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Sacubitril/Valsartan: Breaking barriers in uterine adhesion and boosting pregnancy outcomes by suppressing inflammation and fibrosis

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ABSTRACT

Objective(s): Intrauterine adhesion (IUA) enhances infertility and is primarily driven by inflammation and fibrosis. Sacubitril/Valsartan (Sac/Val), an angiotensin receptor–neprilysin inhibitor, exhibits anti-inflammatory and anti-fibrotic properties; however, its effects on IUA and reproductive outcomes have not been previously studied.

Materials and Methods: A rat model of IUA was established by mechanical endometrial injury. Animals were treated orally with Sac/Val (100 mg/kg/day) for 10 days. Inflammatory cytokine expression, oxidative stress markers, histological changes, and fibrotic indices were evaluated. Endometrial regeneration, embryonic development, pregnancy outcomes, and extra-uterine adhesion formation were also assessed.

Results: Sac/Val treatment significantly reduced uterine inflammation, oxidative stress, and collagen deposition, as evidenced by decreased expression of pro-inflammatory cytokines and pro-fibrotic markers. Histological analysis demonstrated improved endometrial regeneration, including increased gland numbers and endometrial thickness. In addition, Sac/Val enhanced embryonic development, improved pregnancy rates, increased the number of live offspring, shortened time to conception, and reduced extra-uterine adhesion formation.

Conclusion: Sac/Val enhances regeneration of endometrium and pregnancy outcomes in a rat model of IUA, primarily through suppression of inflammation and fibrosis. Further preclinical and clinical studies are needed to determine the protective functions of Sac/Val for IUA treatment.

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Introduction

Intrauterine adhesion (IUA) induces infertility, affecting 25-30% of infertile women of reproductive age (1). This condition arises from an inflammation-induced fibrosis pathway resulted from postpartum hemorrhage, hysteroscopic interventions, curettage, and myomectomy (2, 3), resulting in uterine fibrosis. IUA not only impacts pregnancy outcomes but also disrupts endometrial regeneration, resulting in menstrual abnormalities, particularly amenorrhea. Fibrosis and inflammation are the principal factors in the adhesion formation (4, 5).

Post-surgical adhesion development is a complex, dynamic process initiated by tissue trauma. This cascade involves fibrin exudate, cytokine release, cellular migration, vascular edema, and reduced fibrinolytic activity (6). The sequence of delayed cellular events encompasses immune responses and fibrinolytic deposition, ultimately leading to the formation of scar tissue rich in fibroblasts, collagen, and extracellular matrix (7). Following tissue injuries, immune cell and fibroblast accumulation at the injury site can activate the Nuclear Factor kappa B (NF-kB) signaling pathway, leading to increased levels of Tumor Necrosis Factor alpha (TNF-α) and promoting Reactive Oxygen Species (ROS) formation.

The Angiotensin II/ Angiotensin II Receptor Type 1 (AngII/AT1R) pathway indirectly activates NF-kB and reduces nitric oxide synthesis through the release of ROS (8). Interleukins contribute to adhesion formation through their involvement in humoral inflammatory responses to injury, elicited by cytokines released from damaged tissue (9). Research shows that cytokine levels, particularly TNF-α and Interleukin 6 (IL-6), correlate directly with adhesion severity (10, 11). After an endometrium injury, inflammatory signaling pathways are activated and increase expression of Interleukin 1 beta (IL-1β), TNF-α, and Interferon gamma (IFN-γ)(10, 12). It is proposed that Interleukin 1 (IL-1) contributes to inflammation while impairing local fibrinolytic capacity.

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Different approaches including hysteroscopic adhesiolysis, the use of intrauterine devices (IUDs), Uterine balloon stent, Foley catheters, anti-adhesion barriers, and estrogen therapy have been used to decrease uterine fibrosis (13, 14). While these methods demonstrated effectiveness in the treatment of IUA, they are invasive (15). Here, the protective responses of Sac/Val against IUA, and the therapeutic effects of this FDA-approved drug on embryonic development is studied.

Materials and Methods

Study design

Rats were divided into 1) Sham Group, IUA Positive Control Group, and Sac/Val-treated Group (16, 17). Animals were allocated to experimental groups using a computer-generated random number list (simple randomization). Regarding sample size determination, key factors including effect sizes, power levels, significance thresholds, attrition rates, variability, and ethical considerations were considered. We performed a power analysis to justify the adequacy of the sample size for achieving reliable and statistically significant results, striking a balance between detecting meaningful effects and minimizing unnecessary animal use. This approach facilitated direct comparisons with previous findings and adhered to established methodologies within the field (18, 19).

