Original Article

Short-Term Benefit From Oral Vancomycin Treatment of Regressive-Onset Autism

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ABSTRACT

In most cases symptoms of autism begin in early infancy. However, a subset of children appears to develop normally until a clear deterioration is observed. Many parents of children with "regressive"-onset autism have noted antecedent antibiotic exposure followed by chronic diarrhea. We speculated that, in a subgroup of children, disruption of indigenous gut flora might promote colonization by one or more neurotoxin-producing bacteria, contributing, at least in part, to their autistic symptomatology. To help test this hypothesis, 11 children with regressive-onset autism were recruited for an intervention trial using a minimally absorbed oral antibiotic. Entry criteria included antecedent broad-spectrum antimicrobial exposure followed by chronic persistent diarrhea, deterioration of previously acquired skills, and then autistic features. Short-term improvement was noted using multiple pre- and post-therapy evaluations. These included coded, paired videotapes scored by a clinical psychologist blinded to treatment status; these noted improvement in 8 of 10 children studied. Unfortunately, these gains had largely waned at follow-up. Although the protocol used is not suggested as useful therapy, these results indicate that a possible gut flora–brain connection warrants further investigation, as it might lead to greater pathophysiologic insight and meaningful prevention or treatment in a subset of children with autism. (*J Child Neurol* 2000;15:429–435).

Autism is a devastating and largely untreatable disorder first described by Kanner in 1943.[1] Currently classified as a pervasive developmental disorder in the *Diagnostic and Statistical Manual of Mental Disorders,* 4th edition (*DSM-IV*),[2] it usually manifests in early infancy, with impairment typically persisting into adulthood.[3] Incidence estimates vary from 10 to 20 per 10,000 children, with boys four times more likely to be affected.[4] Although some children are later found to have chromosomal aberrations or metabolic disorders that might explain their autistic features, no underlying etiology can be identified in the vast majority of cases.[5] "Autistic regression" occurs in approximately one third of cases, with regression typically occurring before 2 years of age, and involving loss of language, social, and play skills.[6]

HYPOTHESIS

Several parents of children with regressive-onset autism reported to us their observation of the following sequence: repeated broad-spectrum antimicrobial use (usually for chronic otitis media), followed by chronic diarrhea, then loss of language, play, and social skills, and subsequent onset of autistic symptoms. Although these observations could be unrelated, they led to speculation regarding a possible etiologic link in this sequence. We developed the hypothesis that repeated antimicrobial use might have disrupted a protective effect of indigenous intestinal organisms and allowed colonization by one or more neurotoxin-producing species.[7] If this were true, then appropriately targeted antimicrobial therapy might reduce autistic symptoms in these individuals.

We therefore performed an exhaustive literature review to search for candidate organisms. The details of this review

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are beyond the scope of this article, but it appeared most plausible that the agent(s), if present, might be a clostridial species. Several members of this genus have been implicated in diarrheal diseases of humans and animals. *Clostridium tetani* and *C botulinum* produce potent, nonnecrotizing neurotoxins that severely disrupt neurotransmitter release.[8] Also, at least one clostridial species (*C difficile*) has demonstrated its ability to proliferate enterically during use of certain antimicrobials.[9]

TREATMENT RATIONALE

If, in fact, this conjecture were correct, therapeutic options would include oral metronidazole, bacitracin, or vancomycin. The latter was chosen for its efficacy, minimal absorption (ie, the antibiotic remains in the intestinal tract and is excreted in the stool), and benign taste (the unpleasant-tasting metronidazole or bacitracin would have required a nasogastric tube for drug delivery). The decision to use vancomycin was not made lightly, however, since this drug is of paramount importance in treating life-threatening antibiotic-resistant bacterial infections, and significant public health concerns exist should its use become widespread in the community.[10]

INDEX CASE

The index case was a 4½-year-old Caucasian boy with chronic diarrhea and autism whose motor, cognitive, and social development was normal until 18 months of age. Diarrhea began at approximately 17 months of age after three 10-day courses of broad-spectrum antimicrobials prescribed over a 6-week period for chronic otitis media. There was no blood or pus in the stool nor associated constitutional symptoms. At 19 months of age there was profound behavioral and developmental deterioration, along with emergence of severe autistic features.

