

Clinical Case Report of a Novel Immunotherapeutic Approach to Autism Spectrum Disorders based on an emulsion of Chondroitin Sulfate, Vitamin D₃ and Oleic Acid

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Abstract: In this clinical case report we describe the effects associated with the administration of a supplement constituted by chondroitin sulfate, vitamin D₃ and oleic acid (Rerum[®]) on the symptoms of Autism Spectrum Disorders (ASD). 44 patients (11 females and 33 males) were administered this supplement for a period of two months and variations of symptomatology were evaluated by the Clinical Global Impression of Improvement (CGI-I). 32 out of 44 subjects (73%), showed improvements in the two months of observation. In the group of female subjects, 9 out of 11 (81%) showed improvements and, among these, 6 out of 9 (66%) showed moderate to very considerable improvements. In the group of male subjects, 24 out of 33 (73%) showed improvements and, among these, 22 out of 24 (91%) showed moderate to very considerable improvements. We attribute these remarkable results to the effects of the supplement on the endocannabinoid system as it relates to neuro-inflammation and neuro-immune alterations.

Keywords: Autism, Endocannabinoid, GcMAF, Chondroitin Sulfate, Vitamin D₃, Oleic Acid

Introduction

The term Autism Spectrum Disorders (ASD) encompasses a wide range of heterogeneous alterations of neural development characterized by three basic features: Impaired social interaction, language and communication abnormalities and stereotypical behavior. Although the etiology of ASD is debated and still largely unclear (Yenkoyan *et al.*, 2017; Geier *et al.*, 2017), the pathogenesis seems to be associated with alterations of the immune system and neuro-inflammation. Thus, we recently hypothesized that infections or inflammation at the level of the deep cervical nodes lead to impaired lymphatic drainage from the brain lymphatic system with consequent stagnation of lymph in the brain, disruption of neuronal connections, accumulation of metabolites and toxicants, neuro-inflammation and alterations of the brain intrinsic microbiota (Bradstreet *et al.*, 2015; Ruggiero, 2016a). Because of the heterogeneity of the symptoms and the lack of certainty surrounding the cause(s), it is no surprise that the very existence of a standard of care for ASD is highly disputed and even reviews of systematic

reviews fail to provide useful information that may help health providers working in the frontlines of this growing epidemics (Lyra *et al.*, 2017).

At the Biomedical Centre for Autism Research and Treatment, several therapeutic strategies targeting the immune system and neuro-inflammation have been implemented over the course of the years (Antonucci *et al.*, 2015). In the recent past, we focused our attention on the role of modulators of the immune system as potential candidates for ASD treatment based on the seminal work by Bradstreet *et al.* (2012) on the Gc protein-derived Macrophage Activating Factor (GcMAF). Since it was recently hypothesized that the results observed by Bradstreet *et al.* (2012) may be due to a glycosaminoglycan, chondroitin sulfate (Ruggiero *et al.*, 2016; Ruggiero, 2016b), we decided to treat ASD subjects with a chondroitin sulfate-containing supplement that has shown remarkable results in the field of immunotherapy (Schwalb *et al.*, 2016; Ruggiero, 2017). Here we report the first series of results observed in 44 ASD subjects treated for two months with this supplement constituted by chondroitin sulfate, vitamin D₃ and oleic acid (Rerum[®]).

Patients and Methods

The immunotherapeutic approach described in this study is similar, in line of principle, to that described in Schwalb *et al.* (2016), that is an approach currently implemented in a number of Clinics. The original clinical records as well as the informed consents given by patients or their parents or legal guardians, are conserved at the Biomedical Centre for Autism Research and Treatment. In the clinical case report described here, 44 patients (11 females and 33 males) were administered the emulsion of chondroitin sulfate, vitamin D₃ and oleic acid mentioned in the Introduction and thoroughly described in a recent paper (Ruggiero, 2017). Such an emulsion, marketed as Rerum®, is manufactured in Germany by the company “dr. reinwald healthcare” and it is classified and registered in Europe and Switzerland as a food supplement since it is composed by well-known supplements that have been in use for decades. Although supplements are usually intended to be administered through the oral route, according to national rules and regulations and under the direct responsibility of the Therapist, they can also be administered through the parenteral route as described in Schwalb *et al.* (2016). The period of observation to which this study refers to spanned two years (2016 and 2017) and each patient was observed for two months. All patients met the diagnostic criteria for ASD and were diagnosed by either a child neurologist or developmental psychologist, in addition to receiving the evaluation of the clinician (NA). No significant changes were made to the participants’ treatments apart from the introduction of Rerum® during the timeframe reported in this study. The initial dose for each patient was 0.01 mL per week. In some subjects, the dose was progressively adjusted to the body weight up to the maximum tolerated dose or up to the minimum efficient dose.

