Hormones (2020) 19:123–133
https://doi.org/10.1007/s42000-019-00149-x

REVIEW ARTICLE

Role of the small proteoglycan bikunin in human reproduction

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Received: 12 July 2019 / Accepted: 21 October 2019 / Published online: 14 November 2019

Abstract

Purpose Female reproductive events, including ovulation, menstruation, implantation, and delivery, are physiologically characterized by deep tissue remodeling and display hallmark signs of inflammation. This review discusses the pleiotropic roles played by bikunin in human reproduction.

Methods A comprehensive literature search of the Medline/PubMed database was performed on the following topics: bikunin structure, roles in pathophysiological conditions and involvement in human reproduction, and usefulness as a marker of gestational complications or as a drug to improve pregnancy outcomes.

Results Bikunin is a small chondroitin sulfate proteoglycan found in blood, urine, and amniotic and cerebrospinal fluids, known for its anti-inflammatory and anti-proteolytic activities. Its levels are usually low, but they can increase several-fold in both acute and chronic inflammatory diseases. Bikunin plays key roles in reproductive events, such as cumulus-oocyte complex formation, pregnancy, and delivery. Its levels have been associated with the most common pregnancy complications such as preterm delivery, pre-eclampsia, and gestational diabetes mellitus. Finally, its intravaginal administration has been reported to reduce the risk of preterm delivery and to improve neonatal outcomes.

Conclusions Because of its pleiotropic roles in several reproductive events and its association with some life-threatening pathological conditions of pregnancy, bikunin may represent a non-invasive marker for improving follow-up and early diagnosis. Studies showing its usefulness as a drug for reducing the risk of preterm delivery and improving neonatal outcomes have yielded interesting results that deserve to be investigated through further research.

Keywords Bikunin · Human reproduction · Extracellular matrix stabilization · Inhibitor of uterine contractions · Anti-inflammatory agent · Anti-proteolytic agent

Introduction

It is well known that the complex physiology involved in female reproduction, including ovulation, menstruation, implantation, and delivery, is characterized by injury and tissue remodeling and display hallmark signs of inflammation [1, 2]. All of these reproductive events are associated with up-regulation of inflammatory mediators, which include cytokines, growth factors, and lipid mediators that influence the growth and function of the immune and vascular compartments [1–3]. Tissue remodeling also involves production of local inflammatory mediators such as kinins, histamine, and eicosanoids which include prostanoids (prostaglandins, prostacyclins, and thromboxanes) and leukotrienes [4].

During the pre-ovulatory phase, deep changes in the oocyte environment occur. It becomes surrounded by several layers of cumulus cells, which, stimulated by the ovulatory gonadotropin surge, are permeable to serum and secrete large amounts of the hyaluronan (HA)-rich extracellular matrix (ECM), leading to a significant expansion of the cumulus-oocyte complex (COC). Ovulation is a complex process that is initiated by the luteinizing hormone surge, and it has been likened to an inflammatory response [5].

Implantation, placentation, and the first and early second trimester of pregnancy look like "an open wound" that requires a strong inflammatory response [6]. The nesting of the blastocyst in the wall of the uterus occurs through a deep

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remodeling of the endometrium, which will provide nutrients to the growing embryo. An inflammatory environment is established to provide adequate repair of the uterine epithelium and the removal of cellular debris. Therefore, this first adaptive phase of fetal development is characterized by deep hormonal and metabolic changes that may also adversely affect maternal well-being. Conversely, the second phase of pregnancy, in which the mother, placenta, and fetus are symbiotic, is characterized by an anti-inflammatory state. Finally, in the last phase, a pro-inflammatory environment promotes the contraction of the uterus, expulsion of the baby, and rejection of the placenta [7].

Several studies have shown that, in this sequence of events, the small proteoglycan bikunin plays pleiotropic roles, from the stabilization of the ECM of the COC during the preovulatory phase [8] to its inhibitory activity of uterine muscle contraction (Table 1) and its anti-inflammatory and anti-proteolytic activities (Table 2) during gestation. Furthermore, its levels in serum, urine, amniotic fluid, or cerebrospinal fluid may represent a useful marker of preterm delivery, pre-eclampsia (PE), and gestational diabetes mellitus (GDM) (Table 3). Finally, several preclinical and clinical studies have also assessed its effectiveness as a drug in reducing the risk of preterm delivery (Table 4).