Extensive genetic, neurologic, gastrointestinal, and immunologic evaluations were all unrevealing. Neither conventional (eg, full-day special education program, speech, and play therapy) nor unconventional interventions (eg, special diets, megavitamin loading) had a significant effect on his autistic symptoms.

A 12-week therapeutic trial of oral vancomycin (125 mg four times per day) was begun with expanded observations by a pediatric neuropsychologist pre- and post-treatment. At baseline, the child was not on a special diet nor was he taking any vitamin supplements. Three days after initiation of the vancomycin therapy, a hyperactivity pattern emerged that lasted 4 days. This was followed by 2 days of lethargy and subsequently by a rapid and dramatic clinical improvement. He became affectionate and relatively calm. He promptly achieved toilet training and increased vocabulary. Follow-up behavioral observations after 8 weeks of therapy noted an increase in on-task performance, compliance with parental requests, awareness of environmental surroundings, and persistence when engaging in positive activities. A significant reduction in repetitive and self-stimulatory behaviors was also noted. The child's educational therapies remained unchanged for both 6 months before and during the vancomycin trial. Shortly after vancomycin discontinuation, behavioral deterioration was observed. Though still improved over baseline, he eventually lost most of the initial gains.

METHODS

Subjects and Study Design

To explore whether our index case's improvement represented a true therapeutic effect, institutional human investigation committee approval was obtained for an open-label trial in a narrowly defined subgroup of autistic children. Eleven children (10 boys, 1 girl; age range, 43 to 84 months) were enrolled. Inclusion criteria for the study are listed in Table 1 and were derived from our central hypothesis and index case characteristics. All children had diarrhea and regressive onset of autistic features (occurring at a mean of 17.7 ± 3.4 months) as previously defined in the literature.[11]

The Developmental Profile II[12] provided descriptive developmental levels to contrast with developmental age. While mean chronologic age of the children was 59.4 ± 12.7 months, the mean developmental age for the domains of communication (23.0 months ± 13.0), socialization (25.6 months ± 12.9), and self-help (34.4 months ± 12.4) is evidence of their significant developmental delay. The Childhood Autism Rating Scale was also administered. This is a 15-item behavioral rating scale developed to identify children with autism, and to distinguish them from developmentally handicapped children without autism syndrome. Based on Childhood Autism Rating Scale diagnostic categories, six children met the criteria for severe autism, two for moderate autism, and three for mild autism.[13] The vancomycin dose was 500 mg/day given orally as a liquid (500 mg/6 mL), divided 2 mL three times per day for 8 weeks. This was followed by 4 weeks of oral treatment with a probiotic mixture of *Lactobacillus acidophilus*, *L bulgaricus*, and *Bifidobacterium bifidum* (40×10^9 colony-forming units/mL).

Psychologic Evaluations

Two measures of potential improvement were examined: (1) Children were videotaped for 30 minutes at baseline and once during therapy in a playroom environment. At each session, the child was directed to play with a series of puzzles, books, blocks, and dolls by the mother and then by the evaluator. At the end of the trial,

Table 1. Study Entry Criteria

1. Meets diagnostic criteria for autistic disorder (*DSM-IV* 299.00)
2. Other genetic and medical diagnoses have been adequately evaluated and ruled out
3. Definable, rapid onset after 12 months of age
4. Antecedent antimicrobial use (2 months or less before autism symptom onset)
5. Persistent loose stool history, with diarrhea onset before autism symptoms
6. Symptoms for 4 years or less
7. Child is between 2 and 8 years of age
8. No evidence of any significant medical problem that might complicate treatment, such as renal, cardiac, or pulmonary disease; severe enterocolitis (visible blood or pus in the stool), or chronic infection (eg, tuberculosis)
9. Clinically static for 3 months or more (no new neuroleptic, seizure, or other medications), with no elective changes during the study
10. No antimicrobial use for at least 2 months prior to entry into the study

Table 2. Scoring System for Videotapes

Observer Rating Analog Assessment Scales for Behavior, Communication, and Social Skills

Child's Name: _____

Does the child appear "better" overall in one tape over the other?
Yes No

If yes, in which tape does the child appear better? _____

Place mark on line where 10 = normal behavior and 0 = horribly abnormal. Note "NR" on scale if not ratable.

