

## Does treatment of premature labor with terbutaline increase the risk of autism spectrum disorders?

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**B**eta-adrenergic agents have been used in pregnant women for the treatment of premature labor and for the treatment of asthma. Concerns have been expressed that exposure to terbutaline, which is a beta-2 adrenergic agonist, may increase the risk of autism spectrum disorders (ASDs) in the offspring. This hypothesis deserves critical review, given the number of patients who have been exposed to the drug in the last 2 decades. The results are important to both the obstetricians and patients who weigh the risks and benefits of interventions and to the pediatricians who counsel the families of affected children.

We conducted an examination of the human and animal studies that have been used to support this hypothesis in a recent review,<sup>1</sup> and we have reached a different conclusion. We find no support for the hypothesis that the use of terbutaline as a tocolytic agent is associated with the subsequent development of ASDs.

The ASDs are common neurodevelopmental disorders that occur in approximately 1 of 150 to 1 of 100 children.<sup>2</sup> They are diagnosed by deficits in social interaction and communication and by restricted and repetitive behaviors and interests; the symptoms appear during the first 3 years of life.<sup>3</sup>

Family and twin studies demonstrate that these disorders are heritable, with estimates of heritability approaching 90%.<sup>4</sup> However, the genetic etiology is complex, with many loci involved and none that accounts for more than a small fraction of cases.<sup>5</sup> Many developmental pathways seem to be involved and include those that have roles in initiating or maintaining synapses.<sup>6,7</sup> The penetrance of the genetic liability factors is variable so that symptoms differ, even in pairs of monozygotic twins.<sup>8</sup> The largest genome scan to date has added to this confusing picture the etiologic role of structural chromosomal anomalies (copy number variants), some of which arise de novo.<sup>9</sup> This unexpected result has been verified by other groups.<sup>10</sup>

The known environmental risk factors for ASDs account for a small number of cases but are interesting because they share

critical periods of exposure in the first trimester.<sup>11</sup> The identification of environmental risk factors is especially important because, unlike genetic risk factors, they should be preventable. Thus, it is reasonable to ask whether there is evidence that medications, such as terbutaline, to which pregnant women may be exposed increase the risk for an ASD outcome in the offspring. Our argument is not with the intent but with the quality of the evidence presented in the review by Witter et al,<sup>1</sup> which we now describe.

### Human studies

Is fetal exposure to terbutaline associated with neurobehavioral defects? The review cites Stein et al<sup>12</sup> as the source that shows terbutaline exposure has been associated with expressive language delay; however, that article does not report a study but the presentation of a “Challenging Case” with responses by 4 pediatricians from different backgrounds. None of the commentators suggested that the patient’s language delay might be associated with her prenatal exposure to terbutaline. This source does not offer any evidence to support the hypothesis of a link between ASD and exposure to terbutaline.

The second reference that is cited in the article is to a study that had been reported by several of the same authors of the review.<sup>13</sup> The review states that the earlier paper demonstrated increased concordance for ASDs in dizygotic twins after terbutaline treatment in utero. However, a careful review of the data found no significant association of concordance with terbutaline exposure in the whole twin sample (36 cases). Only when the investigators divided the sample into smaller subgroups and focused on twin pairs who were both male and had no other cases of ASDs in their family were they able to find a significant association between drug exposure and outcome. This group of 16 twin pairs should have been at increased risk for ASDs because they were male and at decreased risk because of the absence of ASDs in their families. It is unclear why such a subgroup would ever be selected for study a priori. If it was selected a posteriori, then the results should be considered “hypothesis-generating” at best.

Another aspect of this publication deserves comment. For all comparisons of “exposed” to “nonexposed” twins, 2 twin pairs with short exposures were counted as “unexposed.” No explanation was given to justify recategorizing these twins. Because both of these pairs were discordant, moving them to the unexposed category had a major effect on the results. For example, the probability value for the whole sample difference in concordance between exposed and unexposed pairs was reported as .157 in the article. Had the briefly exposed pairs been included as “exposed,” the probability value would have been .471. In the selected group of cases that were composed of male

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pairs with no other siblings who were diagnosed with ASDs and in which the authors reported a significant increase in concordance after terbutaline ( $P = .035$ ), the probability value would have been .118. Thus, the only significant effect that was reported for concordance is dependent on the reclassification of briefly exposed twin pairs. The authors might argue that the relative risks for their comparisons are  $>1$ , but they are not significantly  $>1$ . That is the reason confidence intervals are calculated.

