Abstract

Introduction

To describe the relationship or lack thereof, between Beta-agonist drugs such as terbutaline and autism. Relevant scientific publications regarding autism spectrum disorders were reviewed. The data in both human and animal studies regarding Beta-agonist drugs used for treatment of asthma as well as preterm labour were interrogated for potential association with Autism Spectrum Disorders. The likelihood of causality was tested using the appropriate epidemiologic criteria.

Discussion

The prevalence of Autism Spectrum Disorders has increased substantially over past decades. Genetics plays a predominant role in causation, being involved in nearly 90% of known cases. In a minority of patients there is a causal relationship between drugs such as thalidomide or valproic acid, and Autism Spectrum Disorder. The vast preponderance of the literature confirms that the use of beta-agonist drugs, particularly during pregnancy as treatment for preterm labour and asthma are not related in any scientific way to autism spectrum disorders.

Conclusion

It can now be stated conclusively that exposure to beta-agonist drugs during pregnancy does not increase the risk of Autism Spectrum Disorder. Terbutaline and other beta-agonist drugs should be used during the prenatal period based on the physician’s perceived need for the medication because asthma and prematurity issues are of the utmost importance.

Introduction

Prior to 1980, autism was considered rare, being diagnosed in approximately 1/2000 children. However, more recently the Centres for Disease Control Prevention (CDCP) report, lists the prevalence of Autism Spectrum Disorders (ASD) at approximately 9/1000. ASD is a group of associated neurodevelopmental disorders, which are diagnosed by problems in social interaction and repetitive behaviours, which usually appear during the first three years of life. It is accepted that most ASD cases are attributable to genetic causes where the estimates approach 90%. However, the genetic aetiology is quite complex with variable penetrance of several genetic factors. There are a few documented environmental risk factors for ASD, such as valproic acid or thalidomide, which occur during critical periods of exposure during the first trimester. There has been much time, effort, and monetary focus over the last decade on environmental risk factors because, while genetic factors cannot be altered, environmental exposure could potentially be reduced. Indeed, a review of a popular website (Autism Speaks) reveals over 20 pages of awards totalling over four million dollars, granted for research addressing potential environmental risk factors for ASD. There are well established epidemiologic methodologies for evaluating causation between drugs and disease. To confirm causation there are nine criteria to be met (strength, consistency, specificity, temporality, dose-response, biologic plausibility, coherence, experimental evidence, and analogy) and they should have support from multiple authors/publications in the literature in order to establish causation. For example, the strength of evidence, particularly with beta-agonist used so frequently during pregnancy hundreds or even thousands of cases per year of ASD being related to such treatment during pregnancy. Consistency is also critical in that such a relationship should be repeatedly observed by different persons in different places, circumstances, and times. Likewise, there should be a dose response curve so that the more beta-agonist that was used the higher the chance of ASD. These are just examples of the nine requirements and since the use of beta-agonist has been reduced as autism has risen the strength, consistency, and dose-response would seem to argue against such a causal relationship. This epidemiologic examination is critical, as the purported link between the MMR vaccine and autism was first reported in the late 90’s but has been disproved as a cause for autism and its association retracted by the journal.

The use of beta-agonist drugs has been ubiquitous in obstetric practice for many decades. It’s use in various preparations has been a mainstay in the treatment of maternal asthma. Indeed, in a recent study appropriate beta-agonist treatment significantly decreased the risk for worsening asthma during pregnancy and the authors concluded that, even with mild asthma, injudicious discontinuation of the medication resulted in a higher likelihood that the asthma would become significantly worse. Although, the use of intravenous beta-agonists to treat preterm labour has decreased in the past 15-20 years the use of terbutaline to decrease recurrent preterm labour by using continuous subcutaneous infusion has been extensively studied in the literature and clinically was used