Bikunin structure and metabolism

Bikunin is a chondroitin sulfate (CS) proteoglycan (PG) with inhibitory activity against serine proteases found in plasma, urine, and amniotic fluid [36]. It consists of a small polypeptide of 147 amino acid residues, which carries an N-linked oligosaccharide at Asn^{45} and a O-linked low-charge CS chain at Ser^{10} [37–39]. The polypeptide moiety is folded in two Kunitz-type domains (7 kDa each) containing three disulfide bonds, a connecting peptide, as well as N- and C-terminal sequences of 10–25 amino acid residues each [37, 40]. The molecular mass of the whole proteoglycan is about 25–26 kDa, being made up of the protein core, the CS moiety, and oligosaccharide chains 16 kDa, 7 kDa, and 2 kDa, respectively. The CS chain is composed of 12–18 disaccharide repeating units, consisting of glucuronic acid (GlcA) and N-acetyl galactosamine (GalNAc), linked to bikunin through four monosaccharide residues (xylose-galactose-galactose-GlcA). On average, about 25% of GalNAc, more commonly near the reducing end of the CS chain, is 4-sulfated [41] (Fig. 1).

The bikunin protein core is encoded by the α1-microglobulin/bikunin (AMBP) gene as a 352-amino-acid precursor fusion protein with a functionally unrelated serum protein, α1-microglobulin. This precursor is proteolytically processed into mature bikunin, residues from 206 to 352 of the AMBP chain, and α1-microglobulin [42].

Synthesized by hepatocytes, about 90–98% of bikunin occurs in plasma as a subunit of the inter-alpha-inhibitor (IαI) family of molecules, linked via an ester bond between C6 of a non-sulfated GalNAc residue of the CS chain and the α-carbon of the C-terminal amino acid residue of one (pre-alpha-inhibitor, PαI) or two polypeptides (IαI) called heavy chains (HCs) (Fig. 1) [8, 40, 43, 44]. After an inflammatory stimulus, IαI leaves the circulation, and, in extravascular sites, the HCs are transferred from the CS chain to the locally synthesized HA to form the serum-derived hyaluronan-associated protein–hyaluronan complex (SHAP-HA) (Fig. 2). This complex plays important roles in stabilizing ECMs and is often associated with inflammatory conditions [8]. In this respect, bikunin has a central role because it is necessary for transferring HCs to HA, as demonstrated by a bikunin gene knockout mouse model [45].

When bound to HCs, bikunin lacks some of its known activities, and there is evidence that its release, by either partial proteolytic degradation of HCs [46–48] or transesterification of HCs to HA [49–52], may function as a regulatory mechanism. Free bikunin is a weak inhibitor of several Ser proteinases, including trypsin, chymotrypsin, granulocyte elastase, kallikrein, cathepsin G, and acrosin [8, 37]. Among them, it has been shown that bikunin inhibits plasmin on the surfaces of cancer cells during tumor cell invasion and metastasis [53, 54]. Besides its anti-proteolytic activity, bikunin plays a role in several pathophysiological events, such as inhibition of interleukin (IL)-8 gene expression induced by lipopolysaccharide [55],

Table 1 Studies showing bikunin inhibitory activity against uterine muscle contraction during gestation

Tissue/biological fluid	Main results	References
Myometrial biopsies from the term normal pregnant women	Inhibition of uterine muscle contraction by regulation of intracellular Ca^{++}	[9]
Human amnion cells	Inhibition of HA fragment-induced arachidonic acid metabolism	[10]
Amniotic fluid, myometrial cultures in term and preterm deliveries	Higher concentration in amniotic fluid in preterm delivery Suppression of myometrial contraction in term and preterm deliveries	[11]
Fibroblasts derived from human fetal skin	UTI inhibits calcium influx of myometrium and it is effective in preventing uterine contraction UTI is a physiological substance of fetal origin that modulates calcium-dependent and voltage-dependent potassium channels	[12]

Table 2 Studies showing bikunin anti-inflammatory and anti-proteolytic activities during gestation

