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Highly expressed ACE-2 receptors during pregnancy: A protective factor for SARS-COV-2 infection?

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SUMMARY

While previous viral pandemics showed that pregnancy was a risk factor for susceptibility and adverse outcomes, current evidence is conflicting whether SARS-CoV-2 infection during pregnancy is more severe than in the general population, with relatively low maternal and fetal/neonatal mortality rates. SARS-CoV-2 is known to enter host cells via the ACE-2 receptors, competitively occupying their binding sites. In theory, viral invasion can lead to a reduction in available ACE-2 receptors and consequently an unbalanced regulation between the ACE-AngII-AT1 axis and the ACE-2-Ang-(1-7)-MAS axis, thus enhancing pathological vasoconstriction, fibrosis, inflammation and thrombotic processes. We hypothesize that the normal pregnant state of highly expressed ACE-2 receptors leads to higher Ang-(1-7) levels and consequently more vasodilation and anti-inflammatory response to SARS-COV-2 infection. We suggest that this up-regulation of ACE-2 receptors in human gestation may actually be clinically protective and propose a potential research line to investigate this hypothesis, which may lead to future novel therapeutics.

Key points

- SARS-CoV-2 is known to enter host cells via the ACE-2 receptors, competitively occupying their binding sites. In theory, viral invasion can lead to a reduction in available ACE-2 receptors and consequently an unbalanced regulation between the ACE-AngII-AT1 axis and the ACE-2-Ang-(1-7)-MAS axis, thus enhancing pathological vasoconstriction, fibrosis, inflammation and thrombotic processes.
- We hypothesize that the normal healthy pregnant state of highly expressed ACE-2 receptors leads to higher Ang-(1-7) levels and consequently more vasodilation and anti-inflammatory response to SARS-COV-2 infection.
- We suggest that this up-regulation of ACE-2 receptors in human gestation may actually be clinically protective and propose a potential research line to investigate this hypothesis, which may lead to future novel therapeutics.

Introduction

Initial reports of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had suggested that pregnancy negatively impacted on the clinical course of infection [1–3]. Conversely, current evidence is conflicting whether SARS-CoV-2 infection during pregnancy is more severe than in the general population [3–5], with relatively low maternal and fetal/neonatal mortality rates [3–5].

Previous viral outbreaks [1,6] demonstrated detrimental effects on perinatal and maternal outcomes. Rejection of fetal tissues is mitigated by elevation of humoral responses and suppression of cell-mediated immunity throughout pregnancy [7]. These changes are referred to as the T-helper lymphocyte type-1-type-2-type17 (Th1/Th2/Th17) and regulatory T cell (Treg) paradigm [8], and impact directly on the response to viral infections [7,9]. Curiously, SARS-CoV-2 has not, thus far, demonstrated the same pregnancy related adverse effects [4,10,11], rather than those associated to preterm deliveries [12].

Both the immune system and the renin-angiotensin system (RAS) play particularly important roles in mediating SARS-CoV-2 virus entry into human cells [13]. The efficient binding of the SARS-CoV-2 spike (S) viral envelope protein to the angiotensin converting enzyme-2 (ACE-2) receptor, and the transmembrane protease serine 2 (TMPRSS2) for S protein priming, are necessary steps to facilitate the successful viral entry to host cell [14,15].

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It was described that, in individuals with COVID-19, severe multi-organ involvement is related to pathobiological immune alterations, rather than to direct viral response [16]. Inflammatory changes were extensively observed in post-mortem organs of patients that succumbed to severe COVID-19 illness [16]. These organ tissues share common local RAS-autocrine pathways that may have been affected by the immune-inflammatory interaction between SARS-CoV-2 and ACE-2 receptors [13,16].

ACE-2 and Ang-(1-7) expression during pregnancy

The most important role of the ACE-2 membrane-bound enzyme, is the cleavage of angiotensin I or II (Ang I or Ang II) to angiotensin1-7 [Ang-(1-7)] [13,17]. This branch of the RAS cascade has an important counter-regulatory effect on the vasoconstrictor, pro-inflammatory and pro-thrombotic activity of the ACE-AngII-AT1 axis. Ultimately, the ACE-2-Ang-(1-7)-MAS axis results in vasodilatation, natriuresis, anti-inflammatory and anti-thrombotic effects [13,18–21] (Fig. 1).

