wheal sizes, a significant reduction, most likely caused by ibuprofen, was observed (n = 38, **P = 0.0044, calculated using two-tailed paired t-test).

inhibitor SC560 and the more COX2-specific inhibitor celecoxib suggesting that both COX subtypes are involved. These data suggest that the synthesis of prostaglandins in small dermal blood vessels of C57BL/6 in response to activation of B2 is much more important for extravasation and oedema formation than activation of eNOS and subsequent generation of NO. This is a striking difference to other mediators such as vascular endothelial growth factor and platelet activating factor, which have been described to induce fluid extravasation predominantly via generation of NO by eNOS [24]. In addition, studies in bovine aortic endothelial cells (EC) have shown that vascular endothelial growth factor stimulated the production of NO 10-fold stronger than that of prostacyclin [25] while BK produced about 3,5-fold more prostacyclin than NO in porcine aortic EC [26].

Earlier experimental studies in rabbits have shown that prostaglandins such as PGE_2 potentiate the extravasation induced by bradykinin, although PGE_2 itself caused only very little effects. In rabbits, fluid extravasation and oedema formation in response to the combined i.d. injection of prostaglandins and bradykinin was much more pronounced than after i.d. bradykinin alone [27]. This synergistic effect was attributed to the vasodilator activity of PGE_2 increasing blood flow to the site of injection [28]. As expected, the bradykinin/PGE_2-induced increase in permeability was not sensitive to the COX-inhibitors indomethacin and ibuprofen [27,29]. Bradykinin can stimulate generation of prostaglandins in endothelial cells of different vascular origin [1] and releases PGE_2 from isolated skin tissue [30]. Unfortunately, the effect of bradykinin alone on extravasation in PGI_2-receptor (IP) deficient mice was not evaluated, although this study nicely demonstrated a strong inhibition of dermal extravasation induced by the combination of bradykinin/PGI_2 [31]. These studies leave little doubt on the important role of prostaglandins for plasma extravasation under inflammatory conditions, but so far no evaluation of the role of prostaglandins generated in response to bradykinin has been undertaken, e.g. on the relative involvement of different prostaglandins and the different prostaglandin receptor subtypes. According to the suggested vasodilator role of prostaglandins on oedema formation [28], several prostaglandins such as E- and D-prostaglandins as well as PGI_2 and their respective receptors EP_2, EP_4, DP_1 and IP may play a role in oedema elicited by bradykinin in small skin vessels of mice [32,33].

Neither ibuprofen nor diclofenac completely blocked extravasation by bradykinin. As generation of NO by eNOS was found to be rather

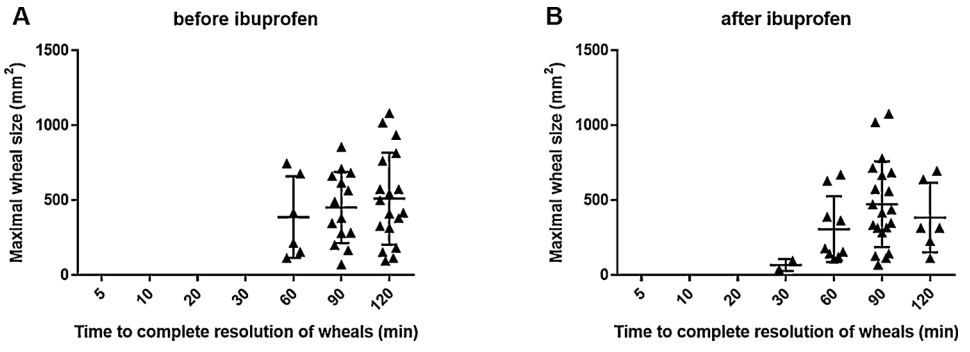

Fig. 6. Illustration of the relation between variation of maximal wheal size and the time to complete resolution of wheals maximal wheals among volunteers before and after ingestion of 600 mg ibuprofen. **A** At each time point there was still a considerable variation of maximal wheal size and there was no significant difference between the groups before ibuprofen (n = 38, P = 0.7278, calculated using Kruskal-Wallis test). **B** At each time point there was still a considerable variation of maximal wheal size and there was no significant difference between the groups after ibuprofen (n = 37, P = 0.0674, calculated using Kruskal-Wallis test after exclusion of the single value at 20 min, because single values cannot be computed by the Kruskal-Wallis test).

