



ORIGINAL ARTICLE

# Improvement of autism spectrum disorder symptoms in three children by using gastrin-releasing peptide<sup>☆,☆☆</sup>



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## KEYWORDS

Gastrin-releasing peptide receptor;  
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## Abstract

**Objective:** To evaluate the safety, tolerability and potential therapeutic effects of gastrin-releasing peptide in three children with autistic spectrum disorder.

**Methods:** Case series study with the intravenous administration of gastrin-releasing peptide in the dose of 160 pmol/kg for four consecutive days. To evaluate the results, parental impressions the Childhood Autism Rating Scale (CARS) and the Clinical Global Impression (CGI) Scale. Each child underwent a new peptide cycle after two weeks. The children were followed for four weeks after the end of the infusions.

**Results:** The gastrin-releasing peptide was well tolerated and no child had adverse effects. Two children had improved social interaction, with a slight improvement in joint attention and the interaction initiatives. Two showed reduction of stereotypes and improvement in verbal language. One child lost his compulsion to bathe, an effect that lasted two weeks after each infusion cycle. Average reduction in CARS score was 2.8 points. CGI was “minimally better” in two children and “much better” in one.

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<sup>☆☆</sup> Study linked to the Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil.

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**PALAVRAS-CHAVE**

Receptor do peptídeo liberador de gastrina;  
Neuropeptídios;  
Autismo;  
Transtorno do espectro autista

**Conclusions:** This study suggests that the gastrin-releasing peptide is safe and may be effective in improving key symptoms of autism spectrum disorder, but its results should be interpreted with caution. Controlled clinical trials—randomized, double-blinded, and with more children—are needed to better evaluate the possible therapeutic effects of gastrin-releasing peptide in autism.

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### Melhoria nos sistemas do transtorno do espectro autista em três crianças, utilizando peptídeo liberador de gastrina

**Resumo**

**Objetivo:** Avaliar a segurança, tolerabilidade e possíveis efeitos terapêuticos do peptídeo liberador de gastrina em três crianças com transtorno do espectro autista.

**Métodos:** Estudo de casuística com administração intravenosa de peptídeo liberador de gastrina na dose de 160 pmol/kg por quatro dias consecutivos. Para avaliar os resultados, foram utilizadas a impressão dos pais, a Escala de Classificação de Autismo na Infância (CARS) e a Escala de Impressão Clínica Global (CGI). Cada criança foi submetida a novo ciclo de peptídeo após duas semanas. As crianças foram acompanhadas por quatro semanas após o término das infusões.

**Resultados:** O peptídeo liberador de gastrina foi bem tolerado e nenhuma criança apresentou efeitos adversos. Duas crianças apresentaram melhora na interação social, com melhora na atenção compartilhada e nas iniciativas de interação. Duas mostraram redução dos estereotípias e melhora na linguagem verbal. Uma criança perdeu sua compulsão por banhos, efeito que durou duas semanas após cada ciclo de infusão. A redução média no escore da CARS foi 2,8 pontos. Quanto à CGI, os resultados foram “minimamente melhor em duas crianças” e “muito melhor” em uma.

**Conclusões:** Este estudo sugere que o peptídeo liberador de gastrina é seguro e pode ser efetivo na melhora dos principais sintomas do transtorno do espectro autista, porém seus resultados devem ser interpretados com cautela. Ensaios clínicos controlados, randomizados, duplo-cego e com maior número de crianças são necessários para melhor avaliar os possíveis efeitos terapêuticos do peptídeo liberador de gastrina sobre o autismo.

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**Introduction**

Autism is a pervasive development disorder characterized by severe impairment in reciprocal socialization, qualitative problems in communication, and repetitive or unusual behavior.<sup>1</sup> The current estimated prevalence of autism is one in every 66 children.<sup>2</sup> Diagnosis is clinical and to date there is no specific treatment.<sup>3</sup> Neurochemical, neuropathological, neuroimaging, and genetic studies suggests disorganization of cortical neurons and cerebral disconnectivity, determined by both genetic and environmental factors.<sup>4</sup>

Over recent years the effects of endocrine peptides, including gastrin-releasing peptide (GRP), on the central nervous system (CNS) have been investigated.<sup>5</sup> GRP is released by glutamatergic neurons and acts as a neurotransmitter that regulates neuronal excitability.<sup>6–8</sup> In the brain, the gastrin-releasing peptide receptor (GRPR) is highly expressed in cerebral regions related to cognitive function and emotional processing, such as the dorsal hippocampus and basolateral amygdala.<sup>6–9</sup> Experimental studies have shown that pharmacological blockade of GRPR in neonatal rats leads to reduced preference for maternal odor and the development of late and permanent deficits in social

interaction, a behavior consistent with animal models of autism.<sup>10–12</sup>

In this experimental study, GRP was given intravenously to three children with autism to test its safety, tolerability, and possible therapeutic effects on autism spectrum disorder (ASD) symptoms. To the best of the authors' knowledge, this is the first report of GRP use in humans with autism.

