

Tumor Necrosis Factor- α Inhibition and VATER Association: A Causal Relationship?

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ABSTRACT. Inflammatory conditions that may require the use of a tumor necrosis factor- α (TNF- α) antagonist often involve women of child-bearing age. TNF- α antagonists are presumed to be safe in pregnancy based on animal data. However, this has never been formally studied in prospective trials involving humans. We describe a patient with psoriasis and psoriatic arthritis who took etanercept 50 mg subcutaneously (SQ) twice weekly throughout her pregnancy. She gave birth to a child with VATER association. Animal and human data exist to suggest a possible causal relationship between the mother's use of etanercept and the child's development of VATER association. We propose that the TNF antagonists, specifically etanercept, be used with caution in pregnant women. Patient registries of women who take TNF- α antagonists during pregnancy also need to be followed to see if there is an increase in the birth defects that are part of VATER association. (J Rheumatol 2006;33:1014-7)

Key Indexing Terms:

PREGNANCY

ETANERCEPT

BIRTH DEFECTS

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VATER ASSOCIATION

Medications that inhibit tumor necrosis factor- α (TNF- α) are quite effective at treating rheumatoid arthritis (RA), psoriatic arthritis (PsA), and other inflammatory conditions. Since many of these same conditions have an increased prevalence in women of child-bearing age, this often raises the question of the safety of these medications in pregnancy. To date there have been no large prospective trials assessing the safety of these drugs in pregnant women. Such trials would have an ethical concern. However, there is a small case series (data obtained from a mailed questionnaire) and one report suggesting they may be safe^{1,2}. There are also 2 studies with infliximab (safety database study and a prospective trial involving 10 patients) that suggest women with Crohn's disease who are exposed to infliximab during pregnancy do not have increased adverse events compared to those who are not^{3,4}. There were, however, adverse events, which will be discussed later. Currently, these drugs are rated "category B" for pregnancy (presumed safe based on animal studies).

We describe a patient who took high-dose etanercept for psoriasis and psoriatic arthritis (PsA) throughout her pregnancy. Her child was born with tracheal atresia and a tracheoesophageal fistula, esophageal atresia, imperforate anus, hypospadias, T12 vertebral anomaly, and patent foramen ovale (PFO). Was the use of etanercept throughout the preg-

nancy related to the birth defects? Our data suggest a possible causal relationship.

CASE REPORT

A 28-year-old Caucasian woman developed psoriasis at the age of 11 years. She then developed inflammatory spinal pain and inflammatory arthritis of her large proximal joints at the age of 19. At age 25, she started etanercept 25 mg SQ twice weekly. She experienced near complete resolution of her arthritis and a partial cutaneous response at that dose. One year prior to presentation, etanercept was increased to 50 mg SQ twice weekly for her cutaneous disease. She experienced complete resolution of her psoriasis and continued that dose for 4 months, then her dose was decreased to 25 mg SQ twice weekly again. Shortly after the dose was decreased, her cutaneous and articular symptoms flared, so the dose was again increased to 50 mg SQ twice weekly. She was taking no other medication with the exception of naproxen as needed.

The patient became pregnant shortly after her etanercept dose was increased back to 50 mg SQ twice weekly. After consultation with her physician, she chose to continue etanercept at her current dose because of the severity of her symptoms. She was initially evaluated at our institution at 26 weeks of pregnancy. We stressed the unknown risks of this drug during pregnancy, especially at high dose, but decided to continue 50 mg twice weekly.

The patient gave birth to a male child at 37 weeks' gestation, who was born with tracheal atresia and a tracheoesophageal fistula, esophageal atresia, imperforate anus, PFO, hypospadias, and a T12 vertebral anomaly. The child had VATER association (V: vertebrae anomalies; A: anal anomalies; T: tracheal problems; E: esophageal problems; R: radius or renal defects) without renal or limb abnormalities. Amniocentesis had previously revealed a 46XY chromosomal analysis.

DISCUSSION

VATER association describes a rare condition characterized by a sporadic association of specific birth defects. VATER association can also encompass other findings such as cardiac abnormalities and hypospadias. Its estimated frequency is 1.6

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cases per 10,000 live births⁵. Although a genetic mutation is suspected, and one has been reported in a patient with macrocephaly and features of VATER association⁶, the etiology is unknown.