As evidence that terbutaline exposure for tocolysis or asthma is associated with autism, the publication cites an oral presentation at a meeting.<sup>14</sup> We were not able to access the presentation at the URL, but the full report of that study is now available.<sup>15</sup> The design of the study was peculiar, in that the 8 terbutaline-exposed recruited children (from just 2 families) had been diagnosed with autism already. Their performance on a battery of neuropsychologic tests was reported to differ from the performance of typically developing children. It is not clear how this result can be interpreted to support a teratogenic effect of terbutaline, because subjects with an ASD diagnosis surely would be expected to differ in many ways from subjects without a diagnosis. The design makes the study uninterpretable.

Another line of evidence that was offered to support the hypothesis that beta-2-adrenergic agonists are harmful to the conceptus is a study that examined the risk of an ASD diagnosis in the offspring of mothers who have autoimmune disorders.<sup>16</sup> The review described this as a study of asthma and suggested that the risk must be related to the use of beta-2-adrenergic agonists by patients with asthma. However, the only autoimmune condition that was a significant risk factor in the cited study was psoriasis, which is a condition that is not treated with these agonists.

The fifth reference is a personal communication about an unpublished study. An abstract of that study is available.<sup>17</sup> The authors of the article under review contended that the data showed that beta-2-adrenergic agonist exposure during early pregnancy produces an increased risk for ASDs. In contrast, the abstract reported no such finding; instead, there was no effect of exposure in the first, second, or third trimester. The only significant effect was from the use of beta-2-adrenergic agonists before conception. These results do not support the hypothesis that terbutaline exposure in the third trimester increases the risk of ASD.

Although the issues that we have identified herein address a fraction of the review article, they suggest a very loose interpretation of the findings in the literature. Although we respect the right of authors to select studies that support their views, the scientific community would expect an accurate description and a balanced report of the studies that are cited.

### Animal studies

The review describes many animal studies to support the hypothesis that terbutaline is teratogenic in late pregnancy, which leads to lasting changes in neurotransmitter function, behavior, and brain morphologic condition.<sup>1</sup> However, it is impor-

tant to note that the studies that are cited come almost exclusively from 1 research group and use the same dose at the same stage of development for the same number of repeated doses. If there is any error in the dose or timing or duration of exposure that was chosen to mimic terbutaline as a tocolytic therapy, it is shared by all the articles that were cited.

The dose that was selected was based on pharmacologic evidence that adult rats clear terbutaline more rapidly than adult humans.<sup>18,19</sup> Therefore, the dose to which rats were exposed was purposely higher than the doses that typically are administered to women in preterm labor. However, it is well known that developing humans and rats do not clear xenobiotics at the same rate as adults.<sup>20</sup> The problem is that no data are available that compare clearance rates in the human fetus and the neonatal rat (a stage of rat development often used to model the third trimester of human pregnancy). Without that information, there is no way to judge whether the doses in the rat studies are equivalent to those used in humans. Similarly, the only data available are from animals that were given repeated doses over 4 days. What would be the corresponding length of exposure in humans? We do not know.

None of the studies provide information on the dose-response curve of terbutaline exposure in neonatal rats for the endpoints that were measured. This information is needed to interpret the risk of teratogenicity after terbutaline exposure. Very few teratogens have been studied extensively for their dose-response characteristics; however, when many laboratories perform studies of the same teratogen, there are often enough differences in method between studies to offer some insights into the importance of dose, timing, and duration of exposure. This information does not exist with terbutaline.

In the review, the neuropathologic evidence of terbutaline-exposed rats is described as similar to the neuropathologic evidence that is reported for human cases with an ASD diagnosis.<sup>21</sup> The accuracy of this claim is not easy to assess because of the brain regions that were studied in the animal model (hippocampus, somatosensory cortex, and cerebellum), only the cerebellum has been observed consistently to be abnormal in human cases.<sup>22,23</sup> The animal finding of reduced Purkinje cell numbers does agree with the human findings. The result of reduced size of cortical neurons in the exposed animals is not similar to anything that has been reported in humans. Finally, there is the result of gliosis that was observed in the CA3 region of the hippocampus in the treated rats. Most pathology studies in cases of ASD have not reported the presence of gliosis. However, Bailey et al<sup>23</sup> did report astrogliosis that was restricted to the cerebellum in 3 of their 6 cases. Vargas et al<sup>24</sup> observed and quantified widespread astrogliosis and microgliosis in each of their human cases. The glial reactivity was greatest in the cerebellum. The animal finding of gliosis that was limited to 1 region of the hippocampus does not appear similar to any of the human findings. However, subsequent to the publication of the article by Vargas et al in 2005, the same group of investigators who reported the very restricted gliosis in 2004 used the immunocytochemical methods of Vargas et al and reported widespread astrogliosis and microgliosis in both grey matter