**Methods**

Children with autism diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM-IV-TR),<sup>13</sup> aged from 3 to 18 years, were considered suitable for selection. Exclusion criteria were serious clinical disorders, psychiatric diseases, increased acid production in gastrointestinal system, secondary autism, and changes in medication or other treatments during the previous four weeks. Parents signed an informed consent and committed themselves to maintaining the current treatment unchanged during the study period.

The sample was selected by convenience from children seen at the ASD Clinic at the Hospital de Clínicas de Porto

Alegre (HCPA), Brazil. The first three children who met the above mentioned criteria were selected.

GRP was administered at a 160 pmol/kg dosage by continuous intravenous infusion over 30 min. Patients were observed for 1 h after infusion. Vital signs were monitored (arterial blood pressure and heart rate) every 15 min. Adverse effects were monitored. Afterwards, the patient's family was encouraged to return normal daily routine. Each child received infusions on four consecutive days.

The results were evaluated throughout the Childhood Autism Rating Scale (CARS) and Clinical Global Impression (CGI) Scale, both administered on the day before starting GRP treatment and on the last day of infusion. The CARS was translated and validated for use in Brazil by Pereira et al. in 2008.<sup>14</sup>

Each child underwent a second cycle of infusions after a two-week interval, to test for cause-effect relationships, and then followed-up weekly for four weeks. The two-week interval was chosen based on the duration of objective improvements observed in the first case.

Children received oral omeprazole during the study, to prevent any possible adverse effects from stimulation of acid production in the gastrointestinal tract.

The human GRP used was synthesized by Biopetide CO (Biotope CO. Inc; CA, USA).

The study was approved by the HCPA Medical Research Ethics Committee (project No. 11-0277).

## Case reports

### Case 1

G is a 4-year-old boy. He is an agitated child and appears to have unlimited energy. He is stubborn and will not accept being told "no" and when he is opposed he attacks himself with blows to the head, but is not aggressive to others. He prefers to be alone and does not seek out social interaction. When people he knows attempt to interact, he makes eye contact sporadically and has serious problems with joint attention. If people he does not know attempt to interact, he is indifferent. He exhibits stereotypical movements, walking on tiptoes and sporadically pointing at his nose with his finger. His only interest is toy cars, which he lines up, arranges by color, or removes the wheels from, without engaging in any type of imaginative play. He takes them with him wherever he goes and is not interested in any other toys. He exhibits unusual fear of the hairdryer, which sends him into a panic. He is only resistant to change with relation to his route to school. He has rituals for going to sleep, and a few months before the study outset, he acquired a compulsion for bathing and must be washed five or six times each day – whenever he becomes anxious, hurts himself, or gets dirty. He only speaks single-word sentences and a few short phrases. He does not have sensory abnormalities. He had been attending school since he was 1 year and 2 months old, and has been seeing a speech therapist since he was 3 years old. He takes 2 mg risperidone per day. CARS 38.5; CGI: moderately ill.

After the infusions, G lost his obsession with bathing and his fear of the hairdryer, stopped exhibiting stereotypical behavior with his hands and reduced stereotypical behaviors

with his body, improved his tolerance of frustration and his irritability, and relaxed his pre-sleep rituals. He exhibited a discrete improvement in the variety of his interests, his play, and the quality of his social interaction, paying more joint attention and making initiatives at interaction. The compulsion for bathing and abnormal fear of the hairdryer disappeared and the stereotypical behaviors were attenuated on the first day of infusion. There was no change to agitation, or verbal or non-verbal communication. CARS: 34; CGI-S (severity scale): moderately ill; CGI-I (improvement scale): much improved.

The improvement in objective symptoms lasted for two weeks. After this period, the boy was administered another cycle of infusions, with similar results. The compulsion for bathing and stereotypical movements with the hands disappeared on the first day of infusions once more, and the improvements were maintained for two weeks after the last infusion.

G did not exhibit any type of adverse effect after either of the two GRP cycles.