unimportant, it appears likely that other arachidonic acid metabolites such as leukotrienes synthetized by lipoxygenases and/or epoxyeicosatrienoic acids (EETs) synthetized by cytochrome P450 2C [16] might be involved. The key lipases providing arachidonic acid as substrates for COX, lipoxygenase or cytochrome P450 2C in cells are the six subtypes of cytosolic PLA_2, i.e. $cPLA_2$-4A - $cPLA_2$-4 F. Binding of Ca^{2+} to the C2 domain of $cPLA_2$ initiates translocation of the enzyme from the cytosol to the cell membrane, where the active site of the α/β hydrolase domain is in the correct orientation to allow substrate molecules to enter the active site [34]. Subsequently, arachidonic acid is released from the cell membrane. One important mechanism of action underlying the anti-inflammatory effects of glucocorticoids is inhibition of $cPLA_2$ by induction of annexin A1 [35]. Therefore, our findings may point to a potential activity of drugs interfering with the release of the COX substrate arachidonic acid, e.g. glucocorticoids. Further research using specific inhibitors of $cPLA_2$ [36] and glucocorticoids is needed to clarify the role of non-prostaglandin arachidonic acid metabolites in non-allergic angioedema.

5.2. ABRASE study

The ABRASE study was initiated to investigate the role of COX activity for bradykinin-induced extravasation in humans. Intradermal injection of bradykinin provides an interstitial reservoir from which the kinin is continuously released to the small dermal blood vessels. This local increase in bradykinin results in the formation of visible and palpable wheals as shown in Results. According to clinical trials using the B2 antagonist icatibant for the treatment of HAE [37] and ACEi-induced angioedema [10], the time to complete resolution of wheals was chosen as the primary endpoint. The resolution of dermal extravasation and angioedema is thought to be mediated by degradation of bradykinin and lymphatic drain [38], but the clinical trials with icatibant in non-allergic angioedema suggest that inhibition of B2 activation by B2 antagonism obviously contributes [10,37]. In this study, unselective inhibition of COX activity with ibuprofen significantly shortened the time to complete resolution of symptoms and resulted in a reduction of maximal wheal size suggesting that inhibition of B2 signal transduction downstream of B2 agonism attenuates both the development and persistence of bradykinin-induced dermal extravasation in humans.

The effect of 600 mg ibuprofen on dermal extravasation in healthy volunteers, which is a recommended single dose to treat pain, fever and inflammation, was smaller than the effect in mice. Beside the higher dose per kg body weight in mice, ibuprofen was administered by i.p. injection and not orally. It is well known, that i.p. injection results in faster absorption associated with a shorter time to reach maximal plasma concentration (t_{max}) and a higher maximal plasma concentration (c_{max}) [39]. This route of administration is rarely used in humans to achieve systemic drug delivery. Therefore, it appears reasonable to assume that the active ibuprofen plasma concentration at the time of bradykinin injection was considerably higher in mice. In addition, the signalling of bradykinin in small dermal blood vessels in mice might differ from the situation in humans inasmuch as there is a greater dependency on COX activity in mice. For example, in large blood vessels of mice such as aortic ring preparations, bradykinin causes a constriction that is completely dependent on COX activity induced by activation of B2 [23,40,41]. In contrast, continuous infusion of bradykinin into the brachial artery of humans causes vasodilation and increases forearm blood flow, but the COX-inhibitor aspirin showed no effect [42]. Furthermore, the NOS-inhibitor NG-monomethyl-L-arginine (L-NMMA) produced a strong inhibition of bradykinin-induced human forearm blood flow [43] while NOS did not contribute to bradykinin-induced dermal extravasation in mice. Interestingly, increased blood flow following bradykinin infusion is largely dependent on vasodilation of resistance vessels suggesting that in these vessels COX activation induced by bradykinin is of less importance in humans. However, the results of ABRASE suggest that in humans, COX activity is significantly involved in bradykinin-induced endothelial hyper-permeability in small dermal vessels suggesting that bradykinin signalling differs between these two vascular beds.