Tissue/biological fluid	Main results	References
Amniotic fluid, first neonatal urine, meconium, adult urine	Higher concentration in neonatal urine Suggested protective effect on the amnion especially against IL-1 beta and TNF-beta	[13]
Rabbit cervix	Inhibition of IL-8 induced softening and dilatation of the cervix	[14]
Mouse cervix and plasma	Inhibition of preterm delivery through the suppression of interleukin-1 alpha, interleukin-6, and tumor necrosis factor-alpha production	[15]
Mouse placenta and serum	Suppression of trophoblastic apoptosis and lower level of tumor necrosis factor-alpha and interleukin-1 alpha	[16]
Mouse ascites and peritoneal macrophages	Suppression of inflammatory cytokines production induced by LPS	[17]
Mouse neutrophils stimulated by lipopolysaccharide	Bikunin can inhibit LPS-induced neutrophil activation and cytokine release playing a major contributory role in abrogation of neutrophil-mediated inflammatory responses, such as preterm delivery	[18]
Human uterine cervical fibroblasts and chorionic cells	Overall suppression of proMMP-1 and proMMP-3 and maintenance of fetal membranes and/or uterine cervix	[19]

smooth muscle contraction [9, 56], neutrophil release of elastase [57], mast cell release of histamine [58], suppression of immune cells [59, 60], and urolithiasis [61, 62], as well as stabilization of lysosomal membranes [63, 64].

Free bikunin (protein core + CS) is rapidly cleared from the circulation by both tissue uptake and renal excretion [65], and it is found in urine as urinary trypsin inhibitor (UTI). In healthy individuals, bikunin concentration is 4–7 μM, mostly as the IαI/PαI subunit (only 2–10% is in free form), while urine UTI levels are about 0.03–0.05 μM [37]. These values may increase up to tenfold in both acute and chronic inflammatory diseases, e.g., bacterial or viral infections, chronic liver disease, arthritis, Crohn's disease, cancer, systemic lupus erythematosus, renal disease, and types 1 and 2 diabetes mellitus [66–74]. It has been reported that the CS chain of Bikunin can also be modified during inflammatory conditions in terms of both sulfation degree and chain length [75]. Therefore, according to a plethora of papers, bikunin can be considered a positive acute phase protein [76].

Bikunin roles in ovarian folliculogenesis

During follicle maturation, the oocyte becomes surrounded by several layers of granulosa cells, differentiated into cumulus cells, which, stimulated by the ovulatory gonadotropin surge, become permeable to serum and secrete large amounts of HA-rich ECM, leading to a significant expansion of the COC [77, 78]. For the correct assembly of this viscoelastic matrix, the serum factor IαI is absolutely necessary [79, 80]. In this respect, the role of bikunin in SHAP-HA complex formation has been independently investigated by two research groups, both using AMBP knockout mice. This animal model showed severe female infertility, characterized by impaired ovulation and fertilization, due to the absence of the SHAP-HA complex, leading to an abnormal ECM organization around the oocyte [45, 81]. Zhuo L et al. [45] demonstrated that, although not directly involved as an ECM component of the COC, bikunin plays a central role by transferring HCs to HA. In fact, although free HCs were present in serum from bikunin-null mice, incubation with HA did not allow for SHAP-HA complex formation. In contrast, intraperitoneal administration of IαI resulted in a correct ECM deposition during COC expansion. Furthermore, Sato H et al. [81] showed that knockout mice were able to maintain pregnancy when transplanted with wild-type embryos and those ovaries from knockout mice functioned normally if transplanted into wild-type mice. Both studies demonstrated that the absence of IαI leads to anomalous ECM deposition around the oocyte and female infertility.

To determine whether, besides bikunin deficiency, other related mechanisms were involved in knockout mice infertility, Suzuki M et al. [82] performed gene expression profile analysis on over 5000 genes from ovaries of both wild-type and knockout mice. They identified a set of up- and downregulated genes encoding for proteins known to be involved in follicle development and/or ovulation, demonstrating that proteins of the IαI family may exert potent regulatory effects on these processes.