Vaginal smears were applied onto a glass slide, allowed to air-dry, and subsequently stained with Giemsa. The assessment of cell type and determination of the stage of the estrous cycle were performed (20). Mechanical induction of IUA was performed by a single individual to minimize the potential bias.

Following model induction, treatment commenced in the drug-receiving group for duration of 10 days. The study proceeded in three phases, including Sacrifice for analyses, fertilization after completing treatment, cesarean section on the 15th day of the gestation, and natural delivery.

Three phases of the study

Phase 1: In this phase 10 days after surgery, rats were sacrificed and the protective responses of Sac/Val on endometrium was studied. Extra-uterine adhesions to internal organs were evaluated using the scoring system for adhesion proposed by Mazuji *et al.* (21).

Phase 2: After 10 days of surgery rats were housed to mate. Males were paired with females continuously for 24 hr (1:1 mating scheme). To minimize potential confounding effects of RAAS-modulating therapy on conception and fetal development, all animals in the Sac/Val-treated group completed the 10-day treatment regimen prior to mating. A drug-free washout interval of 24 hr was applied between the final oral dose and pairing with male rats. No pharmacological intervention was administered during gestation in phases 2 and 3 (22, 23). If no vaginal plaque was observed, the mating process was repeated until all females were confirmed pregnant.

15 days post-mating, rats were subjected to caesarian section, and embryo parameters were compared between groups. Critical organogenesis is well advanced by gestational day 15, and the embryo parameters could be precisely measured (24).

Phase 3: Neonatal outcomes including the number and weight of babies, the number of live births, and conception time were analyzed at this stage. The study design of this manuscript is presented in Figure 1.

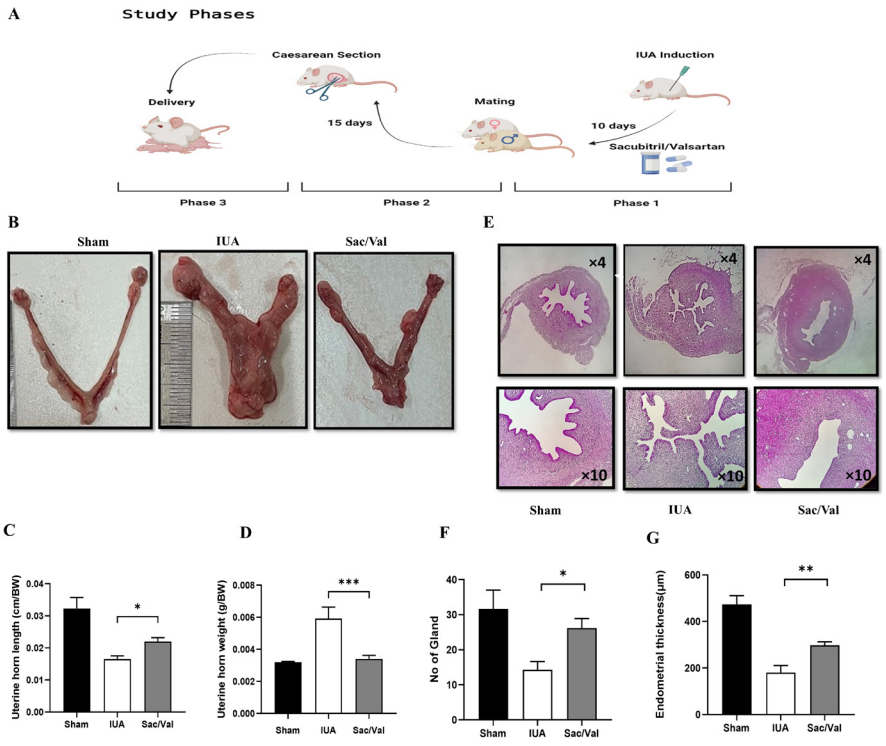

Figure 1. Sacubitril/Valsartan (Sac/Val) decreased adhesion bands and enhanced endometrial regeneration in rats
(A) Schematic presentation of the study. The therapeutic effects of Sac/Val on (B and C) uterine length and (D) weight were investigated in different groups. (E) H&E-stained sections of the uterine in different groups. (F) Number of glands and (G) endometrial thickness were also measured in the H&E-stained sections of the uterine. * P<0.05, ** P<0.01. Data were presented as Mean±SEM