ABRASE has several limitations. Ibuprofen is an unspecific COX-inhibitor leaving the open question whether COX1, COX2 or both contribute to the development and persistence of dermal extravasation. Hence, an extension of ABRASE investigating the effects of the COX2 selective drug etoricoxib is planned for the near future. It remains unknown as well which type of prostaglandin and which prostaglandin receptor mediates the propagation of dermal extravasation. Of note, the results of ABRASE do also not exclude a contribution of NOS-activity to bradykinin-induced hyper-permeability. Finally, intradermal injection of bradykinin covers just aspects of the pathophysiology of non-allergic angioedema and it is uncertain whether COX activity plays a role in this condition. Nevertheless, activation of B2 is a crucial step in the development of non-allergic angioedema [10,37] and our data suggest that COX activity contributes to bradykinin-induced extravasation in humans.

6. Conclusions

Taken together, the findings of this study provide evidence for a contribution of COX activity in bradykinin-induced dermal extravasation in mice and humans. Identification of the prostaglandins as well as the corresponding receptors may open new treatment options for non-allergic angioedema and may point to a potential activity of drugs interfering with the release of arachidonic acid, e.g. glucocorticoids, in severe drug induced non-allergic angioedema such as ACE inhibitor induced angioedema [10,37].

Information on financial support

This study was supported by grants of the Forschungskommission of the Medical Faculty of the Heinrich-Heine University Düsseldorf (Project 9,772,681, to GK) and the Deutsche Forschungsgemeinschaft (KO 1557/14-1, to GK).

Conflicts of interests

The authors declare no conflicts of interest.

Authors' contributions

EGF and MBi did Miles assays and contributed to the collection and assembly of experimental and to the collection of ABRASE data in Düsseldorf, JH contributed to the collection of ABRASE data in Ulm, US contributed to the collection of ABRASE data in München, MK and FK contributed to Miles assays, TKH contributed as study physician in Ulm, TH contributed as study physician in Düsseldorf, JG contributed to the collection of ABRASE data in Ulm, MBa contributed as study physician in München, ST contributed to the collection of ABRASE data in Düsseldorf and contributed in data analysis and writing of the manuscript, GK contributed as principal investigator of ABRASE, did conception and design, administrative support, data analysis and interpretation, raised funding and wrote the manuscript.

Acknowledgements

The authors wish to thank Dieter Hafner, PhD, mathematician and study statistician, Institute of Pharmacology and Clinical Pharmacology, University Hospital, Heinrich Heine University, Düsseldorf, Germany, for his valuable contribution, and the Forschungskommission of the Medical Faculty of the Heinrich-Heine University Düsseldorf (Project 9772681 to GK) and the Deutsche Forschungsgemeinschaft (KO 1557/14-1 to GK) for the financial

support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2019.109797.

References

[1] L.M. Leeb-Lundberg, F. Marceau, W. Muller-Esterl, D.J. Pettibone, B.L. Zuraw, International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences, Pharmacol. Rev. 57 (2005) 27–77.

[2] M. Bas, V. Adams, T. Suvorava, T. Niehues, T.K. Hoffmann, G. Kojda, Non-allergic angioedema. Role of bradykinin, Allergy 62 (8) (2007) 842–856.