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by many thousands of patients per year since 1990. A review of the entire body of this literature reveals 46 peer review papers of which, 44 reveal efficacy, such as significant prolongation of pregnancy, less neonatal morbidity, and enhanced cost savings. Moreover, the maternal and foetal side effects of terbutaline used in this way have been extensively reviewed and have been found to be safe as well as efficacious. Indeed, the benefits of beta-agonist drugs employed during pregnancy for tocolysis lead to significant prolongation of the gestation, which significantly reduces neonatal morbidity related to early delivery. This is important as recent studies have related prematurity itself to ASD. In late 2009, Witter et al. published a review of basic science data in rat pups treated with terbutaline after delivery, which ostensibly caused brain lesions and behaviour disorders in these animals. The authors then associated terbutaline exposure in humans and linked it to ASD. By this convoluted hypothesis, early receptor stimulation by terbutaline might affect receptor function during postnatal life and such changes might have a relationship to ASD. The evidence for such a relationship was reviewed by Rodier et al. and they found terbutaline, used for tocolysis or asthma, either in the animal or in human studies, had no relationship with ASD in the offspring. The animal work was further assessed by Owens et al. who confirmed in the rat model that pharmacologic grade (not for human consumption) terbutaline did indeed result in cerebral lesions in the rat offspring. However, there was an absence of neurotoxicity and behavioural disorders when medical terbutaline (such as that used in patients with asthma and preterm labour) was employed. The purpose of this review is to assess all of the data with regards to whether terbutaline and other beta-agonists can possibly be related to ASD.

Discussion

Animal Studies

The Witter article quotes several publications, which are exclusively from one research group (T. A. Slotkin) showing that terbutaline stimulates the B2AR (beta 2 agonist receptor) which is part of a system of neurotransmitters. By their hypothesis the use of beta-agonists in pregnancy might increase signalling (over stimulation) of the receptor, which might have “wide spread effects in light of the function of these receptors during pre-postnatal life” (Table 1). The researchers from Slotkin’s lab have shown that subcutaneous injection of terbutaline directly into the rat pup on postnatal (not during pregnancy) days 2-5 and 11-14, resulted in abnormal brain development and aberrant behaviour compared to non-exposed rodent pups. Unfortunately, all these animal studies use the same large dose and pharmacologic preparation of terbutaline, at the same time (post-delivery) and are based on the belief that terbutaline is more rapidly metabolized in the rodent then in the human. Work in the late 80’s using x-ray as a teratogen and studying the effects on the developing animal brain, is the basis for dosing the rat pups at 2-5 days after birth and stating the timing is equivalent to the human foetal brain (Table 1). To our knowledge other authors have not replicated this reference. It would seem particularly tenuous for any hypothesizes to use treatment of postnatal rat pups after delivery and extrapolate results to human foetuses treated with beta-agonist during gestation.

The second flaw in the work of Slotkin et al. is the supposition that a much larger dose is necessary in treatment of rat pups compared to that given in a human pregnancy. The work by Tegner et al. from 1984 is purported to be the basis for a much larger dose being required in the rat pup to make it comparable to the dose of terbutaline that the human foetus would receive after maternal treatment for preterm labour (Table 1). The scientific basis for this supposition suggests that rats have a shorter half-life of terbutaline metabolically than humans. Larger and better controlled studies show that there is a equal or even shorter half-life in human pregnancies when compared to rats (Table 2). The dose selected by Slotkin et al. at 10mg/kg/day is 260 times the dose used in humans for subcutaneous terbutaline maintenance therapy during pregnancy (0.04mg/kg/day).