Histological evaluation

Hematoxylin and Eosin (H&E) staining and Trichrome staining were performed to examine endometrial alterations and fibrosis, respectively (25, 26). For histological assessment from each uterine horn, three to four representative cross-sections encompassing the injured and adjacent areas were obtained at 4-5 μm thickness. For quantitative analysis, three non-overlapping fields per section were selected at a fixed magnification in the endometrial region, avoiding areas with technical artifacts or tangential sectioning. All evaluators were blind to the tissue sections. Tissue slides were analyzed for endometrial gland count and thickness using H&E staining. Images from each group were evaluated, and ImageJ software was used to ensure consistency in the assessment.

Quantitative RT-PCR

GAPDH gene was used as the control housekeeping gene (27). Primer sequences are presented in Table 1. qRT-PCR analyses were conducted by investigators blinded to group allocation.

ELISA assay

TNF-α and IL-6 were measured using Zellbio ELISA kits as described previously (28). ELISA measurements were performed by investigators blinded to the experimental groups.

Oxidative stress evaluation

Oxidative stress markers, including Malondialdehyde (MDA) as an oxidative marker, and the enzyme activities of Superoxide dismutase (SOD) and Catalase (CAT) were measured as described (29).

Scoring of extra-uterine adhesions

Adhesions were scored as described by Mazuji *et al.* (21) (Table 2-4) and widely applied in experimental adhesion models. The animals and corresponding photographs were coded by a third person not involved in scoring, so that the observers had no information about whether a given animal belonged to the Sham, IUA, or Sac/Val group. Each observer independently assigned scores for each parameter based on intra-operative findings and photographic documentation. If the difference between the two scores for a given animal exceeded one grade, the case was re-evaluated jointly until consensus was achieved. This procedure was implemented to ensure reproducibility and to minimize subjective bias in adhesion scoring.

Table 1. The sequence of the primers used for qRT-PCR

Gene	Source	Primer	Sequence
GAPDH	Rat	Forward	5'-CTTCTCTTGTGACAAAGTGGACA-3'
		Reverse	5'-TTGACTGTGCCGTTGAACTTG-3'
Interleukin-1 beta (IL-1β)	Rat	Forward	5'-GACTTCACCATGGAACCCGT-3'
		Reverse	5'-GGAGACTGCCCATTCTCGAC-3'
Interferon gamma (IFNγ)	Rat	Forward	5'-TGAGCATCGCCAAGTTCGAG-3'
		Reverse	5'-TCTGGTGACAGCTGGTGAATC-3'
Collagen, type I, alpha 1 (Col 1A1)	Rat	Forward	5'-CCCAGCGGTGGTTATGACTT-3'
		Reverse	5'-AACGGCCACCATCTTGAGAC-3'
Collagen, type III, alpha 1 (Col 13A1)	Rat	Forward	5'-ATATGTGTCTGCGACTCGGG-3'
		Reverse	5'-GGGCAGTCTAGTGGCTCATC-3'

Statistical analysis

Data distributions were assessed for normality using the Shapiro–Wilk test, and homogeneity of variances was examined using Levene's test. To analyze data in more than two groups, one-way analysis of variance (ANOVA) was performed when the assumptions of normality were satisfied, followed by Tukey's honest significant difference (HSD) test. *P*-values<0.05 indicated a significant difference between the given groups. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Results

Sacubitril/Valsartan enhances endometrial regeneration and reduces adhesion bands in rat model of IUA

Sac/Val treatment significantly decreased uterine shortening compared to the untreated IUA group (Figures 1B and C). Uterine length and weight were evaluated as gross morphological indicators of uterine condition. The shortened uterine horns observed in the IUA group are consistent with reports linking severe intrauterine fibrosis and adhesion formation to contraction of uterine tissues; however, these macroscopic changes are considered indirect reflections of the underlying pathological process. Uterine shortening and increased uterine weight in the IUA group likely represent secondary manifestations of severe endometrial fibrosis, extracellular matrix deposition, inflammatory cell infiltration, and tissue edema, as described previously in experimental adhesion models (28). The significant restoration of uterine length and normalization of uterine weight in the Sac/Val group suggest improved uterine integrity, which needs further investigation by the more direct histological and molecular findings to be directly