[3] J.J. McMurray, M. Packer, A.S. Desai, J. Gong, M.P. Lefkowitz, A.R. Rizkala, J.L. Rouleau, V.C. Shi, S.D. Solomon, K. Swedberg, M.R. Zile, Angiotensin-neprilysin inhibition versus enalapril in heart failure, N. Engl. J. Med. 371 (2014) 993–1004.

[4] Z. Hofman, S. de Maat, C.E. Hack, C. Maas, Bradykinin: inflammatory product of the coagulation system, Clin. Rev. Allergy Immunol. 51 (2) (2016) 152–161.

[5] M. Bas, T.K. Hoffmann, G. Kojda, Evaluation and management of angioedema of the head and neck, Curr. Opin. Otolaryngol. Head Neck Surg. 14 (2006) 170–175.

[6] H. Longhurst, M. Cicardi, Hereditary angio-oedema, Lancet 379 (2012) 474–481.

[7] W.J. Kostis, M. Shetty, Y.S. Chowdhury, J.B. Kostis, ACE inhibitor-induced angioedema: a review, Curr. Hypertens. Rep. 20 (7) (2018) 55.

[8] S.J. Kim, J.C. Brooks, J. Sheikh, M.S. Kaplan, B.J. Goldberg, Angioedema deaths in the United States, 1979–2010, Ann. Allergy Asthma Immunol. 113 (2014) 630–634.

[9] D. Sondhi, M. Lippmann, G. Murali, Airway compromise due to angiotensin-converting enzyme inhibitor-induced angioedema: clinical experience at a large community teaching hospital, Chest 126 (2004) 400–404.

[10] M. Bas, J. Greve, K. Stelter, M. Havel, U. Strassen, N. Rotter, J. Veit, B. Schossow, A. Hapfelmeier, V. Kehl, G. Kojda, T.K. Hoffmann, A randomized trial of icatibant in ACE-inhibitor-induced angioedema, N. Engl. J. Med. 372 (2015) 418–425.

[11] R. Sinert, P. Levy, J.A. Bernstein, R. Body, M.L.A. Sivilotti, J. Moellman, J. Schranz, J. Baptista, A. Kimura, W. Nothaft, Randomized trial of icatibant for angiotensin-converting enzyme inhibitor-induced upper airway angioedema, J. Allergy Clin. Immunol. In practice 5 (5) (2017) 1402–1409.e3..

[12] S.A. Hubers, K. Kohm, S. Wei, C. Yu, H. Nian, R. Grabert, D.J. Sexton, N.J. Brown, Endogenous bradykinin and B1-B5 during angiotensin-converting enzyme inhibitor-associated angioedema, J. Allergy Clin. Immunol. 142 (5) (2018) 1636–1639.e5.

[13] E.D. Han, R.C. MacFarlane, A.N. Mulligan, J. Scafidi, A.E. Davis III, Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor, J. Clin. Invest. 109 (8) (2002) 1057–1063.

[14] C. Oschatz, C. Maas, B. Lecher, T. Jansen, J. Bjorkqvist, T. Tradler, R. Sedlmeier, P. Burfeind, S. Cichon, S. Hammerschmidt, W. Muller-Esterl, W.A. Wuillemin, G. Nilsson, T. Renne, Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo, Immunity 34 (2011) 258–268.

[15] A. Blaukat, Structure and signalling pathways of kinin receptors, Andrologia 35 (2003) 17–23.

[16] B. Fisslthaler, R. Popp, L. Kiss, M. Potente, D.R. Harder, I. Fleming, R. Busse, Cytochrome P450 2C is an EDHF synthase in coronary arteries, Nature 401 (1999) 493–497.

[17] E.G. Shesely, N. Maeda, H.S. Kim, K.M. Desai, J.H. Krege, V.E. Laubach, P.A. Sherman, W.C. Sessa, O. Smithies, Elevated blood pressures in mice lacking endothelial nitric oxide synthase, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 13176–13181.