Animal and human studies before the SARS-CoV-2 pandemic have demonstrated that ACE-2 and Ang-(1-7) expression are enhanced during normal pregnancy [18,19,22,23], resulting in increased vasodilation, and a reduction in fibrosis, inflammation, thrombosis and pulmonary damage [13,20,21]. In theory, SARS-CoV-2 viral invasion can induce a relative reduction in unbound ACE-2 receptors and subsequent unbalanced dysregulation between the ACE-AngII-AT1 axis and the ACE-2-Ang-(1-7)-MAS axis, thus contributing to an environment of progressive vasoconstriction, fibrosis, inflammation and thrombo-embolic processes [13]. (Fig. 1).

Comorbidities that are known to be associated with ACE-2 deficiency include older age, diabetes, cardiovascular disease and hypertension [13]. Individuals with these conditions also represent those that are more likely to be infected with SARS-CoV-2 and develop more severe complications of COVID-19 both in the general population and also during pregnancy [5,24,25]. In pregnancies with preeclampsia (PE), plasma ACE-2, Ang-(1-7) levels and ACE-2 activity are lower compared with normotensive pregnant women [22], resulting in the opposite biological consequences of vasoconstriction, inflammation and pro-thrombotic effects [26]. Others have previously described the similar clinical phenotypes of preeclampsia and severe COVID-19 infection during pregnancy [26].

The relationship between the downregulation of ACE-2 receptors in individuals with a reduced baseline ACE-2 phenotype and its effect on worsening SARS-CoV-2 infection, compared to those individuals with normal baseline or enhanced ACE-2 phenotype was previously explored by Verdecchia et al., at early stages of the COVID-19 pandemic [13]. This proposed physiological pathway [13] was the basis for the rationale hereby presented.

Placental and fetal expression of ACE-2 and Ang-(1-7)

Strong expression of ACE-2 receptors in trophoblastic human cells is demonstrated throughout pregnancy, supporting a receptor-mediated mechanism leading to SARS-CoV-2 placental infection [27]. Low levels of ACE-2 and TMPRSS2 have been identified in extra villous trophoblast (EVT) cells at 8 weeks' gestation, whereas ACE-2 and TMPRSS2 placental expression was significantly increased in EVT at 24 weeks' gestation [28]. These results suggest that the placental expression of ACE-2 and TMPRSS2 at the maternal-fetal interface may increase as pregnancy advances [28]. Furthermore, syncytiotrophoblastic ACE-2 expression may regulate Ang-(1-7) release into maternal circulation,

