Inhibins and activins in pregnancy

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Abstract

Human placenta, decidua, and fetal membranes are the major sites of production and secretion of inhibin A and activin A in maternal serum, amniotic fluid, and umbilical cord blood. These tissues also express follistatin-related gene and betaglycan, the binding proteins of activin A and inhibin A, respectively, recently identified. They show a different expression throughout pregnancy, suggesting new functional roles into gestational tissues.

The availability of suitable assays for measuring inhibin A and activin A lead us the possibility to investigate their secretion in healthy pregnancy. In addition, several evidences underline the potential role and the clinical usefulness of their measurement in the diagnosis, prevention, prognosis and follow-up of different gestational pathologies such as: threatened abortion, placental tumors, hypertensive disorders of pregnancy, intrauterine growth restriction, fetal hypoxia.

The measurement of inhibin A and activin A into the biological fluids of pregnancy will offer in the future further possibilities in early diagnosis, prediction, and monitoring pregnancy diseases.

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1. Introduction

Human placenta and fetal membranes produce considerable amounts of activin A and inhibin A in increasing levels in maternal serum and amniotic fluid, and their secretion changes in presence of gestational diseases. Consequently, their measurement may assume relevance with respect to a putative clinical application in the diagnosis, prevention, prognosis and follow-up of different gestational pathologies (Florio et al., 2001; Reis et al., 2002).

The putative clinical usefulness of inhibin A and activin A measurement in pregnancy (early pregnancy loss, placental tumours, hypertensive disturbances of gestation, fetal growth restriction, and fetal hypoxia) will be reviewed.

2. Activin A and inhibin A in pregnant tissues

Pregnant tissues express activin A and inhibin A subunits (Petraglia et al., 1996), activin A-binding protein, namely follistatin (Petraglia et al., 1994) and activin receptors (Act-Rs) (Schneider-Kolsky et al., 2002).

The availability of bioactive activin A on its own receptors is regulated by a binding protein also discovered in the ovarian follicular fluid and therefore named follistatin. This single-chain glycoprotein of 35 kDa is able to inhibit FSH release by neutralizing the effect of activin (Mather et al., 1993), and nevertheless, it is also able to modulate activin biological actions on pregnant tissues, preventing the interaction of activin A to its own receptors (Luisi et al., 2001).

Recently, a new binding protein for activin A has been identified, namely follistatin-related gene (FLRG), a protein of 70 aminoacids, containing two cystein-rich repeats closely related to the follistatin domains common to other members of follistatin family (Hayette et al., 1998). FLRG interacts physically with activin A and, preventing the binding of activin A on ActRs, regulates its functions (Tsukida et al., 2001).

Recently trophoblast, decidua and fetal membranes have been found to express both FLRG mRNA and protein. In particular, immunoreactive FLRG was found in maternal decidua, in syncytiotrophoblast and in epithelial amniotic and chronic cells, with the most intense staining in the decidual and placental blood vessels (Ciarmela et al., 2003a). This
localization differs from that previously shown for follistatin, that is mainly localized in cyto- and syncytiotrophoblast cells (Petraglia et al., 1994), but resembles the ActRs being present in the lining of blood vessels (Schneider-Kolsky et al., 2002; Jones et al., 2002). The finding of FLRG (Ciarmela et al., 2003a), ActRs (Schneider-Kolsky et al., 2002; Jones et al., 2002) and activin A (Schneider-Kolsky et al., 2002) distribution in vascular endothelial cells of decidua and villous blood vessels in term placenta supports the hypothesis that FLRG may act by paracrine/autocrine mechanisms as a modulator of activin A vascular actions, as activin A is known to affect the endothelial proliferation and angiogenesis (McCarthy and Bicknell, 1993; Kozian et al., 1997; Brett et al., 2000).

With respect to inhibin A, the transduction of its pathway into the cells is mediated through ActRII (Gray et al., 2002). However, the inhibin A binding to ActRII is facilitated by betaglycan, a membrane anchored proteoglycan, that allows the formation of a ternary complex including inhibin A, betaglycan and ActRII. Betaglycan also interacts with activin A, because it prevents the binding of activin A to ActRII, thereby antagonising the activin signal (Gray et al., 2002).