Table 2. The extent score of adhesion formation

Grade	Description
0	No uterine adhesion
1	1-25% involvement
2	26-50%
3	51-75%
4	76-100%

Table 3. The severity score of adhesion formation

Grade	Description
0	No adhesion
1	Filmy avascular
2	Vascular or opaque
3	Cohesive attachment of uterine horn to each other or other abdominal organs

Table 4. The degree score of adhesion formation

Grade	Description
0	No adhesion
1	The adhesion could be separated from tissue with gentle traction
2	The adhesion could be separated from tissue with moderate traction
3	Requiring sharp dissection

associated with fibrosis and inflammation, respectively. H&E results showed a marked improvement in endometrial regeneration in Sac/Val-treated rats. Specifically, there was a significant increase in the number of glands (Figure 1F), and endometrial thickness (Figure 1G).

Sacubitril/Valsartan decreased inflammation in rat uterine

Sac/Val treatment suppressed expression of key inflammatory cytokines. Specifically, in mRNA expression levels of IL-1β and IFN-γ (Figures 2A and B). Moreover, the concentrations of TNF-α and IL-6 were decreased in the Sac/Val-treated group, but the difference was statistically significant only for the TNF-α protein (Figures 2C and D). Consistently, Sac/Val exhibited strong antioxidant effects by reducing Malondialdehyde (MDA) concentrations, an oxidant marker, in uterine tissues (Figure 2E). Similarly, Sac/Val treatment enhanced the enzymatic activities of key antioxidants, catalase (CAT)(Figure 2F) and superoxide dismutase (SOD)(Figure 2G), indicating a robust increase in antioxidant defense mechanisms.

Sacubitril/Valsartan decreased fibrosis in uterine tissues

Our study assessed fibrinolytic effects of Sac/Val in uterine tissue samples. Sac/Val treatment suppressed deposition of collagen, as demonstrated by Trichrome staining (Figure 3A). Histological analysis showed a notable reduction in uterine fibrosis treated with Sac/Val (Figure 3B). Consistently, the mRNA levels of COL1a1 and COL3a1 were down-regulated significantly (Figures 3C and 3D). These results support the potent anti-fibrotic effects of Sac/Val, suggesting its potential to reduce fibrosis and adhesion band formation in uterine tissues.

Effect of Sacubitril/Valsartan on the embryonic development

The injury to the untreated uterine tissue led to a decrease in the total number of embryos (Figure 4A). However, oral

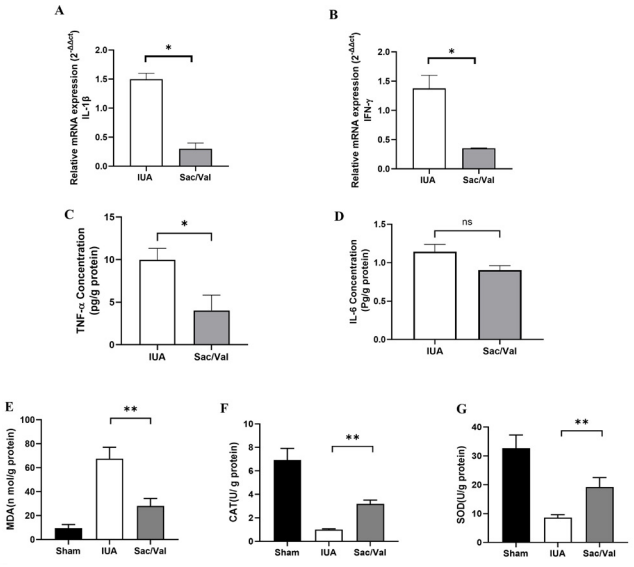

Figure 2. Sacubitril/Valsartan (Sac/Val) attenuated uterine inflammation in rat IUA model

Transcriptional mRNA levels of (A) IL-1β and (B) IFN-γ were measured in the uterine tissue samples. The protein expression levels of (C) TNF-α and (D) IL-6 were evaluated in the tissue homogenates of the uterine samples. (E) Sac/Val attenuated MDA concentration, whereas (F) increased enzymatic activities of CAT and (G) SOD, in uterine tissue samples. * P<0.05, ** P<0.01, *** P<0.001. Data were presented as Mean±SEM