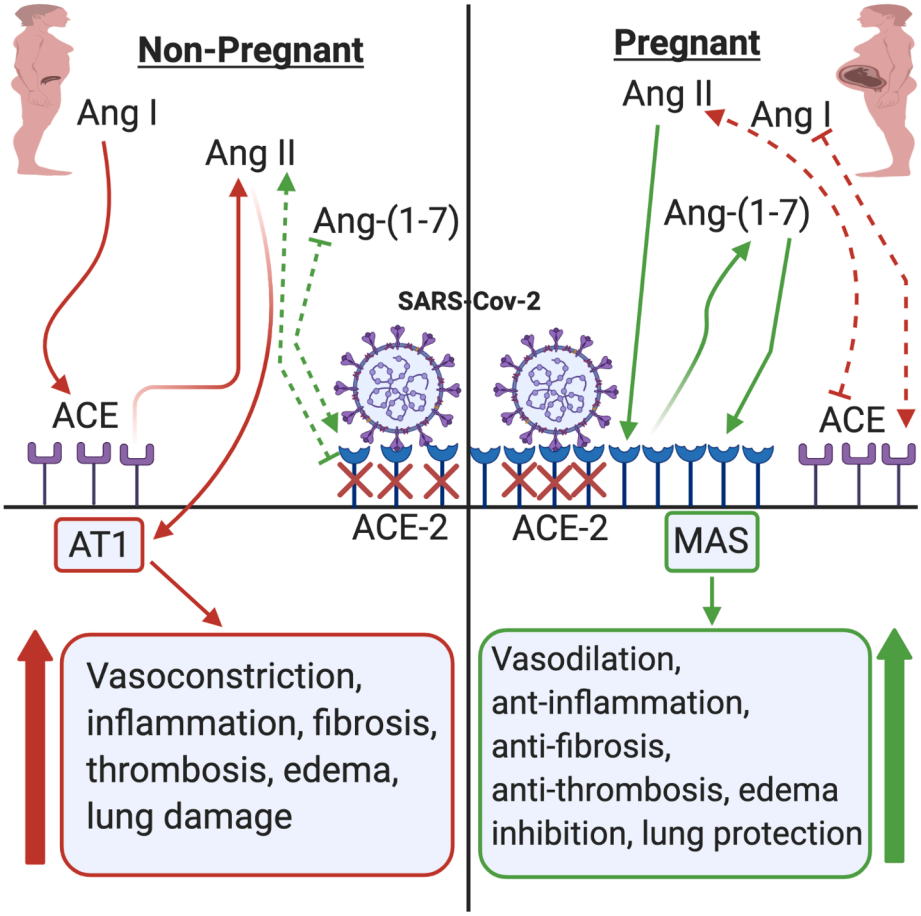

Fig. 1. Angiotensin converting enzyme (ACE) metabolizes angiotensin I (Ang I) to angiotensin II (Ang II) which interacts with AT1 receptors. Angiotensin converting enzyme 2 (ACE-2) contra-regulates Ang II by generating angiotensin 1-7 [(Ang- (1-7)], which then interacts to MAS receptors. During pregnancy, the enhanced expression of ACE-2 leads to vasodilation, less inflammation, less fibrosis, anti-thrombosis, edema inhibition and consequently lung protection. In non-enhanced ACE-2 phenotypes (non-pregnant), when entering cells, SARS-CoV-2 downregulates the expression of ACE-2 (represented by "X"), thus leading to ACE-AngII-AT1 overactivation predisposing to increased vasoconstriction, inflammation, fibrosis, edema and lung damage. Created with BioRender.com.

thus promoting maternal vasodilation [29].

Even before the COVID-19 pandemic, animal studies demonstrated that ACE-2 and Ang-(1-7) are highly expressed on the fetal side of the placenta [30], whereas human and animal studies demonstrated that the maternal side has low expression of ACE-2 and Ang-(1-7) [30,31]. While a recent study reported that ACE-2 receptor expression is negligible on the chorioamniotic membranes in the human placenta [32], other authors described intense signal positivity for SARS-CoV-2 in syncytiotrophoblast lining the chorionic villi (with RNA *in situ* hybridization) [33]. Moreover, fetal vascular malperfusion, intervillous space inflammatory infiltrates, increased villous stromal macrophages and increased inflammatory platelet aggregates were observed on the fetal sides of the same human placentas [33].

Irregular expression of ACE-2 and TMPRSS2 have been described in 19 SARS-CoV-2 infected placentas [34]. The authors concluded that the human placenta is capable of being infected, although the polarized expression of ACE-2 towards the fetal compartment and the scarce expression of TMPRSS2 in trophoblast, remote from maternal blood, may justify the rarity of vertical transmission of COVID-19 [34]. ACE-2 is abundantly present in the heart, lungs, intestine, kidneys, and fetal tissues [35]. In live fetuses, ACE-2 receptors are involved in myocardium growth, lung and brain development [35]. These findings suggest that a potential Placental Barrier against COVID-19 is possible [36].

ACE-2 receptors in Pregnancy: Protective against COVID-19?