Recently, betaglycan was found in the first and third trimester decidual cells and vessel walls, in the chorion, in the syncytiotrophoblast (but not cytotrophoblast), and in the placental endothelial cells only at the first trimester (Ciarmela et al., 2003b). In other words, the new picture emerging from these and other studies is thus that betaglycan (Ciarmela et al., 2003b), FLRG (Ciarmela et al., 2003a) and activin receptors (Schneider-Kolsky et al., 2002) are co-localized in syncytiotrophoblast, and in endothelial cells of trophoblast villi, supporting the hypothesis that these binding sites are involved in modulating the activin A, effects on vascular adaptations of pregnancy. In addition, activin A modulates placental hormonogenesis (Petraglia et al., 1989), uerotonin secretion (Petraglia et al., 1993; Florio et al., 1996), as well as cytotrophoblast proliferation and differentiation (Caniggia et al., 1997).

3. New evidences on the putative clinical usefulness of activin A and inhibin A measurement in pregnancy

Previous studies have well assessed that placental trophoblast is the main source of activin A and inhibin A in maternal circulation throughout pregnancy (Florio et al., 2001). The findings that maternal concentrations of inhibin A and activin A after the removal of feto-placental unit significantly and gradually decreased within the first hour (Muttukrishna et al., 1997), have further reinforced the concept that human placenta is the main source of activin A and inhibin A.

However, the excessive release of these placental hormones has been demonstrated to be associated with several gestational diseases as part of an adaptive response of the placenta to adverse environmental conditions, such as hypertension, hypoxia, and infection, or to malformations of the fetus and placenta (Florio et al., 2001; Reis et al., 2002; Florio et al., 2003a). Therefore, activin A and inhibin A measurement throughout pregnancy may have several clinical usefulness in early detection of the above mentioned gestational diseases.

3.1. Inhibin A and activin A as markers of trophoblast viability

Early pregnancy vaginal bleeding due to the threatened abortion is a common condition. Studies on the role for the inhibin family as markers of early pregnancy viability demonstrated: (i) a very rapid decline of inhibin A in nonviable clinical pregnancies with embryonic failure occur; (ii) that higher activin A levels (detectable 14 days after embryo transfer) are associated with multiple gestations while rapidly falling levels herald embryonic demise and; (iii) that nonviable clinical pregnancies have very low levels of inhibin A (Lockwood et al., 1997, 1998). Taking together, all these findings have suggested that the very rapid decline in inhibin A in pregnancies with embryonic failure may be used as a monitor of early pregnancy viability.

However, all those studies have evaluated the early-pregnancy outcome in women who became pregnant after ART. Only recently inhibin A and activin A concentrations were measured in maternal circulation of healthy spontaneously pregnant women progressing to deliver a healthy term singleton baby, in patients with incomplete miscarriage (carrying a nonviable (absence of heart beat activity) embryo or an anembryonic gestational sac in utero) and with complete miscarriage (an empty uterus with a history of passage of products of conception), in order to ascertain whether their measurement, in comparison to hCG, might provide a rapid and useful marker of early pregnancy viable placenta (Luisi et al., 2003).

Patients with complete miscarriage had the lowest hCG, inhibin A and activin A levels, but only hCG and inhibin A concentrations were lower in complete than in incomplete abortion (Fig. 1). The lack of changes in activin A levels in incomplete miscarriage has been explained by the fact that during the first trimester of pregnancy the ovary may produce activin A and that the early pregnancy corpus luteum revealed intense hybridization with the βA subunit in the granulosa cell compartment (Eramaa et al., 1993).