Tape number:_____	Tape number:_____
GLOBAL IMPRESSION	GLOBAL IMPRESSION
0 5 10	0 5 10
BEHAVIOR SUBDOMAIN RATINGS	
0 5 10	0 5 10
Global Behavior Rating	**Global Behavior Rating**
0 5 10	0 5 10
Perseveration	Perseveration
0 5 10	0 5 10
Noncompliance	Noncompliance
0 5 10	0 5 10
Oppositional Behavior	Oppositional Behavior
0 5 10	0 5 10
Self-Stimulation Behaviors	Self-Stimulation Behaviors
COMMUNICATION SUBDOMAIN RATINGS	
0 5 10	0 5 10
Global Communication Rating	**Global Communication Rating**
0 5 10	0 5 10
Expressive Language	Expressive Language
0 5 10	0 5 10
Receptive Language	Receptive Language
SOCIAL SKILLS SUBDOMAIN RATINGS	
0 5 10	0 5 10
Global Social Scale Rating	**Global Social Scale Rating**
0 5 10	0 5 10
Eye Contact	Eye Contact
0 5 10	0 5 10
Approach Behavior	Approach Behavior
0 5 10	0 5 10
Play Skills	Play Skills

Table 3. Physician Analog Rating Scales

Analog Assessment Scales for Behavior and Communication

Physician Rating

Date: _____

Child's Name: _____

Behavior: 1 5 10

Communication: 1 5 10

1 = Severely impaired, can't be any worse; 10 = Age appropriate

Behavior: Compliance to requests. Mood (temper, outbursts, irritability), eye contact, attention, and alertness. Activity level. Interaction with others present in room. Stereotyped behaviors (degree of severity).

Communication: Pointing or gesturing. Babble and quality of babble. Receptive language. Use of sign language and gesture (prompted/spontaneous). Verbal language (prompted/spontaneous, understandable). Appropriate use of language (single words, two words together, sentences). Verbal perseveration.

a clinical child psychologist (who was provided with a brief explanation of our working hypothesis) compared coded, paired videotapes of 10 of the 11 children studied (video was not available for one child). The psychologist viewed each pair of tapes and scored them using the rating scale shown in Table 2. To diminish the possibility of investigator bias, the tapes were randomly numbered and the psychologist did not have any personal contact with the children. (2) Behavior and communication analog rating scales (Table 3) were completed by the study physician at baseline, during therapy, and at follow-up in a manner similar to previously validated methods for other disease states.[14] Results are presented as median scores to account for potential nonlinear score increment.

Laboratory Evaluations

Extensive medical evaluations were conducted in parallel with the detailed psychologic assessments. Stools were examined for occult blood, inflammatory cells, *Aeromonas hydrophila*, *Cryptosporidium*, *C difficile* toxin, routine bacterial pathogens, and ova and parasites. Blood tests included complete blood cell counts, chemistry panels, and erythrocyte sedimentation rates. Urinalyses were also obtained. Detailed quantitative aerobic and anaerobic fecal microbiologic studies were conducted at the Wadsworth Anaerobic Bacteriology Laboratory on specimens from four children. Each stool was cultured with a total of 27 different media and atmospheric conditions, modified from the procedure described in Summanen et al.[15]

RESULTS

Analog Rating Scales, Videotapes, Treatment Observations, and Laboratory Evaluations

As shown in Table 4, unblinded assessment using an analog rating scale noted improvement for the group as a whole in communication (Wilcoxon Signed Rank Z-score = –2.9, $P = .003$) and behavior (Wilcoxon Signed Rank Z-score = –2.9, $P = .003$). To ensure that changes attributed to intervention were not a reflection of differences at baseline, Spearman correlations were conducted. There were no significant correlations between the baseline measure and scores