Contrary to what was previously inferred [35], and based on the physiological interactions between SARS-CoV-2 and ACE-2 receptors [13,15], along with previous evidence that ACE-2 and Ang-(1-7) levels are enhanced during pregnancy [18,19,22], it is possible to hypothesize that:

1) in normal healthy pregnancies, highly expressed ACE-2 receptors [23] lead to higher Ang-(1-7) levels [19] and consequently more vasodilation and anti-inflammatory response to SARS-CoV-2 infection. We suggest that the up-regulation of ACE-2 receptors in pregnancy may be protective against severe COVID-19 disease.
2) the gestational Th1-Th2 immune shift [7], known as a potential contributor to the severity of viral infections during pregnancy [9], are counter-regulated by the enhanced pregnancy-induced ACE-2-Ang-(1-7) expression [18,22], which may explain the observed improved outcomes of COVID-19 during pregnancy, when compared to previous viral outbreaks in pregnant women.
3) the irregular and unbalanced expression of ACE-2/TMPRSS2 in human placentas [29–34], mostly expressed to the fetal [27,29,30] side but negligible to maternal side [32,34] also play a protective role on vertical transmission of SARS-CoV-2.
4) down-regulation of ACE-2 receptors induced by SARS-CoV-2 cell entry may be detrimental to those with pre-existing ACE-2 deficiencies in pregnancy, explaining the poor outcomes of pregnancies with co-morbidities [25].

Conclusion

We propose to test the hypothesis described above with a case-control design using: a) Non-COVID-19 Control Group (normal, low-risk pregnant women, non-COVID-19 infected, with term deliveries); b) COVID-19 Case Group – Asymptomatic/Symptomatic (pregnant women, COVID-19 infected, without preeclampsia, with term deliveries). Optionally, the investigation could be extended with these groups: c) Non-COVID-19 with Preeclampsia Control Group (pregnant women with preeclampsia, non-COVID-19 infected, with term deliveries); d) COVID-19 with Preeclampsia Case Group – Asymptomatic/Symptomatic (pregnant women, COVID-19 infected, with preeclampsia, with term deliveries).

Table 1 describes the potential maternal, placental, fetal and neonatal investigations to explore the hypothesis. Each of these tests have been previously described [22,32,33,37], attesting to feasibility. The idea of performing these tests upon the different proposed groups, would allow the comparison of ACE-2/TMPRSS2 and Ang-(1-7) expression along the different compartments of the gravid cycle. The dichotomy of the exposed groups in symptomatic and asymptomatic is extremely important to confirm/rule-out the hypothesis described. Additionally, the investigations could be extended to preeclamptic women to test the confounding factor of overlapping clinical phenotypes in COVID-19 and preeclampsia [26].

Table 1
Proposed research investigations to explore the Hypothesis.

Maternal Tests (blood /swabs)	Placental Tests (pathology)	Fetal Tests (cord blood)	Neonatal Tests (blood /swabs)
Plasma ACE-2 levels	Expression of ACE-2	Plasma ACE-2 levels	Plasma ACE-2 levels
ACE-2 Activity Assay	Expression of TMPRSS2	ACE-2 Activity Assay	ACE-2 Activity Assay
Plasma Ang-(1-7) levels	Immunohistochemistry for SARS-CoV-2 S-Protein	Plasma Ang-(1-7) levels	Plasma Ang-(1-7) levels
Nasopharyngeal SARS-CoV-2 swab	Immunohistochemistry for SARS-CoV-2 N-Protein	Anti-SARS-CoV-2 IgG, IgM, IgA serology	Nasopharyngeal SARS-CoV-2 Swabs
Anti-SARS-CoV-2 IgG, IgM, IgA serology			Anti-SARS-CoV-2 IgG, IgM, IgA serology

TMPRSS2: Transmembrane protease serine 2.

It has already been demonstrated that, *in-vitro*, recombinant human soluble ACE-2 significantly blocks SARS-COV-2 in the early stages of cellular infection [38]. If the proposed hypotheses are confirmed, a future potential therapeutic use of soluble recombinant ACE-2, angiotensin1-7 and angiotensin II type 1 receptor blockers might be beneficial in treating severe COVID-19 infections [39].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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