In any case, in presence of complete miscarriage, a condition associated to the trophoblast dismissal, maternal serum levels of the three proteins -hCG, activin A, and inhibin A- are the lowest, indicating the presence of a failed trophoblast. In fact, in patients who underwent voluntary pregnancy interruption, maternal inhibin A and activin A concentrations quickly decreased to low levels after the removal of the placenta (Muttukrishna et al., 1997), reinforcing the concept that the feto-placental unit significantly contributes to the maternal levels. Thus, when evaluated in a larger population, according to the different gestational ages (Luisi et al.,...
Healthy controls Incomplete Complete

0.00 0.25 0.50 0.75 1.00 1.25

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Fig. 1. Levels of hCG, inhibin A and activin A [expresses as Multiples of the Median (MoM)] in patients with complete and incomplete miscarriage (modified by Luisi et al., 2003). *P < 0.001 vs. healthy controls. **P < 0.001 vs. healthy controls and patients with incomplete abortion.

Fig. 2. Levels of hCG, inhibin A and activin A [expresses as Multiples of the Median (MoM)] in women with threatened abortion who progressed to deliver a healthy term baby (ongoing) or whom pregnancy failed (failing) (modified by Florio et al., 2003b). *P < 0.001 vs. healthy controls and ongoing pregnancies.

2003), or longitudinally in the same patients (Muttukrishna et al., 1997) inhibin A, activin A and hCG levels were found in significantly lower levels in miscarriage than in healthy patients.

The measurement of inhibin A and activin A during the first trimesters of pregnancy could be useful in the diagnosis of trophoblast dysfunction, and, therefore be helpful in the management of early pregnancy problems, to predict the first trimester pregnancy outcome in patients with early pregnancy vaginal bleeding due to threatened abortion. In fact, patients with early pregnancy bleeding and an intrauterine sac with fetal cardiac activity (threatened abortion) progressing till term have higher inhibin A and activin A than those with threatened abortion, but whose pregnancy failed later (Florio et al., 2003b) (Fig. 2), and may add significant prognostic information for predicting the first trimester pregnancy outcome. In fact, using the best cut-offs indicated by the ROC analysis, only inhibin-A at the cut-off 0.553 MoM achieved the best accuracy for prediction of failing...
Inhibin A concentrations were also evaluated in women with a history of unexplained recurrent miscarriages: levels were lower in those patients who had a subsequent miscarriage compared with those who had a live-birth (Muttukrishna et al., 2002).

Taking together, all these findings would support the utility of serum inhibin A measurement in the prediction of poor pregnancy outcome, or perhaps even as a marker of placental dysfunction and damage both in presence and before the onset of the clinical symptoms of recurrent miscarriage.

### 3.2. Inhibin A and activin A as markers of trophoblast tumors

Partial hydatidiform mole is a triploid gestational trophoblastic tumor, occurring in about three per 1000 pregnancies (Seckl et al., 2000). Inhibin/activin subunits are increased in the serum of women with preeclampsia previously demonstrated that activin A and inhibin A levels are increased in the serum of women with preeclampsia compared with normotensive gestational age-matched gravidas (reviewed by Florio et al., 2001; Reis et al., 2002). Both mRNA and protein for activin/inhibin subunits are expressed in higher amounts by preeclamptic trophoblast (Florio et al., 2002b; Silver et al., 2002; Bersinger et al., 2002; Manuelpillai et al., 2001).

The use of activin A and inhibin A has been proposed as useful markers to predict the risk of preeclampsia (Florio et al., 2001; Reis et al., 2002) both in all pregnant women indiscriminately and among high-risk pregnant women, i.e., patients with chronic hypertension or a history of preeclampsia, those with a very low probability of presenting preeclampsia in their present gestation.

With respect to the studies evaluating second-trimester inhibin A and activin A measurements to predict preeclampsia in pregnant women indiscriminately, it was summarized that these placental markers have limited sensitivity and low positive predictive value when applied to low-risk populations (Reis et al., 2002). Indeed, several studies have shown that inhibin A is elevated several weeks before the onset of clinical signs of preeclampsia and that women who had at mid-trimester inhibin A levels exceeding two MoM were more likely to develop preeclampsia, and the performance (sensitivity) of that measurement in predicting the onset of the disease range from 18 to 48.6% (95% Coefficient Interval) (Reis et al., 2002) (Table 1). The addition of hCG data to inhibin A did not improve the sensitivity for pre-eclampsia compared to inhibin A alone (Aquilina et al., 2000; Lambert-Messerlian et al., 2000). Interestingly, inhibin A levels tended to be higher when the onset of preeclampsia occurred within a shorter interval after collection of the second-trimester screening sample. These observations suggested that second-trimester inhibin A would be more effective in predicting early-onset than later onset disease (Lambert-Messerlian et al., 2000).