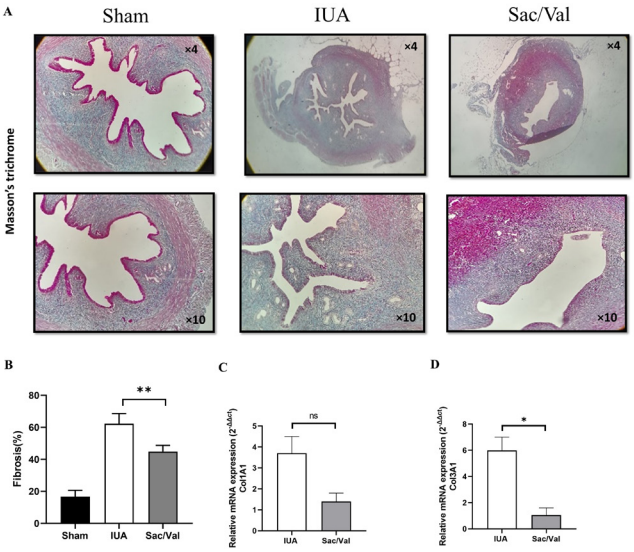

Figure 3. Sacubitril/Valsartan (Sac/Val) elicited anti-fibrotic effects in rat uterine tissues

(A) Sac/Val decreased collagen deposition in the uterine tissue sections as visualized by Trichrome staining. (B) Fibrosis percentage was compared and quantified in the uterine histological sections of rats. mRNA levels of COL1a1 and COL3a1 were decreased in uterine tissue of SAC/Val-treated group, when compared to the untreated counterpart. * P<0.05, ** P<0.01. Data were presented as Mean ± SEM

administration of Sac/Val post-injury significantly increased the embryo number (Figure 4B). Consistently, the embryos' size (Figure 4C) was increased, compared to the untreated group. The percentage of live embryos was markedly improved in the Sac/Val-treated group (Figure 4D). The viability assessment involved extracting and evaluating embryos under anesthesia, distinguishing live from necrotic embryos by color. Injury-induced reductions in fetus implantation factors, such as placental size was significantly ameliorated with Sac/Val treatment (Figure 4E).

Effect of Sacubitril/Valsartan on pregnancy

All the animals in the sham group, with no uterine injury,

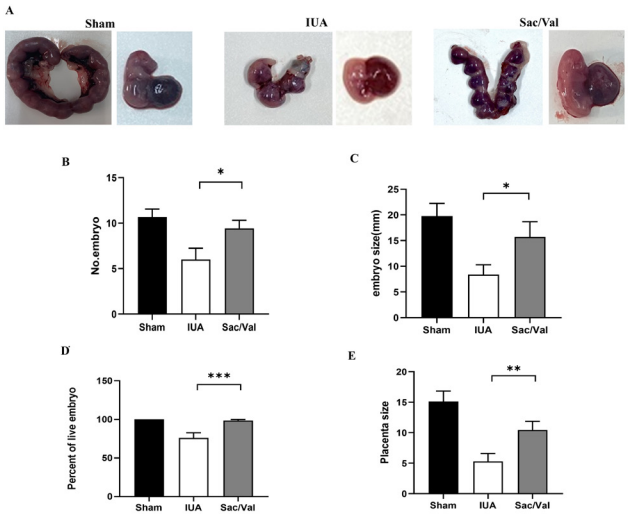

Figure 4. Sacubitril/Valsartan (Sac/Val) enhanced embryonic development in the rat model of IUA

(A) Macroscopic comparison of uterine, embryo, and placenta in different study groups. Gavage administration of Sac/Val post-injury enhanced embryonic development as evidenced by an increase in (B) number of total embryos, (C) embryo size, (D) the percent of live embryos, and (E) placenta size. * P<0.05, ** P<0.01, *** P<0.001. Data were presented as Mean ± SEM

had successful pregnancies by the first mating attempt. Oral administration of Sac/Val improved pregnancy rate during the 1st and the 2nd attempts. As shown in Figure 5A, about 75 percent of Sac/Val-treated rats were made pregnant during these two attempts, whereas in the IUA group, about 50 percent of rats had successful pregnancy during the 3rd mating attempt (Figure 5A). The total number of babies (Figures 5B and C), the percentage of live babies (Figure 5D) and weight of babies (Figure 5E) were enhanced in the Sac/Val-treated group. Maternal body weight was recorded longitudinally. No significant differences in maternal weight trajectories were detected among the groups, indicating that the reproductive outcomes observed were not attributable to systemic maternal illness, malnutrition, or treatment-related toxicity. Embryonic and neonatal measurements were analyzed on a litter-based basis to minimize the confounding influence of litter size on fetal and pup body weight. Moreover, Sac/Val reduced the time to conception compared with the IUA control group (Figure 5F). "Time to conceive" was defined as the number of days between the first co-housing with a fertile male and confirmation of mating by the presence of a vaginal plug, as previously established (22, 30). Because all animals underwent identical anesthesia, surgical manipulation, handling, and postoperative care, including a Sham group allowed us to isolate the effects of IUA and the Sac/Val intervention from those attributable to surgical stress.