Later studies demonstrated that the transfer of HCs from the CS chain of bikunin to the C6-hydroxyls of the N-acetyl glucosamine residues of cell-secreted HA, by a transesterification reaction to form the SHAP-HA complex, requires the formation of a covalent intermediate between HC and the transferase tumor necrosis factor-stimulated gene-6 protein (TSG-6), followed by the release of bikunin [49–51]. In this process, the bikunin CS chain sulfation pattern has been reported to be crucial for the transesterification of HCs to HA [52]. Accordingly, Lord MS et al. [52], following purification of bikunin-containing fractions by anion exchange chromatography, showed that IαI in the 0.5–0.8 M NaCl elution fraction, which has highly sulfated bikunin, was able to promote

Table 3 Studies showing bikunin as marker of preterm delivery, pre-eclampsia, and gestational diabetes

Tissue/biological fluid	Main results	References
Blood and urine samples from pregnant women at low-risk of complications	Significant increase of UTI/CS value with gestation followed by a reduction after delivery	[20]
Amniotic fluid	Significant negative correlation between UTI level and gestational age (hypothesis that parturition may occur through the downregulation of UTI, which may decrease uterine contractility and maintain the uterus in a state of quiescence during pregnancy)	[21]
Amniotic fluid	The ratio polymorphonuclear elastase/UTI as a reliable index to estimate the occurrence of rupture of the membranes	[22]
Plasma samples from pregnant and postpartum women	UTI level was unchanged from the first trimester of pregnancy to the postpartum period UTI level in vaginal delivery group was significantly higher than that in caesarean section group.	[23]
Serum samples from pregnant women with severe PE vs normal pregnant women	Higher expression of α-1-microglobulin/bikunin in patients with PE	[24]
Cerebrospinal fluid of patients with PE and normotensive pregnant women	Higher AMBP levels in PE patients	[25]
Amniotic fluid of patients in preterm labour	Higher expression of AMBP in women without intra-amniotic infection/inflammation (IAI) who delivered at term compared to both women without intra-amniotic IAI who delivered a preterm newborn and women with IAI	[26]
Urine and plasma samples from first trimester pregnant women with increased risk of developing PE	UTI as early predictor of pregnancy complications such as PE, proteinuria, and hypertension	[27]
Serum and urine samples from pregnant women	Association between high fasting glucose level and altered UTI and GAGs metabolism in the first trimester of pregnancy	[28]

the transesterification of HCs to HA in the presence of TSG-6, whereas the 0.1–0.5 M NaCl fraction, mainly containing non-sulfated bikunin, had reduced ability to transfer HC proteins to HA. SHAP-HA complexes are thus cross-linked by multiple interactions between HCs and pentraxin-3, an octameric protein essential for HA incorporation into the COC matrix [83, 84].

The fate of bikunin is to be released into the blood and subsequently excreted into urine as UTI (Fig. 2), which may represent a useful non-invasive marker of ovulation, as demonstrated by our studies [20, 85]. Preliminary data by Maroclo et al. [86] showed a significant increase in total urinary glycosaminoglycan (GAG) excretion in the first half of the menstrual cycle in healthy young women, concurrent with the physiological increase in serum estrogen levels that occurs in this phase. From our side, we analyzed both plasma [85] and urinary [20] GAGs/PGs in fertile women at three time points of the menstrual cycle (days 2–3, menstrual phase; days 12–13, ovulatory phase; days 23–24, luteal phase), reporting significant differences among the ovulatory, menstrual, and luteal phases. In particular, in plasma, we identified a significant

Table 4 Studies that assessed bikunin effectiveness as drug in reducing the risk of preterm delivery

Tissues/Patients	Main results	References
Patients at preterm delivery	UTI is more effective than ritodrine in inhibition of recurrent uterine contraction and elongation of pregnancy	[29]
Mouse uterine muscle strips	Decreased incidence of preterm delivery in a dose-dependent fashion Suppression of in vitro uterine contractions	[30]
Mouse muscle tissue	Decreased incidence of preterm delivery in a dose-dependent fashion Inhibition of in vitro uterine contraction and calcium influx induced by LPS	[31]
Patients with a bulging membrane	UTI therapy is very effective in patients at risk for premature labor with a moderately developed bulging membrane during the second trimester	[32]
Patients in preterm labor with increased levels of granulocyte elastase in cervical secretions	Intravaginal treatment with urinary trypsin inhibitor reduces the risk of preterm delivery and improves neonatal outcomes	[33]
Preclinical and clinical trials	Bikunin reduces preterm labor exacerbations: usefulness and safety considerations related to this novel therapy	[34]
Pregnant women with a short cervical length at risk of preterm delivery	Administration of transvaginal UTI with vaginal irrigation showed no apparent benefit	[35]