Compared with inhibin A, activin A seems to be a more sensitive marker at mid-trimester (Muttukrishna et al., 2000). Indeed, in a low-risk population at 15–19 weeks serum activin A concentrations could discriminate preeclampsia with a sensitivity of 41%, whilst of 59% at 21–25 weeks (Muttukrishna et al., 2000). Similar sensitivity was also reported by Silver and Canick (2000) (Table 1).

With respect to high-risk populations, inhibin A reached a sensitivity of 38% (Zeeman et al., 2003), whilst activin A of 18% (Blackburn et al., 2003), thus suggesting that the contribution of these hormones measurement to the screening may be modest if it does not accumulate other risk factors, such as impaired uterine arteries blood flow. In fact, preeclampsia is associated with abnormal placentation, due to the altered cytotrophoblast proliferation and invasion of endometrium, causing a reduced placental perfusion, the impairment of placental angiogenesis with the insufficiency and failure of spiral arteries remodeling (Roberts and Cooper, 2001). Uterine artery Doppler velocimetry provides indirect demonstration of this process, because the persistence of high resistance placental vessels increases the arterial resistance index and
Table 1
Performance (sensitivity) of inhibin A and activin A measurement to predict the third trimester onset of pre-eclampsia

<table>
<thead>
<tr>
<th>Study Subjects (n)</th>
<th>Cut-off points</th>
<th>Gestational age (weeks)</th>
<th>Sensitivity (%)</th>
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<tr>
<td>Inhibin A</td>
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<tr>
<td>Aquilina et al. (2000) All women 640 &gt;2.0 MoM 15–19 48.6</td>
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<tr>
<td>Nulliparous 313 &gt;2.0 MoM 15–19 47.6</td>
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<tr>
<td>Lambert-Messerlian et al. (2000) 359 &gt;1.9 MoM 15–21 18</td>
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<tr>
<td>Muttukrishna et al. (2000) 297 95th centile 15–19 23</td>
<td>95th centile 21–25 27</td>
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<tr>
<td>Florio et al. (2003c) &gt;1.8 MoM 24 39</td>
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<tr>
<td>Zeeman et al. (2003)a 232 Mean log concentration + 2S.D. Third trimester 38</td>
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<tr>
<td>Activin A</td>
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<tr>
<td>Muttukrishna et al. (2000) 297 95th centile 15–19 41</td>
<td>95th centile 21–25 59</td>
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</tr>
<tr>
<td>Blackburn et al. (2003)a 80 90th centile 21–25 18</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Florio et al. (2003c) &gt;1.7 MoM 24 61</td>
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a High risk pregnancy.

Creates a typical waveform called diastolic notch (Schneider and Schulman, 1995), and has been proposed as a screening test for pre-eclampsia (Campbell et al., 1986). Thus, recently it has been evaluated whether the measurement of maternal serum activin A and inhibin A may add any clinically relevant information for the prediction of preeclampsia in women at risk to develop the disease, as with altered uterine artery Doppler velocimetry at 24 weeks of gestation. Briefly, activin A and inhibin A levels were higher in patients who developed preeclampsia than in those who did not present with preeclampsia at follow-up. Furthermore, activin A at the cut-off 1.7 MoM achieved a sensitivity of 61% and a specificity of 89%, whereas inhibin A at the cut-off 1.8 MoM combined a sensitivity of 39% with a specificity of 92% for prediction of preeclampsia. The probability of preeclampsia was 31% in the whole study population, 86% if both activin A and inhibin A were elevated and 17% if both hormone markers were unaltered (Florio et al., 2003c) (Table 1). In conclusion, activin A was found to be more sensitive than inhibin A at a fixed false-positive rate, and the combination of both hormones may improve the predictive value of the test. Also, the positive predictive value resulting from high activin A and inhibin A levels in this selected group of patients was substantially higher than the positive predictive values previously described for the same hormone alterations in women at low risk for developing preeclampsia (reviewed by Florio et al., 2001; Reis et al., 2002). Thus, activin A and inhibin A may be used as markers for fine-tuning the prediction of preeclampsia in patients with abnormal uterine artery Doppler velocimetry, which has been used as a first step screening test for the general population.