Next, the method of injection may play a considerable role in determining whether the rat pup data by Slotkin et al. can be comparable to a human pregnancy (Table 1). In the rat pups, the large dose 10mg/kg was directly injected into the animals and while in humans the terbutaline is injected subcutaneously into the mother where, after absorption, it is diluted in her vascular volume and then subsequently transferred in even smaller amounts across the placenta to the foetus. Lastly, and probably most important, is the type of terbutaline used. The terbutaline employed in the rat pup experiments by Slotkin et al. was pharmacologic grade (Sigma Chemical Corporation, St. Louis Missouri). This type of terbutaline is not used in humans and is limited to in vitro studies in laboratory animals because it is not USP (United States Pharmacopeia) grade (Table 1). The medicinal grade of terbutaline used in all human pregnancies, regardless of the route of administration, is obtained from pharmacies worldwide, is pure, and is of consistent quality. Indeed, Owens et al. has studied this difference by injecting rat pups (similar to the Slotkin studies with 10mg/kg/d, of pharmacologic terbutaline) as well as the same amount of medicinal terbutaline and placebo. In this study, cerebral pathology, as well as their postnatal biologic function (ambulation, distance travel, resting time, rotarod balance, etc.), were similar between the medicinal grade of terbutaline and placebo group while the 10mg/kg/d pharmacologic grade of terbutaline exhibited abnormal psycho-biologic functioning as well as the brain lesions as described by Slotkin et al. Their data was further supported by GLC – mass spectrometry which revealed differences in the two types of terbutaline. The conclusion was that medicinal terbutaline, even in these large doses,

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did not have a deleterious effect on the foetal rat pups (Table 1).\textsuperscript{15}

The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed. Animal care was in accordance with the institution guidelines.

From a teratology standpoint the studies supposedly linking terbutaline exposure and ASD through exposure of neonatal rats do not offer any dose – response curves and therefore, the risk of a teratogenic effect cannot be calculated. In addition, the neuropathologic evidence in Slotkin et al.\textsuperscript{16,17,18,19} in the terbutaline exposed rats is stated to mimic those in the human condition with autism. However, of the brain lesions studied by these investigators\textsuperscript{16,17,18,19} (hippocampus, somatosensory cortex and cerebellum), only the cerebellum has been shown to be abnormal in human cases of ASD.\textsuperscript{24} Furthermore, the gliosis as well as the reduced size of cortical neurons found in the exposed animals in Slotkins rat studies\textsuperscript{16,17,18,19} have not been reported in humans. Only the findings of reduced Purkinje cell numbers agree with the human findings. The results involving gliosis are not clear because this finding is noted in many disease processes other than autism. Finally, the hypothesis that abnormal behavioural effects noted in rat pups exposed to terbutaline during the neonatal period was similar to those in human autism\textsuperscript{25} is likewise untrue. While the Slottkin group\textsuperscript{16,17,18,19} observed increased activity and sensitivity to noise, Owens et al.\textsuperscript{15} noted similar findings only when the pharmacologic grade of terbutaline was used in the rats. In her study, rat pups receiving the medicinal grade of terbutaline reacted similar to those in the placebo group and showed normal psychophysiologic testing. Also, pulse inhibition, which has been noted to be depressed in human cases of ASD\textsuperscript{26} was not affected in terbutaline, exposed animals even at large dosages. However, in spite of all of these critical flaws associated with the post-delivery rat pup-terbutaline model the Slottkin research group continues to link work in neurotransmitters to ASD in humans.\textsuperscript{27} In contrast, the preponderance of animal literature from all other authors, reveals that terbutaline exposed animals do not exhibit behaviours that are similar to those observed in human ASD.