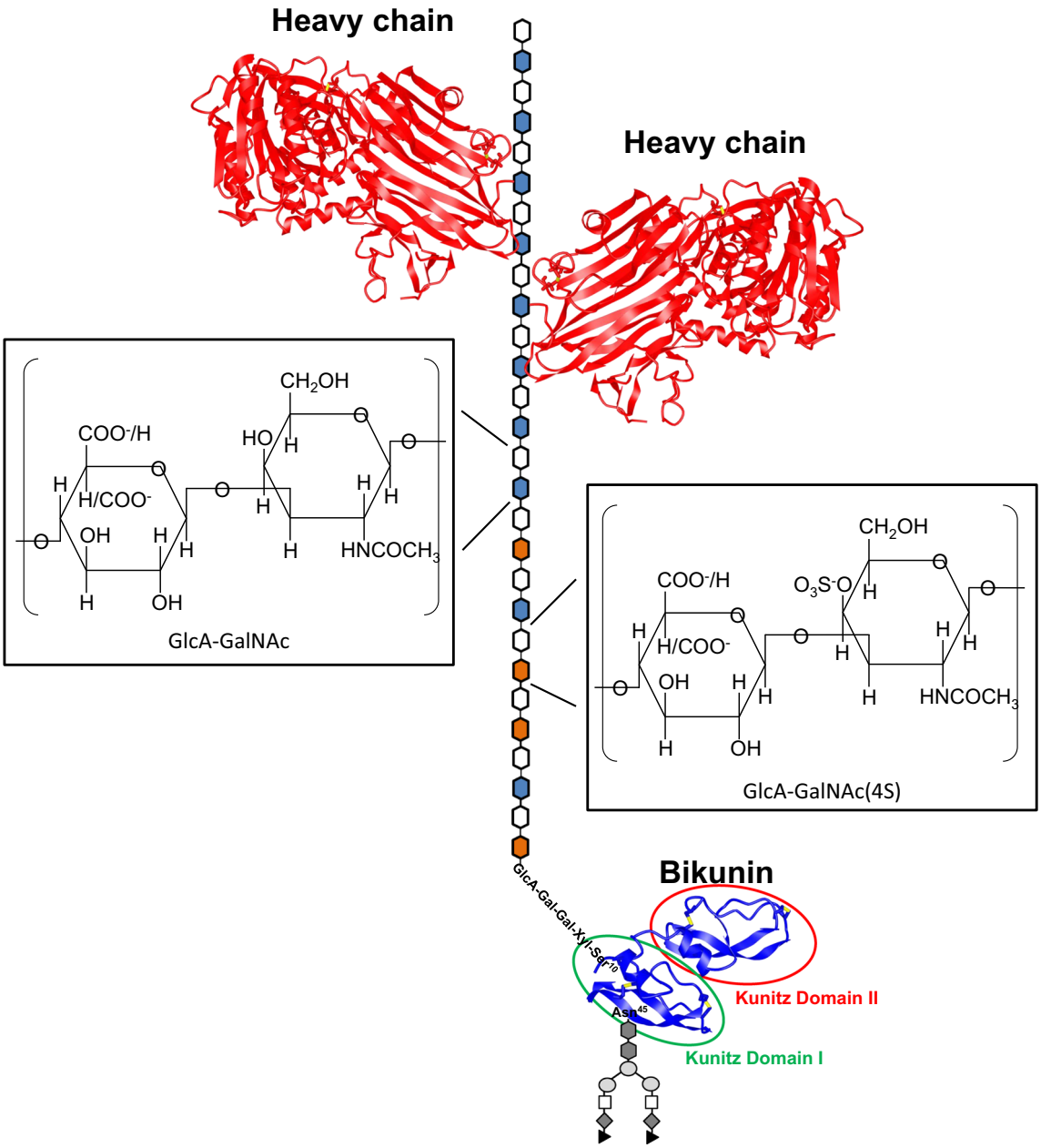

Fig. 1 Schematic drawing of inter-alpha-inhibitor. Protein cores of both bikunin and heavy chains are shown as ribbon 3D structures [40]. Structures of both non-sulfated (left) and 4-sulfated (right) chondroitin sulfate disaccharides units are reported. The N-linked oligosaccharide at Asn^{45} and the O-linked low-charge chondroitin sulfate chain at Ser^{10} to bikunin Kunitz domain I are reported according to the following:

increase of bikunin (assessed as CS levels) in the ovulatory phase, in comparison to both the menstrual and luteal phases. In urine, the UTI/CS ratio showed a similar trend, with a significant increase during the ovulatory phase (Table 5). Furthermore, both UTI and its slow migrating derivatives were observed during the ovulatory and luteal phases. Conversely, no changes in total GAG/UTI concentration and CS isomer content in fertile women with an anovulatory cycle (progesterone levels < 10 ng/mL in the luteal phase) were detected. These findings have illustrated for the first time, to our knowledge, the excretion pattern of UTI and its derivatives during the ovulatory phase in both physiological and anovulatory cycles and represent a further confirmation of bikunin's pivotal role in COC formation during the pre-ovulatory and ovulatory phases.

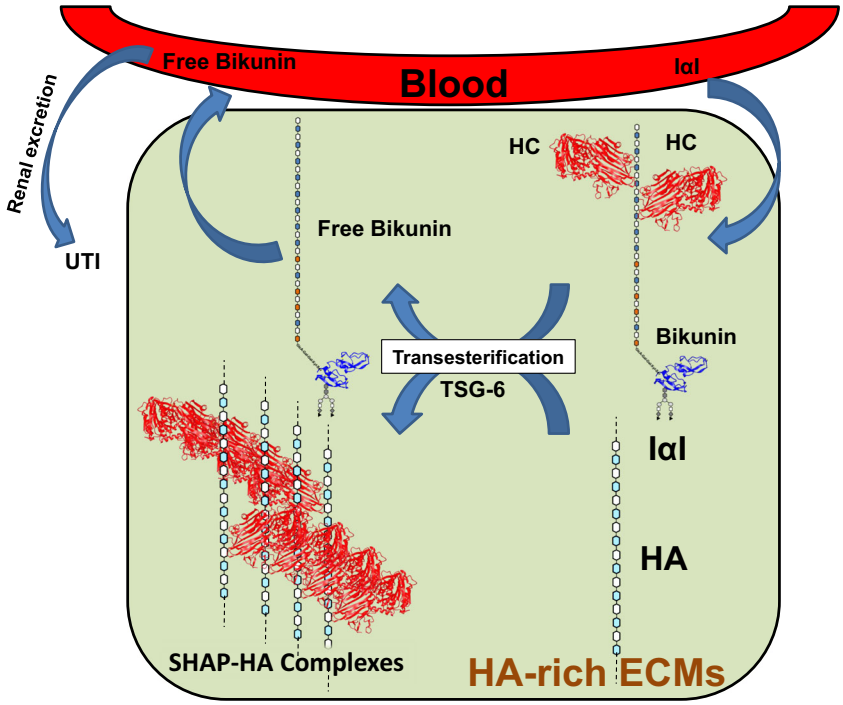

Fig. 2 Schematic representation of postulated inter-alpha-inhibitor (IαI) mechanism of extra cellular matrix (ECM) stabilization. IαI leaves the circulation and, in hyaluronan-rich ECMs, heavy chains (HCs) are transferred from chondroitin sulfate (CS) to the locally synthesized hyaluronan (HA) to form the serum-derived hyaluronan-associated protein–hyaluronan (SHAP-HA) complex by a transesterification reaction catalyzed by tumor necrosis factor-stimulated gene-6 protein (TSG-6). SHAP-HA complexes are, in turn, cross-linked by multiple interactions between HCs and pentraxin-3 (not shown). Following its release into the blood, free bikunin can be excreted into the urine (urinary trypsin inhibitor, UTI)

Bikunin roles in pregnancy

As mentioned above, several studies have shown that this small proteoglycan plays pleiotropic roles not only in the stabilization of the new-synthesized ECM during ovarian folliculogenesis [8] but also in several events during pregnancy and parturition (Tables 1, 2 and 3).