### Case 2

LO is 4-year-old boy. He has verbal language and exhibits many echolalias, both immediate and delayed. He is calm, becoming agitated only when in situations that are out of the ordinary or in strange places. When opposed, he screams and is aggressive to himself and others (biting). He exhibits many stereotypical behaviors, such as swaying, flapping, swinging his hands in front of his eyes, walking on tiptoes, or running in short starts. He does not play imaginatively and is not interested in toys. He is fascinated by brands, logos, letters, and numbers. He does not become fixated on routines, rituals, or manias. He has poor social interaction, completely ignoring his peers. He makes eye contact with adults for short periods and sometimes seeks them out. He has little emotional response and often laughs without reason. His parents report that he has little reaction to pain and sporadically places objects in his mouth or smells them. LO has regular speech therapy, psychological and psychopedagogical care, plus occupational therapy and horse riding therapy. He was taking 1.5 mg/day of risperidone and 625 mg/day of sodium valproate. CARS: 42; CGI-S: moderately ill.

After GRP infusions, LO exhibited discrete improvements in the quality of his verbal communication. He became more talkative, making a greater number of comments and producing unrehearsed utterances. He became more tolerant of frustration and more sensitive to pain. Social interaction and stereotypical behavior were unaltered. CARS 40.5; CGI-S: moderately ill; CGI-I: minimally improved. LO's parents were unable to determine the effect's duration. After two weeks he was given another cycle of infusions and the results lasted until the end of follow-up. He did not exhibit any adverse effects from GRP.

### Case 3

L is boy aged 4 years and 3 months. His interpersonal relationships are highly compromised; he seeks isolation and will only interact with members of his family after persistent and vigorous attempts. His emotional response is highly compromised and he displays indifference and laughs

without reason. He walks on tiptoes, performs little jumps, and exhibits flapping and other stereotypical behaviors with his hands. He is uninterested in toys, does not play imaginatively, and does not play with children his own age. He is an agitated boy, he often places objects in his mouth, and he appears not to feel pain. He is fascinated by the opening titles of soap operas and previews of the news on television. He says few words, is not aggressive, and does not exhibit manias, rituals, or fixation with routines. He receives regular psychological and psychopedagogical care, as well as speech therapy and occupational therapy. He is not on medication. CARS: 41; CGI: moderately ill.

After the infusions, L exhibited an attenuation of stereotypical behaviors with the hands and laughter without reason. His social interaction improved, with more social smiling, greater acceptance of interactions initiated by peers, better joint attention, and better eye contact. He became more affectionate, allowing more physical contact. His play became more creative, taking in a wider variety of interests. He became more tolerant of frustration and more sensitive to pain. He became more talkative and uttered some new words. CARS: 38; CGI-S: moderately ill, CGI-I: minimally improved. His parents were unable to determine the duration of the effect. After two weeks, L was given another cycle of infusions and the results persisted until the end of follow-up. The patient did not exhibit any adverse effects.

Fig. 1 illustrates which ASD symptoms improved after the GRP infusions, by patient.

### Discussion

Over recent decades, there has been an explosive growth in the number of studies and publications on the subject of ASD.<sup>15</sup> Despite this volume of work, to date no biological marker nor any treatments capable of curing ASD have been identified. The available drug treatment only acts on secondary maladaptive symptoms. The majority are prescribed off label, since only risperidone and aripiprazole have been approved by the Food and Drug Administration (FDA) for treatment of aggression, irritability, and screaming fits in patients with autism.<sup>16-18</sup>

Evidence has suggested a link between GRP and neuropsychiatric disturbances, but the association with autism has received little attention in the literature so far. Genetic studies in humans have suggested a possible link between GRPR and regulation of social behavior and bonding. An X;8 translocation on the first intron of the GRPR gene has been identified in women with multiple osteochondroma and autism being treated for mental retardation and epilepsy, indicating that the GRPR gene is one of the candidate genes for autism.<sup>19</sup> Recent preclinical studies have raised the hypothesis that some ASD symptoms, such as social interaction deficits and reduced interest in bonding, may be caused by a lack of GRP action at some early point in development.<sup>10,12</sup>

GRP has previously been administered intravenously in humans to test its effects on nutritional intake and vascular dilation. In one study it induced transitory vasodilator effects, with pressures returning to normal in around 20 min.<sup>20</sup> In another test, GRP was administered at doses of 10, 40, or 160 pmol/kg per hour to healthy male volunteers