### Human Studies

There are several human studies referenced by Witter et al.\textsuperscript{13} regarding the linkage of terbutaline treatment for preterm labour to ASD which deserve close scrutiny. First is a reference by Connors et al.\textsuperscript{28} which purports to demonstrate an increased concordance for ASD in dizygotic twins whose mothers were treated with terbutaline for preterm labour (Table 2). The authors begin with known twin pregnancy pairs (n=36) that had at least one child diagnosed with ASD. There was no difference between the twin sets treated with maternal terbutaline and those not exposed (p=0.157). The authors then apparently removed several twin sets until statistical significance between drug exposure and ASD was obtained. Obviously, there are concerns with this methodology generating bias and also because the gestational age at delivery (number of preterm deliveries), treatment duration, co-morbid conditions and the dosages were not specified. Finally, two of the twin sets who were exposed for short periods of time to terbutaline were classified as non-exposed, which again would have a major effect on biasing the results (Table 2). Another publication (Kilburn et al.\textsuperscript{29}) was referenced as supporting a link between terbutaline treatment and autism. The design of the study compared eight terbutaline exposed, known autistic children from only two families who also had high total chemical exposures. They were compared to a control group of 145 children with no chemical exposure and with no ASD. A battery of tests offered different results in the autistic children compared to the control group. It is not stated in the article how this helps support a link between ASD and treatment with terbutaline. Indeed, the study design renders this publication un-interpretable (Table 2). A study by Stein et al.\textsuperscript{30} is purported to show that terbutaline exposure is associated with expressive language delay. This article is a simple case presentation with comments from four paediatricians. In reading the article carefully, there is no suggestion that the language delay might be associated with prenatal exposure to terbutaline. Likewise, there is clearly no support for a linkage between autism and terbutaline exposure. Another study that is said to support the hypothesis that beta-agonist drugs cause ASD is one by Croen et al.\textsuperscript{31} who studied the risk of ASD in the offspring of mothers with autoimmune disorders. However, only psoriasis in the mother was linked to autism in the offspring and none of these patients were treated with terbutaline. Croen et al.\textsuperscript{32} have now published a more recent study which expanded on the “personal communication” mentioned in Witter et al.\textsuperscript{13} In the published work\textsuperscript{32} the authors significantly expanded their database. In this study, children born over a 5 year period with ASD (n=291) were compared to children without autism (n=284) and the two groups were matched for sex, birth year and delivery hospital. The frequency of exposure to beta-agonist drugs was similar for mothers of children with ASD versus those of controls (18.9% vs. 14.8%, p=0.19). While the authors stated that there was a “four-fold risk of ASD with terbutaline exposure”, the results were actually not significant between the two groups (OR 4.4, 95% CI, 0.8-24.6). Tested

Table 1: Bradford Hill Criteria* for Causal Association.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Strength</th>
<th>Consistency</th>
<th>Specificity</th>
<th>Temporality</th>
<th>Dose-Response</th>
<th>Biologic Plausibility</th>
<th>Coherence</th>
<th>Experimental Evidence</th>
<th>Analogy</th>
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* All 9 criteria must be met to establish causation

### Critical review

Competing interests: None declared. Conflict of interests: None declared.
do state correctly that their study does not offer evidence linking beta-agonist exposure in pregnancy with autism risk.

Older studies, using a variety of beta-agonists, other than terbutaline, was also cited by Witter et al. as showing that such treatment was related to abnormal neurobiological functioning. Pitzer et al. was said to show poor cognitive and motor performance in babies several years after beta-agonist treatment. However, when one closely reviews the article, this retrospective study revealed that the patients receiving tocolysis for preterm labour could not be differentiated from those who were not exposed to terbutaline. Only the children delivered at term after tocolysis had a higher rate of “psychiatric disorders” when compared to the control group. The authors stated that those delivered at term had a higher incidence of hypertension and other co-morbid factors and they recommended prospective evidence before they could associate beta-agonist drugs with long term neonatal effects. Another reference is said to show that tocolytic exposure resulted in “poor school performance” compared to non-exposed children. There was no difference between the two groups in head circumference, general developmental, and motor performance” could no more be attributed to terbutaline and delivered prematurely, but none of them had growth or neurobehavioral problems related to treatment. Other studies have found similar results regarding the lack of beta-agonist therapy as a cause of poor neurodevelopment in children and rather related this to prematurity. Other data, so far unpublished, is supportive of the concept there is no linkage between beta-agonist treatment in the mother and ASD in children. Three nationally known organizations (Side lines, Moms of Super Twins, and Triplet Connection), who provide support for women with high risk and multi-foetal pregnancy; collaborated to perform a web based survey of high risk pregnancy. Of 11,717 e-mail invitations 18.9% (n=2217) responded. Overall, 23.6% (523/2217) reported having at least one child with a disability or chronic medical disorder. Of the total population, 43.5% were exposed to terbutaline and overall 128 children had ASD. There was no difference in ASD diagnosis (6.3% versus 5.1%, p=.233) between those children, whose mothers were treated with terbutaline for preterm labour versus those with no terbutaline exposure (Elliott – personnel communication). Finally, in the United States it has been 20 months since the maker of terbutaline such as showing that terbutaline, as well as any other beta-agonist, could not be attributed to terbutaline and the MMR vaccine there is no data to serve as a cautionary tale for those attempting to connect autism with any environmental cause where, like with terbutaline and the MMR vaccine there is no data supporting an association between them nor is there a plausible mechanism. Since the aetiology of ASD is complex and multi-factorial, it is unlikely that a single chemical agent such as terbutaline, even if used in a large population (such as preterm labour and asthma), would have a linkage to ASD. First, there is no evidence that terbutaline and autism are linked through epidemiologic methodology. Second, terbutaline has