[18] G. Kojda, J.B. Laursen, S. Ramasamy, J.D. Kent, S. Kurz, J. Burchfield, E.G. Shesely, D.G. Harrison, Protein expression, vascular reactivity and soluble guanylate cyclase activity in mice lacking the endothelial nitric oxide synthase: contributions of NOS isoforms to blood pressure and heart rate control, Cardiovasc. Res. 42 (1999) 206–213.

[19] J.C. McGrath, G.B. Drummond, E.M. McLachlan, C. Kilkenny, C.L. Wainwright, Guidelines for reporting experiments involving animals: the ARRIVE guidelines, Br. J. Pharmacol. 160 (7) (2010) 1573–1576.

[20] C. Kilkenny, W. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Animal research: reporting in vivo experiments: the ARRIVE guidelines, Br. J. Pharmacol. 160 (7) (2010) 1577–1579.

[21] A.A. Miles, E.M. Miles, Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs, J. Physiol. (Paris) 118 (1952) 228–257.

[22] S.I. Shimuta, A.M. Barbosa, A.C. Borges, T.B. Paiva, Pharmacological characterization of RMP-7, a novel bradykinin agonist in smooth muscle, Immunopharmacology 45 (1999) 63–67.

[23] M. Bisha, V.T. Dao, E. Gholamreza-Fahimi, M. Vogt, M. van Zandvoort, S. Weber, M. Bas, F. Khosravani, G. Kojda, T. Suvorava, The role of bradykinin receptor type 2 in spontaneous extravasation in mice skin: implications for non-allergic angio-oedema, Br. J. Pharmacol. 175 (10) (2018) 1607–1620.

[24] W.N. Duran, J.W. Breslin, F.A. Sanchez, The NO cascade, eNOS location, and microvascular permeability, Cardiovasc. Res. 87 (2) (2010) 254–261.

[25] H. He, V.J. Venema, X. Gu, R.C. Venema, M.B. Marrero, R.B. Caldwell, Vascular endothelial growth factor signals endothelial cell production of nitric oxide and prostacyclin through flk-1/KDR activation of c-Src, J. Biol. Chem. 274 (1999) 25130–25135.

[26] K. Takeuchi, H. Watanabe, Q.K. Tran, M. Ozeki, D. Sumi, T. Hayashi, A. Iguchi, L.J. Ignarro, K. Ohashi, H. Hayashi, Nitric oxide: inhibitory effects on endothelial cell calcium signaling, prostaglandin I2 production and nitric oxide synthase expression, Cardiovasc. Res. 62 (2004) 194–201.

[27] T.J. Williams, J. Morley, Prostaglandins as potentiators of increased vascular permeability in inflammation, Nature 246 (1973) 215–217.

[28] T.J. Williams, M.J. Peck, Role of prostaglandin-mediated vasodilatation in inflammation, Nature 270 (1977) 530–532.

[29] M. Rampart, T.J. Williams, Suppression of inflammatory oedema by ibuprofen involving a mechanism independent of cyclo-oxygenase inhibition, Biochem. Pharmacol. 35 (1986) 581–586.

[30] S.K. Sauer, D. Schafer, M. Kress, P.W. Reeh, Stimulated prostaglandin E2 release from rat skin, in vitro, Life Sci. 62 (1998) 2045–2055.

[31] T. Murata, F. Ushikubi, T. Matsuoka, M. Hirata, A. Yamasaki, Y. Sugimoto, A. Ichikawa, Y. Aze, T. Tanaka, N. Yoshida, A. Ueno, S. Oh-ishi, S. Narumiya, Altered pain perception and inflammatory response in mice lacking prostacyclin receptor, Nature 388 (1997) 678–682.

[32] S. Narumiya, Y. Sugimoto, F. Ushikubi, Prostanoid receptors: structures, properties, and functions, Physiol. Rev. 79 (1999) 1193–1226.

[33] A.N. Hata, R.M. Breyer, Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation, Pharmacol. Ther. 103 (2004) 147–166.