In addition to meeting diagnostic criteria for ASD, the clinician supervising this study (NA) used an in-house severity scoring system that is based on the Clinical Global Impression of Improvement (CGI-I). According to this system, the score was as designated follows:

- 5: Very considerable improvement
- 4: Considerable improvement
- 3: Moderate improvement
- 2: Slight improvement
- 1: Very slight improvement
- 0: State unchanged
- 1: Very slight deterioration
- 2: Slight deterioration
- 3: Moderate deterioration
- 4: Considerable deterioration
- 5: Very considerable deterioration
- 6: Maximum deterioration

Age, gender, body weight and CGI-I score of participants are summarized in Table 1.

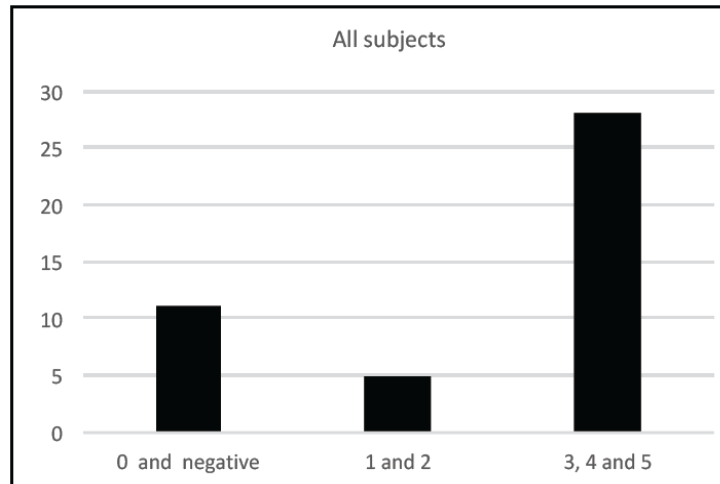
Results

Table 1 and Fig. 1 (upper panel) show that 32 out of 44 subjects (73%) showed improvements in the two months of observation. Of these, 28 out of 32 (87%) showed moderate to very considerable improvements. In the group of those who showed improvements, 16 subjects (50%) showed significant or very significant improvement. In the group of female subjects (Fig. 1, middle panel), 9 out of 11 (81%) showed improvements and, among those who showed improvements, 6 out of 9 (66%) showed moderate to very considerable improvements. In the group of male subjects (Fig. 1, lower panel), 24 out of 33 (73%) showed improvements and, among those who showed improvements, 22 out of 24 (91%) showed moderate to very considerable improvements.

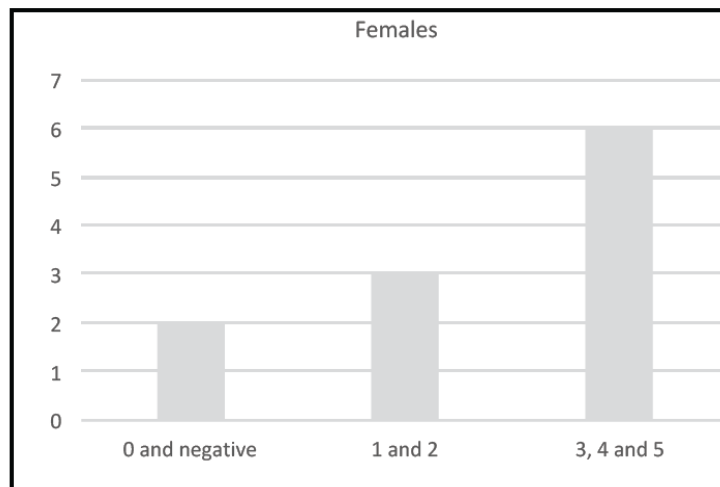
Table 1. Age, gender, body weight and CGI-I score