Sacubitril/Valsartan suppressed extra-uterine adhesion

We compared the extra-uterine adhesion between groups (21). Sac/Val reduced extra-uterine adhesion to other organs (Figure 6A). Moreover, the adhesion extent score (Figure 6B), severity score (Figure 6C), degree score (Figure 6D), and total adhesion score (Figure 6E) were decreased in the Sac/Val-treated rats, as well.

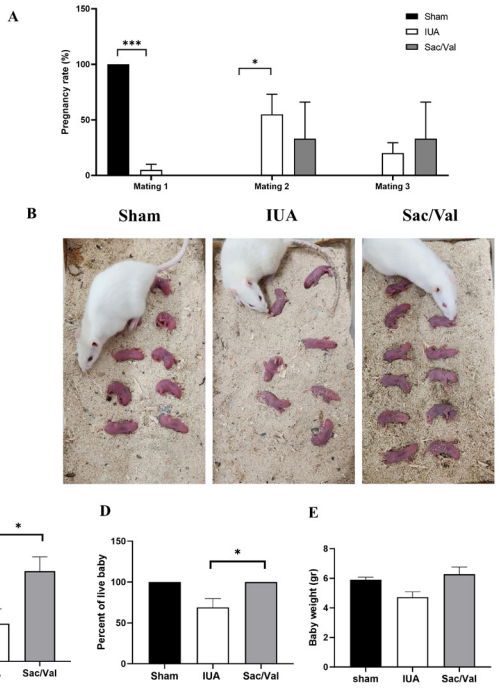

Figure 5. Pregnancy outcomes were enhanced by Sacubitril/Valsartan (Sac/Val) treatment

(A) The rate of pregnancy is compared between groups. Oral administration of Sacubitril/Valsartan (Sac/Val) enhanced the pregnancy outcomes as (B-C) the number of babies, (D) the percent of live babies, and (E) the weight of babies were increased in the Sacubitril/Valsartan (Sac/Val)-treated rats. (F) Mean conception time is compared between groups. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Data were presented as Mean ± SEM

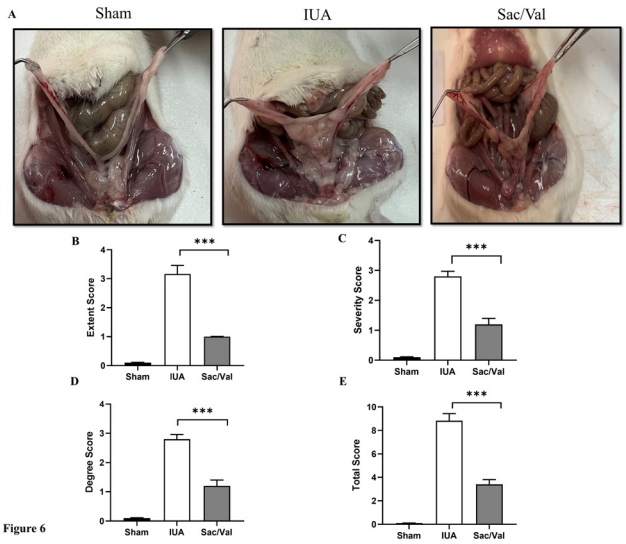

Figure 6. Sacubitril/Valsartan (Sac/Val) decreased extra-uterine adhesions

(A) Compared to the IUA group, the Sacubitril/Valsartan (Sac/Val) attenuated extra adhesion of uterine to the internal organs. (B) The extent, (C) severity, (D) degree, and (E) Total score of extra-uterine adhesions were compared between different groups. $^{***}P<0.001$. Data were presented as Mean ± SEM

Impact of Sacubitril/Valsartan on histopathological alterations in rat organs

Rat liver, kidney, and heart tissues were taken out and stained with H&E. Comparing the Sac/Val-treated group to the sham group, revealed that there were no morphological changes linked to toxicity, no hepatic or renal inflammatory cell infiltration, and no cardiac myofiber reorganization in the Sac/Val-treated group (Figure 7).