[34] E.A. Dennis, J. Cao, Y.H. Hsu, V. Magrioti, G. Kokotos, Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention, Chem. Rev. 111 (2011) 6130–6185.

[35] Y.H. Yang, E. Morand, M. Leech, Annexin A1: potential for glucocorticoid sparing in RA, Nat. Rev. Rheumatol. 9 (10) (2013) 595–603.

[36] S. Bovens, E.A. Schulze, M. Kaptur, D. Reinhardt, M. Schafers, M. Lehr, 1-(5-Carboxyindol-1-yl)propan-2-one inhibitors of human cytosolic phospholipase A2alpha: effect of substituents in position 3 of the indole scaffold on inhibitory potency, metabolic stability, solubility, and bioavailability, J. Med. Chem. 53 (2010) 8298–8308.

[37] M. Cicardi, A. Banerji, F. Bracho, A. Malbran, B. Rosenkranz, M. Riedl, K. Bork, W. Lumry, W. Aberer, H. Bier, M. Bas, J. Greve, T.K. Hoffmann, H. Farkas, A. Reshef, B. Ritchie, W. Yang, J. Grabbe, S. Kivity, W. Kreuz, R.J. Levy, T. Luger, K. Obtulowicz, P. Schmid-Grendelmeier, C. Bull, B. Sitkauskiene, W.B. Smith, E. Toubi, S. Werner, S. Anne, J. Bjorkander, L. Bouillet, E. Cillari, D. Hurewitz, K.W. Jacobson, C.H. Katelaris, M. Maurer, H. Merk, J.A. Bernstein, C. Feighery, B. Floccard, G. Gleich, J. Hebert, M. Kaatz, P. Keith, C.H. Kirkpatrick, D. Langton, L. Martin, C. Pichler, D. Resnick, D. Wombolt, D.S. Fernandez Romero, A. Zanichelli, F. Arcoleo, J. Knolle, I. Kravec, L. Dong, J. Zimmermann, K. Rosen, W.T. Fan, Icatibant, a new bradykinin-receptor antagonist, in hereditary angioedema, N. Engl. J. Med. 363 (2010) 532–541.

[38] J.A. Nagy, L. Benjamin, H. Zeng, A.M. Dvorak, H.F. Dvorak, Vascular permeability, vascular hyperpermeability and angiogenesis, Angiogenesis 11 (2008) 109–119.

[39] R.A.M. Salama, N.H. El Gayar, S.S. Georgy, M. Hamza, Equivalent intraperitoneal doses of ibuprofen supplemented in drinking water or in diet: a behavioral and biochemical assay using antinociceptive and thromboxane inhibitory dose-response curves in mice, PeerJ 4 (2016) e2239.

[40] F. Khosravani, T. Suvorava, V.T. Dao, N. Brockmann, O. Kocgirli, F.F. Herbst, S. Valcaccia, M.U. Kassack, M. Bas, G. Kojda, Stability of murine bradykinin type 2 receptor despite treatment with NO, bradykinin, icatibant, or C1-INH, Allergy 70 (2015) 285–294.

[41] S.A. Felipe, E.S. Rodrigues, R.P. Martin, A.C. Paiva, J.B. Pesquero, S.I. Shimuta, Functional expression of kinin B1 and B2 receptors in mouse abdominal aorta, Braz. J. Med. Biol. Res. 40 (2007) 649–655.

[42] N. Benjamin, J.R. Cockcroft, J.G. Collier, C.T. Dollery, J.M. Ritter, D.J. Webb, Local inhibition of converting enzyme and vascular responses to angiotensin and bradykinin in the human forearm, J. Physiol. (Paris) 412 (1989) 543–555.

[43] J.R. Cockcroft, P.J. Chowienczyk, S.E. Brett, J.M. Ritter, Effect of N^G-monomethyl-L-arginine on kinin-induced vasodilation in the human forearm, Br. J. Clin. Pharmacol. 38 (1994) 307–310.