3.4. Activin A and inhibin A levels in fetal growth restriction

Fetal growth restriction (FGR, also referred to as intrauterine growth restriction/retardation), is a complex condition for which definition has not reached a consensus. From a pathological point of view, FGR is characterized by a disrupted fetal growth and should not be confounded with low birth weight, which encompasses preterm infants with normal development, or even with small-for-gestational-age fetuses, a broad concept that embraces FGR but also normal fetuses with a familial tendency to growth below the population average (Magnus et al., 1997). In addition to ultrasound protocols, maternal serum screening is under extensive investigation, and activin A and inhibin A measurement is emerging as a new method for early detection of FGR. In fact, normotensive women with idiopathic small for gestational age (SGA) pregnancies do not have markedly elevated circulating levels of activin A and inhibin A (Keelan et al., 2002), and the presence of FGR did not significantly modify these concentrations in patients with preeclampsia (D’Antona et al., 2000; Florio et al., 2002b). Furthermore, also in fetal circulation levels do not change in presence of FGR (Debieve et al., 2000). However, very high maternal serum activin A levels were found in FGR with placental ischemia (Greenwood et al., 2001), suggesting that activin A may be a useful marker of fetoplacental compromise. Thus, comparing activin A levels in constitutionally small fetuses, FGR fetus or FGR complicating pre-eclampsia, Wallace et al. (2003) found that levels were significantly lower in constitutionally small pregnancies. However, it seems that activin A is increased in pregnancies carrying a FGR fetuses, mainly in presence of abnormal umbilical artery Doppler waveforms (Bobrow et al., 2002), thus suggesting that maternal serum activin A levels are increased in association with chronic fetal compromise.

3.5. Activin A and fetal hypoxia

Feto-placental hypoxemia is an acute trigger for increased activin secretion in the sheep from the fetoplacental unit in late pregnancy. Indeed, the induction of hypoxia by restricting blood flow through the maternal uterine arteries acutely
increased activin A levels in both sheep amniotic fluid and the fetal circulation, with values rapidly returning to baseline increased activin A levels in both sheep amniotic fluid and umbilical artery pH (Wallace et al., 2003), suggesting that the feto-placental hypoxia may trigger activin A secretion from the placenta and/or the fetus. However, in vivo data reporting that hypoxia inhibits activin A release from cultured human placental cells (Blumenstein et al., 2002; Mansel-Pilail et al., 2003) have suggested that increased activin A levels in the fetal circulation originate from the several fetal source expressing activin A mRNA (Florio et al., 2003d). These observations prompted to test the hypothesis that fetal hypoxia induces activin-A secretion in preterm newborn infants. Preterm newborns with signs of perinatal hypoxia at birth have increased activin-A levels, and umbilical cord concentrations were significantly related with biochemical indexes of hypoxia (hypoxanthine and xanthine), with NRBCs, with pH and base deficit (Florio et al., 2003e), suggesting that activin A may reflect indirectly intrauterine hypoxia.

4. Conclusions

During the decade, the studies conducted on activin A and inhibin A suggested their possible involvement in the pathogenesis of several gestational diseases. Whether their altered secretion is the cause or simply reflects placental problems is still far to be assessed, however it has been assumed that the local changes in inhibin-related proteins processing throughout gestation may be important not only in the paracrine control of the feto-maternal communication required to maintain pregnancy, but also as specific marker of a derangement of that function. Indeed, the measurement of these proteins in maternal and fetal serum will offer new possibilities in the early diagnosis, prediction, and monitoring of gestational diseases.

References