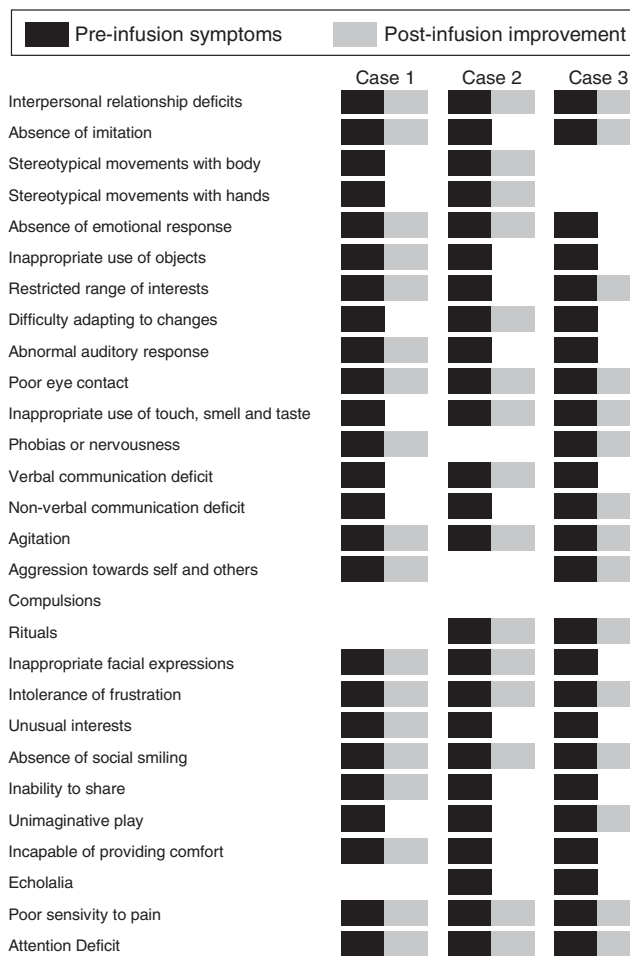


Figure 1 Symptoms of autism disorder spectrum before and after intravenous infusion of gastrin-releasing peptide (GRP).

in order to evaluate its effect on eating behavior and satiety; the results showed a significant reduction in calorie intake and a reduction of approximately 19% in food intake.<sup>21</sup> The 160 pmol/kg dose used in the present case series is larger than in any of these studies.

The few previous studies of GRP in humans have shown that it is safe, which was also confirmed in the present study, since none of the patients exhibited any adverse effects.<sup>20,21</sup>

The results of this case series suggest that GRP may be capable of improving key childhood autism symptoms, for which there is not currently any specific drug treatment, especially for stereotypes.

In the present study, two of the three patients exhibited improvements in social interaction, with discreet changes in joint attention and interaction initiatives. The same patients also exhibited a reduction in stereotypical behavior, and increased variety of interests and improved quality of play, which until then had been entirely stereotyped.

Two cases exhibited improvements in verbal language, with more spontaneous speech and use of new words, became more sensitive to pain and more tolerant to frustration, and exhibited fewer extreme responses such as screaming fits and aggression to self and others.

Although these improvements were discreet and were evaluated subjectively, in one case there was immediate

improvement in a compulsion for bathing, which is an objective variable that is easy to measure and which had a clear cause-effect relationship, demonstrated when the effect was repeated after the second cycle of GRP.

The symptoms that appeared to have improved after GRP infusions varied across the three cases, which may be explainable by the great variety of symptoms observed in each patient prior to the infusions, which is of course the reason for the term "autism spectrum." While the improvements were discreet, it is important to point out that this is a pioneering study in which patients were given GRP for just four days at a time. To date there are no published studies that have investigated the safety of long-term administration of GRP to humans.

Although all three families' responses indicate an improvement in the CGI scores, there was a very small reduction in CARS scores, which suggests that the scale may not have been sensitive enough to detect changes observed in the study.

In this study, GRP proved safe over the short- and medium-term, in common with published data.

It is important to point out that since this is a report on a case series, this study has inherent methodological limitations and the results should be interpreted with caution. The number of cases was small, there were no controls or blinding, and the results were assessed on the basis of parents' subjective impressions, with no way to test for the placebo effect.

This study has suggested that GRP may have an effect on key symptoms of childhood autism, particularly compulsions and stereotypies. Further studies are needed to better evaluate these results, with larger numbers of patients and greater methodological rigor, since administration of GRP to children with autism proved promising.

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## Conflicts of interest

MMB, LO, RSR, GS, and RR are inventors in a patent application (WO2013185187 A1) claiming the use of gastrin-releasing peptide for the treatment of neuropsychiatry and neurodevelopmental disorders.

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