### Table 2: Obstetric Factors.

<table>
<thead>
<tr>
<th>N=92</th>
<th>Indomethacin</th>
<th>MgSO₄</th>
<th>Nifedipine</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA at del</td>
<td>32</td>
<td>33</td>
<td>27</td>
<td></td>
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<tr>
<td>30-34 weeks</td>
<td>30.3 ± 3.6</td>
<td>30.8 ± 2.9</td>
<td>30.1 ± 3.1</td>
<td>.771</td>
</tr>
<tr>
<td>&lt;30 weeks</td>
<td>11</td>
<td>17</td>
<td>11</td>
<td>.368</td>
</tr>
<tr>
<td>Days Gained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;48 hrs.</td>
<td>15.7 ± 20.6</td>
<td>9.9 ± 13.1</td>
<td>9.5 ± 10.9</td>
<td>.923</td>
</tr>
<tr>
<td>&gt;72 hrs.</td>
<td>20 (62.5%)</td>
<td>20 (60.6%)</td>
<td>16 (59.3%)</td>
<td>.968</td>
</tr>
<tr>
<td>&gt;7 days</td>
<td>17 (53.1%)</td>
<td>15 (45.4%)</td>
<td>15 (55.5%)</td>
<td>.791</td>
</tr>
<tr>
<td></td>
<td>14 (43.7%)</td>
<td>12 (36.4%)</td>
<td>10 (37.0%)</td>
<td>.802</td>
</tr>
</tbody>
</table>

Critical review

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been used for many decades in millions of patients with preterm labour as well as those with asthma and the preponderance literature shows good efficacy for prolonging pregnancy and very little in the way of maternal/foetal side effects, particularly when used in a continuous subcutaneous fashion for maintenance tocolysis. It is also important to realize that ASD is linked to prematurity with a rate five times that reported in the general population. While it is unlikely that new environmental toxins will emerge as an important cause of autism the progress in genetics is startling. For example, there is new data that pathogenic structural chromosomal variants have a functional impact in ASD at a genome-wide level. Using these techniques as well as gene pathway analysis and other modalities seem to offer implications for, not only detection and intervention, but perhaps prevention of ASD.

**Conclusion**

In conclusion, there is no creditable or reproducible evidence linking terbutaline treatment of preterm labour or asthma to any developmental problems or ASD. On the other hand, the preponderance of the literature shows that the number one cause of long term disabilities in children is related to preterm delivery. While clinicians may not be able to prevent preterm birth, they can certainly use tocolytic drugs to prolong pregnancy. In concert with corticosteroid treatment, progesterone therapy, cervical cerclage, and other techniques, the burden of prematurity can be reduced. Therefore, the clinician can use beta-agonist treatment or any other tocolytic drug to prolong pregnancy and improve the gestational age at delivery without fear that these therapies might be associated with later ASD in the offspring.

**References**