The TNF receptor (TNFR) family is a group of receptors involved in immune and inflammatory responses. Each receptor binds to a ligand from the TNF family, which results in signaling responses ranging from proliferation and differentiation to apoptosis. A group of proteins called TNFR-associated factors (TRAF) that bind to the cytoplasmic tail of several TNFR family members have now been identified. To date 6 TRAF have been described. The importance of one of these, TRAF4, has remained elusive. In order to define the function of TRAF4, a TRAF4 knockout mouse has been developed. TRAF4-deficient mice are born with tracheal atresia (at the site of the tracheal junction with the larynx) and pulmonary inflammation^{7,8}.

Not only was the child in our case report born with tracheal atresia, but he was also found to have hazy bilateral pulmonary infiltrates and wheezing at birth. TRAF4 knockout mice also experience wheezing secondary to pulmonary inflammation at birth. Further, our patient's tracheal atresia and tracheoesophageal fistula was noted to be proximal, near the junction of the larynx, similar to TRAF4-deficient mice.

Certainly the tracheal and pulmonary defects seen in this child and TRAF4-deficient mice could be coincidental. However, these same mice are also born with malformations of the axial skeleton, including the vertebral arches: TRAF4 is highly expressed in vertebral bodies⁸. The child we describe had a T12 anomaly. Further, TRAF4-deficient mice exhibit a high incidence of spina bifida⁸. This child also had spina bifida occulta.

While it is possible for TRAF4 to be expressed differently in humans versus mice, its expression in humans is also well defined. The basal cell layer of most epithelia in the body is strongly TRAF4-positive, including the respiratory and gastrointestinal tracts and the prostate⁹. This is true in adults and in 12–18-week human fetal tissue⁹, potentially explaining the imperforate anus and hypospadias in this child.

It is not known if any of the TNF- α antagonists (including etanercept, infliximab, or adalimumab) interact specifically with any of the TRAF proteins, including TRAF4. However, TRAF4 preferentially binds the dimeric form of the 75 kDa (p75) common neurotrophin receptor (NTR), rather than the monomeric form¹⁰. Overexpression of TRAF4 by dimeric p75NTR inhibits induction of neural apoptosis, potentially explaining the axial defects that these mice experience. Interestingly, etanercept is a dimerized p75 TNF receptor. Infliximab and adalimumab are TNF antibodies. Further, TNF- α has also been shown to rapidly upregulate TRAF4 mRNA via the nuclear factor-kappa B (NF- κ B) pathway¹¹. TRAF4 also associates with lymphotoxin- β receptor, which binds lymphotoxin- α ⁷. Etanercept also binds and inhibits lymphotoxin- α , whereas the other 2 TNF inhibitors do not. Thus,

etanercept avidly binds and inhibits the very ligands that would activate TRAF4, potentially creating a situation very similar to TRAF4-deficient mice.

Given the theoretical TRAF4-deficient state that the TNF- α antagonists, particularly etanercept, could create, are there other properties that these drugs possess that could counterbalance this deficiency? TRAF4 expression is regulated by the tumor suppressor gene p53 in addition to TNF- α . The murine TRAF4 promoter contains a p53 DNA-binding site that initiates expression of TRAF4¹². Infliximab activates this same p53 gene, whereas etanercept does not¹³. Therefore, infliximab may be able to overcome this TRAF4-deficient state, whereas etanercept may not.

It is also important to note that TRAF4-deficient mice have differential expression of these congenital defects. About 20% of these mice exhibit respiratory defects⁷. Many of the TRAF4-deficient mice are born normal. VATER association also displays variable expression. It most often exists in incomplete forms. Could the amount of TNF- α inhibition (and possibly lymphotoxin- α) be important? Our patient was taking high-dose etanercept (50 mg SQ twice weekly) throughout her pregnancy.

