2-1-2011

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

Patricia Rodier
University of Rochester School of Medicine and Dentistry

Richard K Miller
University of Rochester School of Medicine and Dentistry

Robert L Brent
Thomas Jefferson University, rbrent@nemours.org

As submitted to:

American Journal of Obstetrics and Gynecology

And later published as:

Editorial Comment: Does Treatment of Premature Labor with Terbutaline Increase the Risk of Autism Spectrum Disorders?

Volume 204, Issue 2, Pages 91-94, February 2011

doi:10.1016/j.ajog.2010.11.030

Patricia Rodier, Ph.D.

Professor of Obstetrics/Gynecology, University of Rochester

School of Medicine and Dentistry, Department of Obstetrics and Gynecology

601 Elmwood Avenue, Rochester, New York 14642-8668 USA

patricia_rodier@urmc.rochester.edu

585 275 4789

Richard K. Miller, PhD

Professor of Obstetrics / Gynecology, of Environmental Medicine and of Pathology, University of Rochester, School of Medicine and Dentistry

Department of Obstetrics and Gynecology, 601 Elmwood Avenue

Rochester, New York 14642-8668 USA

richardk_miller@urmc.rochester.edu

585 275 2520

Robert L Brent MD, PhD, D.Sc.

Distinguished Professor of Pediatrics, Pathology, & Radiology

Jefferson Medical College, duPont Hospital for Children
Box 269, Wilmington, DE 19899
rbrent@nemours.org
302-651-6880

Corresponding author:
Dr. Robert L Brent, duPont Hospital for Children
Box 269, Wilmington, DE 19899
rbrent@nemours.org
302-651-6880

Introduction

Beta-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, 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 exposed to the drug in the last two 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 have conducted an examination of the human and animal studies that have been used to support this hypothesis in a recent review[1], and we reach 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, occurring in about 1/150 to 1/100 children[2]. They are diagnosed by deficits in social interaction and communication and by restricted and repetitive behaviors and interests; the symptoms appear during the first three years of life [3].

Family and twin studies demonstrate that these disorders are heritable, with estimates of heritability approaching 90%[4]. However, the genetic etiology is complex, with many loci involved and none that account for more than a small fraction of cases[5]. Many developmental pathways seem to be involved, including those that have roles in initiating or maintaining synapses [6,7]. The penetrance of the genetic liability factors is variable, so that symptoms differ, even in pairs of monozygotic twins[8]. The largest

genome scan to date has added to this confusing picture the etiological role of structural chromosomal anomalies (copy number variants), some of which arise *de novo* [9]. This unexpected result has been verified by other groups[10].

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[11]. 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. [1], which we now describe.

Human Studies

Is fetal exposure to terbutaline associated with neurobehavioral defects? The review cites Stein et al.[12] 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 four 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 cited in the article is to a study reported by several of the same authors of the review. [13]The review states that the earlier paper demonstrates 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, but 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 "non-exposed" twins, two twin pairs with short exposures were counted as "unexposed". No explanation was given to justify re-categorizing 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 p value for the whole sample difference in concordance between exposed and unexposed pairs was reported as p = 0.157 in the paper. Had the briefly-exposed pairs been included as "exposed," the p value would have been 0.471. In the selected group of cases composed of male pairs with no other siblings diagnosed with ASDs, in which the authors reported a significant increase in concordance after terbutaline (P =.035) that p value would have been 0.118. Thus, the only significant effect reported for concordance is dependent on the re-classification of briefly-exposed twin pairs. The authors might argue that the relative risks for their comparisons are greater than one, but they are not significantly greater than one. 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. [14]The full report of that study is now available. [15] The design of the study is peculiar, in that the eight terbutaline-exposed recruited children (from just two families) had already been diagnosed with autism. Their performance on a battery of neuropsychological 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, as subjects with an ASD diagnosis would surely be expected to differ in many ways from subjects without a diagnosis. The design makes the study uninterpretable.

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

The fifth reference is a personal communication about an unpublished study. An abstract of that study is available. [17] The authors of the article under review contend that the data show that beta-2-adrenergic agonist exposure during early pregnancy produces an increased risk for ASDs. In contrast, the abstract reports 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 preconception. These results do not support the hypothesis that terbutaline exposure in the third trimester increases the risk of ASD.