Subject	Age	Gender	Body weight (Kg)	CGI-I
1	5.5	M	23.0	5
2	5.0	M	23.0	5
3	9.0	M	53.0	5
4	5.0	F	19.0	4
5	5.3	F	15.0	4
6	6.0	M	24.0	4
7	5.5	M	20.0	4
8	4.5	M	15.5	4
9	3.0	M	19.0	4
10	3.8	M	15.0	4
11	6.5	M	20.0	4
12	6.5	M	20.0	4
13	5.0	M	21.0	4
14	3.0	M	14.5	4
15	4.0	M	17.5	4
16	4.0	M	20.0	4
17	14.0	F	40.0	3
18	4.5	F	24.0	3
19	7.5	F	24.0	3
20	6.5	F	30.0	3
21	4.5	M	20.0	3
22	6.0	M	20.0	3
23	6.0	M	16.0	3
24	2.5	M	14.0	3
25	4.5	M	17.0	3
26	5.0	M	24.0	3
27	4.0	M	16.0	3
28	6.5	M	20.0	3
29	3.0	F	15.0	2
30	4.0	F	14.5	2
31	3.0	M	13.0	2
32	4.5	M	21.0	2
33	4.0	F	16.0	1
34	2.5	M	14.0	0
35	3.5	M	11.5	0
36	3.0	M	15.0	0
37	9.0	M	36.0	0
38	3.0	M	14.0	0
39	3.5	M	18.0	0
40	3.0	M	13.5	0
41	3.0	M	16.0	0
42	5.5	F	19.0	-1
43	11.0	M	51.0	-1
44	3.0	F	18.0	-4

Upper panel



Middle panel



Lower panel

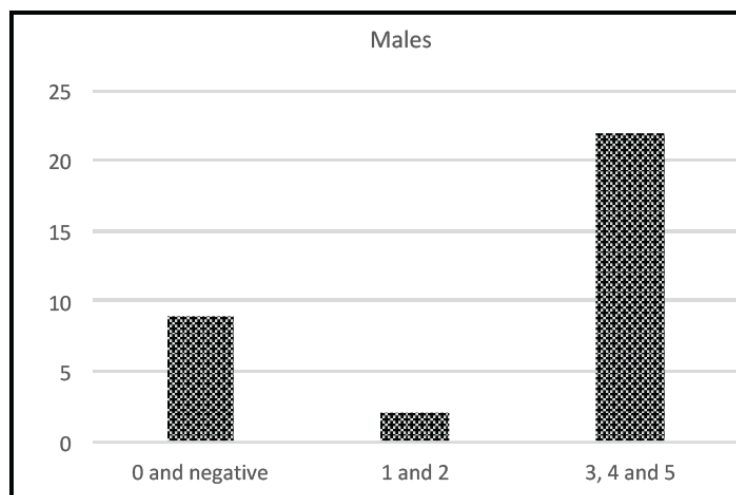


Fig. 1. Clinical Global Impression of Improvement (CGI-I) responses. Y-axis represents the number of subjects in each group. 0 and negative, indicates subjects who showed no improvements or deterioration. 1 and 2 indicates subjects who showed very slight or slight improvements. 3-5 indicates subjects who showed moderate, considerable or very considerable improvements. Upper panel refers to the entire group of subjects; middle panel refers to female subjects; lower panel refers to male subjects

The 3 subjects who showed very significant improvements (CGI-I score 5) belonged to the group of male subjects. In the group of 12 subjects who showed significant improvements (CGI-I score 5), 10 (83%) were males. In the group of those subjects who did not show any improvement 9 out of 11 were males. We could not find association between the degree of improvement and age or body weight.