Table 4. Results of Two Measures of Short-Term Improvement

Demographics			Measures of Improvement				
				Subjective Visual Analog Rating Scales			
				Behavior*		Communication*	
Subject	Age at Onset of Autism, months	Age at Initiation of Treatment, months	Paired Videotapes	Baseline	Post-treatment	Baseline	Post-treatment
1	14	78	+	1.5	4.7	1.7	2.2
2†	16	61	+	5.2	5.6	5.1	5.6
3	16	43	–	1.9	2.1	1.8	2.1
4	15	57	Not available	1.5	3.3	1.4	1.7
5	19	47	+	2.3	4.6	1.2	4.3
6	18	70	+	2.2	6.0	1.7	5.1
7	27	84	+	2.4	5.0	2.0	4.6
8	18	63	+	2.3	7.7	1.7	5.3
9	18	44	±‡	1.4	6.7	1.0	5.2
10	16	56	+	2.2	3.3	2.2	2.8
11	18	56	+	2.5	5.5	2.8	5.6
Mean	17.7 (±3.4)	59.9 (±13.3)	Median§	2.2/1.5, 2.4§	5.0/3.3, 6.0§	1.7/1.4, 2.2§	4.6/2.2, 5.3§

*Behavior scale rated compliance to requests, mood, eye contact, attentiveness, activity level, and severity level of stereotyped behaviors. Communication scale rated receptive and expressive language (10 = "normal," 1 = "worst" autistic features).
†Testing instruments were relatively insensitive at measuring improvement observed in this subject, who was "high functioning" at baseline (eg, communication skills shifted from "need-based" language to "conversational" while on therapy).
‡Although blinded rater did not observe clear difference between paired tapes, study physician, psychologist, coordinator, and parents unanimously agreed that child clearly improved while on therapy.
§Results are presented as median, 25th and 75th percentiles. Subjective analog rating scale post-treatment scores were significantly improved over baseline rating ($P \approx .003$ for *both behavior and communication scales*).
+ = on-therapy videotape rated as clearly better than baseline; – = baseline videotape rated as clearly better than on-therapy;
± = no clear difference between the two tapes.

during intervention for either communication ($\rho = .35$, $P = .28$) or behavior ($\rho = .22$, $P = .51$). Blinded assessment of the coded, paired videotapes noted an improvement during therapy in 8 of 10 children studied, no change in one, and a possible deterioration in one.

As previously observed in the index case, a brief (1 to 4 days) period of hyperactivity was noted in six children within 3 days of initiating antibiotic treatment. One subject then experienced a day of marked lethargy. Otherwise, aside from obvious autistic features, all children had normal physical examinations at baseline and throughout the study, as well as unremarkable basic blood, stool, and urine tests as outlined in Methods.

Long-Term Follow-Up

Although improvement was clear by several measures, unfortunately these gains did not endure. One child who had responded significantly to treatment deteriorated toward the end of the study while still on vancomycin therapy. During telephone follow-up (conducted weekly during the probiotic therapy), most parents reported substantial behavioral deterioration within 2 weeks of discontinuance of vancomycin treatment. Because of difficulty in disguising the taste, probiotic treatment compliance was very poor in several children. Behavioral deterioration appeared to occur whether or not the child was compliant with the probiotic therapy regimen. Therefore, it would appear that the probiotic therapy used as an adjunct after vancomycin treatment had no discernible beneficial or adverse effect. All children were observed in follow-up ranging from 2 to 8 months after discontinuance of vancomycin. In all but one child, the analog ratings returned toward baseline (Figure 1).

Quantitative Fecal Flora

Given the extreme labor intensiveness of such studies, it will be some time before detailed microbiologic analysis of all pre- and post-therapy stool specimens is completed. Stool specimen data from four autistic children prior to vancomycin therapy were compared to those of 104 normal adult subjects from previously published studies (performed under the supervision of the same principal investigator).[16] Anaerobic cocci, chiefly peptostreptococcal species, were present in 93% of the adults' specimens, comprising some 10% of the stool microorganisms. In stark contrast, these