and white matter of cortex and cerebellum in terbutaline-exposed rats.<sup>25</sup> The meaning of these results is not clear because gliosis is not a finding that is restricted to autism.

The authors of the review describe the behavioral effects that were reported in rats that were exposed in the neonatal period to terbutaline as “similar to those found in autism.”<sup>25</sup> The significant effects that were observed included increased activity in an open field and increased reactivity to noise. These effects are so nonspecific that they can hardly be described as confirming parallelism with autism. The same study included 1 measure that is a much better test of parallelism.<sup>25</sup> Prepulse inhibition was measured in terbutaline-exposed rats and control animals. This neurophysiologic measure clearly is depressed in human cases of ASD<sup>26,27</sup> and in a popular animal model of autism that was based on exposure of rat embryos to valproic acid at the time of neural tube closure.<sup>28</sup> In contrast, terbutaline exposure had no effect on prepulse inhibition. Thus, the idea that terbutaline-exposed animals exhibit behaviors similar to those observed in autism is not convincing.

### Comment

An important issue not addressed by Witter et al<sup>1</sup> is the fact that premature birth itself is associated with an increase in the risk of neurobehavioral problems that include ASDs. For example, a recent article by MacKay et al<sup>29</sup> examined the length of gestation and the later need for special educational services. Delivery at <40 weeks of gestation was associated with a significantly increased risk of educational problems over the whole range of gestation. A recent paper by Buckmayer et al<sup>30</sup> analyzed the effect of prematurity on ASDs in an unusually large number of cases. The result was that the significant odds ratio for the whole sample disappeared if analyses controlled for indications of morbidity during gestation (eg, cases with congenital malformations) or at the time of birth (eg, cases with low Apgar scores). Thus, it remains unclear whether prematurity is an etiologic factor in the development of ASDs or whether it is simply an indication that the conceptus has sustained harm in some way.

Indeed, one of the studies that Witter et al<sup>1</sup> describe is an excellent demonstration of the idea that even threatened premature delivery may be evidence of injury. Pitzer et al<sup>31</sup> compared 4 groups for neurologic and psychiatric morbidity in childhood. Two groups were born prematurely, one group without treatment and one group with unsuccessful tocolysis. Compared with children who were born at term without incident, both groups who were born early had elevated rates of neurobehavioral problems, and they did not differ from each other. The fourth group included children who were born at term after successful tocolytic treatment. The rate of neurobehavioral problems in this group was much higher than that seen in children who were born at term without treatment, but it was similar to the 2 groups who were born prematurely. Witter et al<sup>1</sup> interpret the study as supporting the idea that longer duration of therapy is critical to an adverse outcome, which assumes that the children who came to term were ex-

posed for longer than those who were born prematurely. However, Pitzer’s group<sup>31</sup> examined a subset of cases for whom the duration of exposure and cumulative dose were known. The authors reported that the group that was born prematurely was treated for a median of 18 days, with a median cumulative dose of 66 mg; the group that was born at term was treated for a median of 10 days, with a median cumulative dose of 29 mg. The simplest conclusion to be drawn from this study is that tocolysis has no effect on neurobehavioral outcomes, but threatened premature labor may have a substantial effect, even if it is treated successfully.

Several publications have addressed the use of beta-2 adrenergic agonists, including terbutaline, for the treatment of preterm labor. Terbutaline readily crosses the human placenta.<sup>32</sup> In review articles by Lam et al<sup>33</sup> and Goldenberg,<sup>34</sup> the benefits of beta-2-adrenergic agonists in general, and terbutaline in particular, are discussed. Surely, larger human studies with long-term follow up might raise new concerns, but the present literature does not support the hypothesis that beta-2 adrenergic agonists that are used for tocolysis are associated with ASDs in the offspring. There is no reason to make recommendations for clinical care based on an unsubstantiated hypothesis. ■

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