Although the issues 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 cited.

Animal Studies

The review[1] describes many animal studies to support the hypothesis that terbutaline is teratogenic in late pregnancy, leading to lasting changes in neurotransmitter function, behavior, and brain morphology. However, it is important to note that the studies cited come almost exclusively from one research group, using 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 chosen to mimic terbutaline as a tocolytic therapy, it is shared by all the papers cited.

The dose selected is based on pharmacologic evidence that adult rats clear terbutaline more rapidly than adult humans. [18,19] Therefore, the dose to which rats were exposed was purposely higher than the doses typically delivered 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.[20] 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 3rd 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 given repeated doses over four 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 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, but 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 neuropathology of terbutaline-exposed rats [21] is described as similar to the neuropathology reported for human cases with an ASD diagnosis. The accuracy of this claim is not easy to assess, because, of the brain regions studied in the animal model (hippocampus, somatosensory cortex and cerebellum), only the cerebellum has been observed to be consistently abnormal in human cases.[22,23] 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 reported in humans. Finally, there is the result of gliosis 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.[23]did report astrogliosis restricted to the cerebellum in three of their six cases. Vargas et al.[24] 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 limited to one region of the hippocampus does not appear similar to any of the human findings. However, subsequent to the publication of the Vargas paper in 2005, the same group of investigators who reported the very restricted gliosis in 2004 used the immunocytochemical methods of Vargas and reported widespread astrogliosis and microgliosis in both grey matter and white matter of cortex and cerebellum in terbutaline-exposed rats.[25] The meaning of these results is not clear, because gliosis is not a finding restricted to autism.

The authors of the review describe the behavioral effects reported in rats exposed in the neonatal period to terbutaline [25] as "similar to those found in autism." The significant effects 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[25] included one measure that is a much better test of parallelism. Pre-Pulse Inhibition was measured in terbutaline-exposed rats and controls. This neurophysiological measure is clearly depressed in human cases of ASD[26,27] and in a popular animal model of autism based on exposure of rat embryos to valproic acid at the time of neural tube closure.[28] In contrast, terbutaline exposure had no effect on Pre-Pulse 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.[1] is the fact that premature birth itself is associated with an increase in the risk of neurobehavioral problems, including ASDs. For example, a recent paper by MacKay and others[29] examined the length of gestation and the later need for special educational services. Delivery before 40 weeks was associated with a significantly increased risk of educational problems over the whole range of gestation. A recent paper by Buckmayer and others[30] 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 (e.g., cases with congenital malformations) or at the time of birth (e.g., cases with low APGAR scores). Thus, it

remains unclear whether prematurity is an etiological 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 and others[1] describe is an excellent demonstration of the idea that even threatened premature delivery may be evidence of injury. Pitzer et al.[31] compared four groups for neurological and psychiatric morbidity in childhood. Two groups were born prematurely, one without treatment and one with unsuccessful tocolysis. Compared to children born at term without incident both groups 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 born at term without treatment, but it was similar to the two groups born prematurely. Witter et al.[1] interpret the study as supporting the idea that longer duration of therapy is critical to an adverse outcome, assuming that the children who came to term were exposed for longer than those born prematurely. However, Pitzer's group[31] examined a subset of cases for whom duration of exposure and cumulative dose were known. The authors reported that the group born prematurely was treated for a median of 18 days with a median cumulative dose of 66 mg, while the group 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 utilization of beta-2 adrenergic agonists, including terbutaline, for the treatment of preterm labor. Terbutaline readily crosses the human placenta [32]. In review articles by Lam et al. [33] and Goldenberg [34], the benefits of beta-2-adrenergic agonists in general, and terbutaline in particular, are discussed. Surely, larger human studies with long–tern follow up might raise new concerns, but the present literature does not support the hypothesis that beta-2 adrenergic agonists 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.

Conflict of Interest Statement: Dr. RK Miller and Dr. PM Rodier are serving as expert witnesses in litigation involving the use of terbutaline during pregnancy. No other Conflict of Interest is noted for Drs Brent, Miller, or Rodier.

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