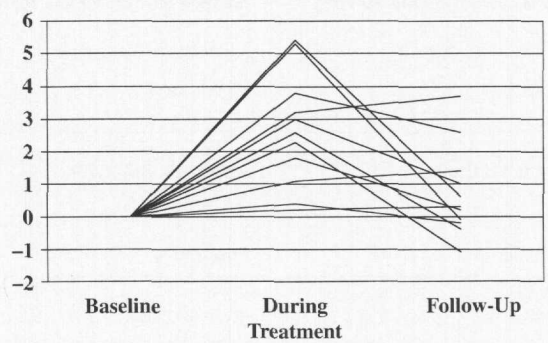

Figure 1. Analog Behavioral Rating Scale. Shown is a comparison of analog behavioral ratings for each of the 11 study children at three points: baseline, during vancomycin treatment, and after longer-term follow-up (2 to 8 months). Scores for each child are adjusted for differences in baseline. Positive scores indicate improvement; negative scores indicate worsening relative to baseline. Ratings for communication skills showed a similar pattern and are not shown separately.

Table 5. Fecal Flora Data

Organism	*Autistic Patient A*	*Autistic Patient B*	*Autistic Patient C*	*Autistic Patient D*	*Adults (N = 104*)*
Enterobacteriaceae	6	7	7	7	9
Streptococcus	3	5	0	4	9
Enterococcus	0	6	0	0	8
Bacteroides fragilis group	8	8	9	8	11
Bacteroides, other	8	0	9	8	11
Anaerobic gram negative rod, other	6	4	7	5	8
Peptostreptococcus species	0	0	0	0	10†
Anaerobic cocci, other	0‡	0	0§	0	11†
Lactobacillus species	9	9	10	8	10
Bifidobacterium species	7	9	9	8	10
Eubacterium species	8	0	9	8	11
Clostridium species	9	7	8	8	10

Units are $\log_{10}$ colony-forming units per gram dry weight.
*Mean of positive specimens. Subjects were normal adults on various diets (vegetarian, traditional Japanese, or standard Western); there were no statistically significant differences in results between these various groups.
†93% of the 104 subjects had *Peptostreptococcus* species or other anaerobic cocci.
‡Ethanol and heat-resistant coccoid forms were present (probably clostridia).
§Heat-resistant coccoid forms were present (probably clostridia).

species were absent from the stools of each of the four autistic children tested (Table 5).

DISCUSSION

The apparent, though short-term, improvement during treatment with this minimally absorbed antibiotic is not explainable using current conventional genetic hypotheses[17] alone for autism. Results of this preliminary study, along with previous reports of increased intestinal permeability[18] and a "nonspecific colitis" in children with autism,[19] suggests a possible "gut-brain" etiologic connection in a subset of these children.

Although the hypothesis that autism (in a defined subset of children) might be a sequela to the colonization of the intestinal tract by one or more neurotoxin-producing bacteria is novel, published data along several paths could lend credence to the notion that an alteration in colonic flora contributes to autism symptoms. The first line of evidence is from the infant botulism literature. This condition was first recognized as a distinct clinical entity in 1976.[20] It differs from classic (foodborne) botulism in that the intestinal tract becomes colonized by *Clostridium botulinum* and elaboration of the neurotoxin occurs in vivo.[21] Age is a primary risk factor for the development of infant botulism; diagnosis of the disease is rare after 1 year of age.[22] Studies in animals have demonstrated a similar age-dependent susceptibility.[23] However, the colonization resistance observed in mature animals is greatly diminished when they are treated with broad-spectrum antimicrobials.[23] Similarly, antimicrobial use has been identified as a risk factor for the development of botulism related to intestinal colonization with *C botulinum* in older children and adults.[24]

The second line of evidence is from human and animal studies that have demonstrated repeatedly that intestinal colonization by opportunistic pathogens (eg, *Escherichia coli*,[25,26] *Klebsiella pneumoniae*,[25] *Pseudomonas aeruginosa*,[26,27] *Salmonella enteritidis*,[28] *Shigella flexneri*,[29] and *Vibrio cholerae*[29]) is greatly enhanced when protective intestinal microbiota are disrupted by broad-spectrum antimicrobials. In humans, the best-documented example of opportunistic colonization of the intestinal tract following antimicrobial use is that by *C difficile*, the causative agent of pseudomembranous colitis.[9]

Another potentially relevant condition is D-lactic acidosis, in which associated psychiatric symptoms are well documented. D-lactic acidosis, a complication of short bowel syndrome or intestinal bypass surgery for obesity, is a condition caused by a change in bacterial flora to an acid-tolerant, aciduric (*Lactobacillus*, *Bifidobacterium*, *Eubacterium*, and *Streptococcus*) flora.[30] Patients present with a range of behavioral changes such as hostility, slurred speech, stupor, altered mental status, dizziness, asterixis, and ataxia.[31] Treatment is with oral antimicrobials, resulting in rapid cessation of neurologic signs.