Throughout gestation, uterine smooth muscle cells are in a quiescent state, but they switch to an active contractile state during labor. Parturition occurs following an upregulation of a group of pro-labor genes and a downregulation of other groups of genes, such as those for nitric oxide synthase and bikunin, which may decrease uterine contractility, thereby maintaining the uterus in a state of quiescence during pregnancy [10]. Some studies have focused on the mechanisms by which bikunin is effective in inhibiting muscle contraction (Table 1). Kanayama N et al. [9] demonstrated, via an isometric uterine contraction test, that bikunin inhibits the effects of lipopolysaccharide (LPS), oxytocin, or prostaglandin F2α on myometrial samples from pregnant women by regulating Ca^{2+} influx in muscle cells. The same research team demonstrated that bikunin is able to inhibit the production of prostaglandin E2 in myometrial cell cultures stimulated by IL-1 and LPS [11] and of both prostaglandin F2α and prostaglandin E2 induced by low molecular weight HA in human term amnion cell cultures [10]. In a further study, they reported that bikunin could modulate the membrane excitability of the fetal and myometrial cells in contact with amniotic fluid by regulating both calcium-dependent and voltage-dependent potassium channels [12].

Pregnancy is associated with deep tissue remodeling and activation of inflammatory and immune mechanisms. Bikunin has proven to be very effective in preventing the effects of pro-inflammatory cytokines, such as IL-1β, TNF-β [13], IL-8 [14], IL-1α, IL-6, and TNF-α [15], on amnion cells and the cervix and in suppressing trophoblastic apoptosis

Table 5 Plasma and urinary glycosaminoglycans/proteoglycans in fertile women during the spontaneous menstrual cycle

	Menstrual phase (days 2 to 3)	Ovulatory phase (days 12 to 13)	Luteal phase (days 23 to 24)
Bikunin (CS levels, μg/mL)*	2.2 ± 0.6	3.1 ± 0.7[a]	2.3 ± 1.1
UTI/CS**	0.42 (0–1.03)	1.00 (0–3.60)[a]	0.23 (0–1.50)

[a] p value < 0.05, ovulatory phase vs both menstrual and luteal phase

*Data reported as mean (standard deviation) [85]

**Data reported as median (range) [20]

induced by TNF-α [16]. Furthermore, it has been shown that bikunin can inhibit LPS-induced neutrophils and macrophage activation, thus playing a major role in counteracting inflammatory responses leading to preterm delivery [17, 18]. Interestingly, it has been reported that bikunin suppresses the production of pro-matrix metalloproteinase-1 (proMMP-1) and proMMP-3, thereby participating in the maintenance of fetal membranes and preventing their premature rupture and cervical ripening [19].

Bikunin levels assessed in blood, urine, and amniotic fluid have shown that they are high during physiological gestation and rapidly decrease after delivery, in accordance with the suggested roles of bikunin in inhibiting contractions and maintaining the uterus in a quiescent state during pregnancy [21–23, 83]. Some studies have also pointed to an association between bikunin levels in blood and cerebrospinal fluid and PE [24, 25], as well as its amniotic levels and/in preterm labor [26]. In this respect, we found that pregnant women at 11–13 weeks' gestation, with some risk factors for PE, have higher levels of UTI as well as lower levels of both heparin sulfate (HS) and CS, suggesting that UTI may represent an early marker of this pathological condition [27]. Interestingly, we also found an association between high fasting glucose levels and altered bikunin/GAG metabolism in the first trimester of pregnancy [28]. GDM is defined by the American Diabetes Association (ADA) as diabetes diagnosed in the second or third trimester of pregnancy, which was not clearly diagnosed as overt diabetes prior to gestation [87]. In 2017, it was estimated that 21.3 million births (16.2%) worldwide were affected by hyperglycemia in pregnancy, and of these, 86.4% were due to GDM, ranging with significant regional variation from 9.5% in Africa to 26.6% in Southeast Asia [88]. GDM is associated with adverse pregnancy and neonatal outcomes, as well as with increased maternal risk for type 2 diabetes or GDM in subsequent pregnancies [89]. Furthermore, babies born to mothers with GDM also have a higher lifetime risk of obesity and of developing type 2 diabetes [88].

Early identification of hyperglycemia along with good glycemic control during pregnancy can reduce these risks. To our knowledge, this is the first study on the association between GAG/PG urinary excretion and the risk for GDM in early pregnancy. We grouped patients according to their fasting glycemic levels: 65–89 mg/dL, 90–99 mg/dL, and 100–125 mg/dL. Groups were homogeneous for the main demographic and clinical data, except for body mass index (BMI), which was significantly higher in pregnant women with fasting glycemic levels above 90 mg/dL. We observed alterations in both HS and CS excretion, with an increased HS/CS ratio, even in women with blood glucose levels lower than 100 mg/dL. Of note, a positive correlation between fasting blood glucose levels and the urine relative contents of HS, as well as HS/CS and UTI/CS ratios, was noted. This preliminary study shows, for the first time, to the best of our knowledge, changes in GAGs/PG metabolism at an early stage of pregnancy and suggests that, at initial screening for GDM, fasting blood glucose values of 90–99 mg/dL should be taken into consideration as a risk factor.