Discussion

In the context of IUA, inflammation and fibrosis are central drivers of endometrial damage and impaired regeneration. Excessive inflammatory responses following endometrial injury promote extracellular matrix deposition and collagen accumulation, ultimately leading to adhesion formation and

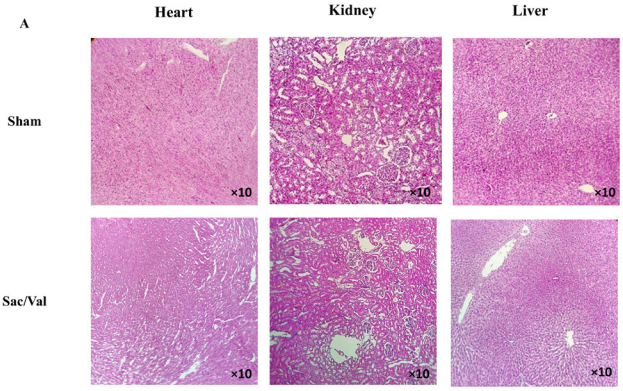

Figure 7. Oral administrations of Sacubitril/Valsartan (Sac/Val) is safe to internal organs

The Hematoxylin and Eosin (H&E) staining of heart, liver, and kidney tissues showed no histo-pathological changes, infiltration of inflammatory cells to liver or kidney, nor re-arrangement of myofibers in heart in Sacubitril/Valsartan (Sac/Val)-treated group

reduced uterine receptivity. In the present study, Sacubitril/Valsartan significantly attenuated inflammatory and fibrotic responses within uterine tissues, which was accompanied by improved endometrial architecture and reproductive outcomes.

Sacubitril/valsartan containing both angiotensin II receptor blocker and neprilysin inhibitor is therapeutically effective in the treatment of heart failure (31). The renin-angiotensin-aldosterone system (RAAS) can contribute to fibrosis through angiotensin II, which leads to extracellular matrix accumulation (32). AngII/AT1R interactions facilitate fibrosis-related molecular mechanisms, including increased inflammation, oxidative stress, TGF-β/Smad axis activation, and NF-κB induction (33). Research also shows that ACE-I and angiotensin receptor blockers decrease TGF-β-mediated mesothelial fibrosis *in vitro* (34). These findings collectively suggest that targeting angiotensin system could decrease adhesion formation. In line with this, in a study, Sac/Val reduced myocardial fibrosis and inflammation in a rat model of HFpEF. Previous studies have confirmed the anti-fibrosis effect of ARBs in different models (35, 36). Beyond angiotensin II receptor blockade, sacubitril exerts its pharmacological effects through inhibition of neprilysin, a zinc-dependent endopeptidase responsible for the degradation of several bioactive peptides, including bradykinin, and adrenomedullin. Neprilysin inhibition enhances bioavailability of natriuretic peptides, which activate the cGMP–PKG signaling pathway, resulting in potent anti-inflammatory, anti-fibrotic, vasodilatory, and pro-angiogenic effects. Emerging evidence indicates that natriuretic peptides suppress TGF-β/Smad signaling, inhibit fibroblast activation, reduce extracellular matrix deposition, and modulate local immune responses. Given the central role of inflammation, fibrosis, and impaired vascular remodeling in IUA pathogenesis, neprilysin inhibition may represent a previously unexplored mechanistic axis for promoting endometrial regeneration and adhesion resolution. Importantly, these effects cannot be achieved by angiotensin receptor blockade alone, suggesting that the dual mechanism of Sac/Val offers a unique therapeutic advantage over conventional RAAS inhibitors in the context of IUA and endometrial repair. Consistent with these findings our results showed that Sac/Val treatment significantly reduced uterine inflammation and oxidative stress in uterine tissues.

Moreover, oral administration of Sac/Val led to significant improvements in embryonic development parameters, enhancing embryo implantation and pregnancy outcomes. Improved uterine perfusion and angiogenesis may partially explain the increased endometrial thickness, gland number, and improved implantation outcomes observed in the Sac/Val-treated group.