While hypospadias is not part of the VATER association, it is a frequent finding in male offspring who display features of VATER. About 40% of male neonates with VATER association have urethral abnormalities, including hypospadias¹⁴. The etiology of hypospadias in humans was originally thought to be secondary to a primary androgen deficiency, but more recent studies have implicated increased levels of müllerian-inhibiting substance (MIS) as the cause¹⁵. MIS is a hormone that is produced in the Sertoli cells and it suppresses androgen biosynthesis in Leydig cells. TNF- α decreases MIS via NF- κ B¹⁶. Therefore, TNF- α inhibition during pregnancy would result in increased MIS levels, predisposing the fetus to development of hypospadias.

Valproic acid is a known teratogen. It can cause neural tube defects and hypospadias in human offspring^{17,18}. It has also been associated with tracheomalacia¹⁹. Thus, the birth defects are similar to that seen with VATER association. Interestingly, recent findings have shown that valproic acid significantly inhibits TNF- α via NF- κ B²⁰. Further, the risk to the human fetus exposed to valproic acid is variable. It is estimated to confer a 1–2% risk of spina bifida aperta in humans¹⁷. These defects also vary in mice. In regard to hypospadias, C57BL/6J mice are resistant to this birth defect²¹. The TRAF4-deficient mice were engineered from this same strain, potentially explaining why these mice developed many features of VATER association without hypospadias.

The lack of teratogenicity of the TNF antagonists in animal testing does not preclude the possibility of birth defects in humans. Animal testing does not always mirror human response. As with any potential teratogen, it is important to consider the timing and duration of exposure during pregnancy. Etanercept, specifically, was tested at 60–100 times the

peak dose in rabbits and rats²². However, the area under curve systemic exposure levels were estimated to be 4 times the normal human dose (25 mg biweekly)²². This is only twice the dose our patient took. It is known that the TRAF4 sequence varies widely, even in different mouse strains²³. Compared to the other TRAF, TRAF4 and TRAF6 precursor genes arose much earlier during evolution, causing much less sequence homology²³. This lack of homology could explain why certain mouse strains are resistant to some birth defects, such as C57BL/6J mice resistant to developing hypospadias. This variable murine expression could also explain different findings in other species, such as the lack of birth defects in rabbits and rats exposed to etanercept during pregnancy. Indeed, as described, valproic acid causes hypospadias in human offspring, yet this same drug does not cause hypospadias in rats, even at higher doses¹⁸. TRAF4 sequencing was not performed in this child.

A database of 96 women exposed to infliximab during pregnancy has been published³. Fifty-eight of the 96 women (60%) received infliximab during their first trimester and none received the drug throughout the duration of pregnancy. Five infants were born with complications. One was born with Tetralogy of Fallot to a mother who received infliximab during her first trimester. Tetralogy of Fallot is among the most common cardiac defects seen in infants with VATER association. A second child was born with intestinal malrotation. Other gastrointestinal defects, including intestinal malrotation, are seen in 16% of infants born with esophageal atresia²⁴. The mother of this child also received infliximab during her first trimester. The third child was born with respiratory distress of undetermined etiology to a mother who also had received the drug during her first trimester. It is important to remember that 20% of TRAF4-deficient mice are born with pulmonary inflammation and respiratory distress. The child in our case report had similar complications. Infliximab has also been prospectively studied in 10 pregnant women⁴. One of the infants was born with severe respiratory distress that required admission to the neonatal intensive care unit.

A novel mutation has been described in a patient with features of VATER association⁶. This mutation is in the phosphatase and tensin homolog deleted from chromosome 10 (PTEN) gene. PTEN functions in embryonic development, intestinal cell proliferation and differentiation, and tumor suppression. Interestingly, PTEN helps regulate TNF- α expression via the NF- κ B pathway²⁵. Could a PTEN-deficient (or aberrant) state during embryonic development mimic a TNF deficiency? PTEN has also been shown to modulate many different gene expressions. The TNF receptor family, specifically TRAF4, is among the signal transduction molecules that PTEN most significantly upregulates²⁶. Therefore a mutation in the PTEN gene could lead to a TRAF4-deficient state.

While it is impossible to draw firm conclusions from a single case report, questions can certainly be raised. If significant animal and human data exist to corroborate the findings, a

cause and effect is certainly possible. Our case and the supporting data together suggest that etanercept should be used with extreme caution during pregnancy. Large patient registries need to be followed to see if there is an increase in the birth defects that are part of VATER association in women who take TNF- α antagonists, particularly etanercept, during pregnancy.

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