Finally, some preclinical and clinical trials have also evaluated bikunin effectiveness as a drug for inhibiting inflammatory responses and uterine contractions and for counteracting preterm delivery, this showing the utility and safety of this novel therapy [29–35] (Table 4). Among them, Kanayama N et al. [29] performed a study on 132 pregnant Japanese women, who had preterm labor with intact membranes at 22–34 weeks of gestation. They compared the effects of UTI administration by vaginal suppositories with the β-adrenergic agonist ritodrine, a tocolytic agent. Besides the shorter-course treatment, they found that UTI was more effective than ritodrine in reducing the recurrence rate of preterm uterine contraction due to its inhibitory effects on myometrial contraction [9]. Matsuda Y and Yunohara N [32] evaluated the effects of UTI administration in 43 patients with either a moderately developed bulging membrane or a membrane prolapsed into the vagina during the second trimester of pregnancy. Although no positive effect in the second group of patients was noted, in the first group, they observed an improvement in gestational age at delivery, birth weight, and neonatal mortality rate, suggesting that UTI therapy may improve the outcome in patients at risk for premature labor with a moderately developed bulging membrane. Hayashi M et al. [33] enrolled 73 patients with preterm labor, with high granulocyte elastase concentrations in cervical secretion, from 16 to 33 weeks of gestation. Intravaginal administration of UTI increased gestational age at delivery and reduced the rates of premature delivery and neonatal hospitalization. Very recently, Otsuki K et al. [35] examined the preventive effect of UTI administration by vaginal douche on preterm delivery in 70 women with singleton pregnancies and both cervical shortening and lower genital infections between 16 and 26 weeks of gestation. This multicenter, randomized, controlled trial did not demonstrate any improvement in the incidence of preterm delivery or in perinatal outcomes, showing no apparent benefits of vaginal irrigations with UTI. The same authors underlined some limitations of their study, including the limited number of participants and the method of administration, suggesting that future research should evaluate different modes of UTI application.

Conclusions

Bikunin/UTI plays key roles in reproductive events, such as COC formation, pregnancy, and delivery and has been associated with the most common pregnancy complications, such

as preterm delivery, PE, and GDM, of which it may represent a non-invasive marker. In this respect, improving follow-up and early diagnosis of such pathologies is mandatory given that they represent the most frequent life-threatening pregnancy complications and may affect the future life and health of the mother and offspring. According to both in vitro and in vivo studies, bikunin/UTI may represent a new class of tocolytic agents with anti-inflammatory and anti-proteolytic effects. Administered as suppositories, bikunin/UTI therapy is relatively cheap and free of side effects, and in Japan, it is becoming widely used for reducing preterm delivery in combination with ritodrine. Regrettably, only a few clinical trials have been conducted to date, but the potential of this pleiotropic proteoglycan as a therapeutic agent surely invites further, more robust clinical research.

Acknowledgments AJ Lepedda thanks Regione Autonoma della Sardegna for its financial support (POR - FSE 2014–2020 - Asse Prioritario 3 "Istruzione e Formazione" – Obiettivo tematico: 10, Priorità d'investimento: 10ii, Obiettivo specifico: 10.5, Azione dell'Accordo di Partenariato 10.5.12 - C.U.P. J86C18000270002).

Ph. Doctor School in Biomedical Sciences, Address in Gender Medicine, Men, Woman and Child, Sassari University, Italy, supported the study

The authors thank the University of Sassari for its financial support (fondo di Ateneo per la ricerca 2019).

Author contribution A.J. Lepedda: project development, data collection, manuscript writing/editing, and figure drawing/editing

P. De Muro: data collection, manuscript editing, and figure editing

G. Capobianco: project development, data management, and manuscript editing

M. Formato: manuscript writing/editing

Compliance with ethical standards

Conflict of interest The authors (Antonio J. Lepedda, Pierina De Muro, Giampiero Capobianco, and Marilena Formato) declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not needed for review manuscript.

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