Despite the development and clinical application of anti-adhesion membranes, a comprehensive understanding of adhesion formation mechanisms is unknown. Seprafilm has been subject to the most extensive research as an adhesion prevention barrier (37). Seprafilm is a water-gelatinizing, absorbable, and temporary barrier material derived from sodium hyaluronate or carboxymethylcellulose (38). However, Seprafilm has certain drawbacks: it may rupture when bent sharply and can adhere to surgical instruments or tissues upon exposure to moisture. These limitations can pose challenges in laparoscopic applications and may cause film ruptures during introduction into the abdominal cavity via laparoscopic ports (39). Continued research is essential for the development of efficient strategies to prevent adhesion formation. Research by LeGrand *et al.* demonstrated promising outcomes using various NSAIDs like tolmetin, ibuprofen, indomethacin, and aspirin in animal studies (40). These anti-inflammatory drugs have been shown to interfere with multiple inflammatory mediators and enzymes, including prostaglandins, interleukins, and cytokines. Studies indicate that anti-inflammatory drugs can potentially minimize adhesions (41, 42).

Similar to these findings, our results showed that Sac/Val exerted potent anti-inflammatory and fibrinolytic responses and has therapeutic potential for reducing adhesion formation. The observed protective effects of Sac/Val can be largely attributed to its ability to inhibit key processes such as inflammation, oxidative stress, and fibrosis. Sac/Val not only decreased the formation of adhesion bands but also promoted a healthier uterine environment conducive to improved reproductive properties, including the number of total embryos, the percentage of live embryos, and embryo size and weight. These findings support a uterus-specific interpretation of Sac/Val activity, rather than extrapolation from non-gynecological disease models, and emphasize the relevance of anti-inflammatory and anti-fibrotic modulation in the pathophysiology of IUA. While these results are promising, further investigation is necessary to underline the drug's therapeutic effects in treating uterine adhesions. Additionally, conducting clinical trials in humans will contribute to more robust conclusions and potential advancements in the field of uterine adhesion treatment.

Study limitation

Our study's reliance on an animal model is a notable limitation, as translating results to humans may not be straightforward. Despite this limitation, rats serve as a valuable model for assessing IUAs, as demonstrated by their widespread use in related experimental studies. Prescription drug usage during pregnancy is common, yet there remains a limited understanding of the associated maternal-fetal safety and effectiveness, largely due to the traditional exclusion of pregnant individuals from clinical trials. This lack of data contributes to potential health risks for both mother and baby, underscoring the need for more comprehensive research that includes pregnant individuals in clinical studies. However, this finding may be influenced by the presence of maternal hypertensive disease, as chronic hypertension in pregnant women is linked to negative outcomes for the fetus. It is advised that women of childbearing age should abstain from this combination as ARBs have been linked to fetal kidney problems and low amniotic fluid levels. Recognizing the potential risks associated with drug use during pregnancy, the second and third phases of the study were designed to ensure that rats completed their treatment period prior to mating and fertilization. Throughout gestation, the pregnant rats did not receive any medication to safeguard maternal and fetal health. One limitation of the present study is the absence of a comparator group treated with a conventional anti-inflammatory agent. Direct comparison with standard anti-inflammatory therapies may provide additional insight into relative efficacy and specificity; however, such comparisons were beyond the scope of the current experimental design. Future studies should address this limitation by

incorporating established anti-inflammatory agents as comparator controls.

Conclusion

Our study is one of the pioneers in assessing the effect of Sac/Val on a rat model of IUA. Despite these promising findings, further research is needed to fully elucidate the precise biological mechanisms underlying the protective effects of Sac/Val. Future studies should focus on long-term safety, optimal dosing strategies, and the potential benefits of Sac/Val in combination with other therapeutic interventions. Sacubitril/Valsartan has been shown to effectively enhance endometrial regeneration and pregnancy outcomes through its anti-inflammatory and anti-fibrotic properties. However, further research in other animal models and eventually human clinical trials is necessary to confirm the findings and support clinical applications.

Acknowledgment

None.

Availability of Data and Materials

The data that support the findings of this study are available upon reasonable request.

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Consent for Publications

All authors declare their consent for the publication of this work in all formats and agree to abide by the journal's terms and conditions.

Authors' Contributions

SA P designed and performed animal experiments. SM H analyzed data and contributed to the clinical interpretation of the results. M K and A A designed the study plan and supervised the project. All authors discussed the results and contributed to the final manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest.

Declaration

We acknowledge the use of ChatGPT AI tool to revise some sections of the manuscript during the 1st round of the revision.

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