Discussion

Since this clinical case report is an open-label, non-controlled, retrospective analysis, caution must be employed when ascribing cause and effect to any treatment outcome. However, the response to Rerum[®] was robust with regard to symptomatic improvements as shown by the CGI-I score and we attribute these remarkable results to the effects of the supplement on the Endocannabinoid (EC) system as it relates to neuro-inflammation and neuro-immune alterations. Thus, we recently described the role of the EC system in the pathophysiology of ASD and we highlighted the involvement of the EC system in autism-associated immunological disruptions (Brigida *et al.*, 2017). Preliminary results obtained in our laboratory seem to indicate that Rerum[®] acts on the EC system *in vitro* (manuscript in preparation). This is not surprising considering that the components of Rerum[®] are known to interact with the metabolisms of 2-arachidonoyl glycerol (2-AG), the physiological ligand for both cannabinoid receptors CB1 and CB2 (Sugiura *et al.*, 1999; Gonsiorek *et al.*, 2000). Thus, 2-AG derives from the hydrolysis of 1,2-Diacylglycerol (DAG) (Reisenberg *et al.*, 2012), a second messenger that is formed either as consequence of phospholipase C hydrolysis of inositol lipids or phosphatidylcholine (Michell, 1983; Lacal, 1990), or as consequence of *de novo* synthesis through the glycolytic pathway (Chiarugi *et al.*, 1989). More than thirty years ago, we demonstrated that glycosaminoglycans similar in structure to chondroitin sulfate interact with the metabolism of inositol lipids (Ruggiero *et al.*, 1984) or phosphatidylcholine (Vannucchi *et al.*, 1985), thus leading to DAG and 2-AG endogenous production. Also the other component of Rerum[®], vitamin D, is known to stimulate the formation of DAG through stimulation of inositol lipid metabolism (Doroudi *et al.*, 2012), thus converging on the same EC pathway thanks to the production of 2-AG from DAG.

It is acknowledged that the EC system is a promising therapeutic target in the field of ASD research (Yeh and Levine, 2017) and our observations seem to indicate that Rerum[®], by stimulating the endogenous production of 2-AG, may play a role in this field.

Conclusion

A recent systematic review of the scientific literature states that "the use of dietary supplements in

children with autism seems to be a safe practice with encouraging data about their clinical efficacy" (Gogou and Kolios, 2017). Our observations appear to be consistent with this statement in that they suggest that the use of Rerum[®], a supplement composed of chondroitin sulfate, vitamin D₃ and oleic acid, may be associated with significant improvements of the symptoms of the disease.

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The Authors wish to thank Dr. Heinz Reinwald and Kerstin Reinwald for their contribution to the manufacture of Rerum[®] and for the organization of the Fourth International Congress on Integrative Medicine that was held in Fulda, Germany, on April 1 and 2, 2017.

Author's Contributions

Nicola Antonucci: Performed all the diagnostic and therapeutic procedures described in this study.

Marco Ruggiero and Stefania Pacini: Wrote the first draft of this paper, provided critical input and assisted in revising and improving the paper.

Conflict of Interest

Nicola Antonucci is the founder of the Biomedical Centre for Autism Research and Treatment, a private clinic.

Marco Ruggiero is the inventor of Rerum[®] and consults for the company "dr. reinwald healthcare". He had no prior knowledge of the therapies being used nor of the names of any patient whose data were being analyzed and described in this study. Neither he, nor any employee of company "dr. reinwald healthcare", had any prior knowledge of the clinical records or the patients' names used in this study. Marco Ruggiero is member of the Editorial Board of The American Journal of Immunology and is waived from the Article Processing fee for this contribution; he receives no remuneration for his editorial work.

Stefania Pacini declares no conflict of interest.

Ethics

This article is original and contains material that has not been published in any other scientific journal. Preliminary reports of some of the results described in this study have been presented at the Fourth International Conference on Integrative Medicine (Fulda, Germany, April 2017) and at the AutismOne 2017 Conference (Colorado Springs, USA, May 2017). The corresponding author confirms that the other authors have read and approved the manuscript.

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