No validated instrument is currently available for quantitative measurement of improvement in autistic symptomatology and there is an urgent need to correct this deficit for use in future autism intervention trials. In the absence of a preexisting standardized method, the current study used two independent assessment tools. Although the analog rating scales were completed by the study physician, who was aware of the children's treatment status, the formal videotape ratings were performed in a blinded manner. The improvement observed after vancomycin intervention appeared to be significantly greater than could normally be attributable to the characteristic waxing and waning of autistic symptomatology.[32]

A substantial deterioration of the behavioral improvements made while on therapy was reported by most parents within 2 weeks of ending the vancomycin trial. While the cause is not known for either the apparent improvement or the later decline, it is possible that the deterioration is due to the offending organism being spore forming, and hence surviving therapy to germinate after vancomycin discontinuation, as has been documented with *C difficile* infection.[33] An additional possibility is that the therapy was sublethal because of antimicrobial choice, dosage regimen,

or both, permitting emergence of an antimicrobial-resistant bacterium.

Since vancomycin is not absorbed, it appears likely that the behavioral improvement was related, in some way, to the drug's effect on the intestinal-tract flora (and not a "drug effect" per se on the central nervous system). Although we theorize that the short-term benefit from vancomycin treatment might be due to the temporary elimination of a neurotoxin-producing pathogen, there are other possible mechanisms. For example, autoantibodies to neuron-axon filament protein,[34] glial fibrillary acidic protein,[34] and myelin basic protein[35] have been reported in autism and it has been postulated that these autoantibodies might contribute to autistic symptomotology.[34,35] It is, at least, theoretically possible that the production of these autoantibodies is related to the presence of an infectious pathogen, as has been postulated for rheumatoid arthritis.[36]

The significance of the possible fecal flora changes in these autistic children is unknown. It is unlikely that specimen collection or shipping contributed to the absence of *Peptostreptococcus* and other anaerobic cocci as other equally oxygen-sensitive organisms were recovered. Although all of the children previously had received broad-spectrum antimicrobials (capable of severely disrupting intestinal flora), fecal bacterial counts typically return to their pretreatment composition within 2 weeks of discontinuance of the antimicrobial agent.[37] Therefore, since none of the children, at baseline, had a history of antimicrobial treatment for at least 2 months prior to entering our study, it is unlikely that the absence of these species reflects a transient alteration in the children's fecal flora. An uncharacterized *Peptostreptococcus* species has been documented to inhibit certain organisms, including clostridia, in vitro and in animals,[38] and it is intriguing to speculate that the absence of such organisms in certain autistic children might permit growth of clostridial or other toxin-producing bacteria through loss of competitive inhibition.[39]

The fecal flora of pediatric subjects has been studied extensively.[40–42] Use of normal adult control fecal specimens in the present study, though not ideal, is justifiable given documented similarity to pediatric stool flora. For example, one recent review of bacterial colonization patterns states that "by 12 months (of age) the anaerobic fecal populations begin to resemble that of adults in number and composition as the facultative anaerobes decrease. By two years of age, the profile resembles that of the adult."[43]

CONCLUSIONS

In this study, an open-label vancomycin trial indicates the possibility of a gut flora–brain connection in a subset of children with autism and diarrhea. If such a connection is later verified, it might be possible to identify an offending organism, and from this, effective prophylactic (eg, vaccine) or therapeutic measures.

Given the devastating nature of autism and the current lack of effective medical treatments, parents are understandably anxious to try newly reported therapies. However, we must emphasize that the benefit of oral vancomycin appears to be only short-term, and the potential threat of vancomycin-resistant organisms must be seriously considered. Therefore, we urge that vancomycin not be used to treat autistic symptomatology outside of a study protocol, and that further research of a possible gut–brain connection be vigorously pursued.

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