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REGULAR ARTICLE

The diuretic bumetanide decreases autistic behaviour in five infants treated during 3 months with no side effects

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ABSTRACT

The inhibitory transmitter GABA has been suggested to play an important role in infantile autistic syndrome (IAS), and extensive investigations suggest that excitatory actions of GABA in neurological disorders are because of a persistent increase of $[Cl^-]_I$.

Aims: To test the effects of the chloride co-transporter NKCC1 diuretic compound Bumetanide that reduces $[Cl^-]_I$ on IAS.

Methods: Bumetanide was administered daily (1 mg daily) during a 3 -month period and clinical and biological tests made. We used 5 standard IAS severity tests - Childhood Autism Rating Scale, Aberrant Behaviour Checklist, Clinical Global Impressions; Repetitive and Restrictive Behaviour and the Regulation Disorder Evaluation Grid.

Results: We report a significant improvement in IAS with no side effects.

Conclusion: Bumetanide decreases autistic behaviour with no side effects suggesting that diuretic agents may exert beneficial effects on IAS and that alterations of the actions of GABA may be efficient in IAS treatment calling for large scale randomized trials.

INTRODUCTION

Infantile autistic syndromes (IAS) is a pervasive developmental disorder characterized by impaired social interaction, deficits in verbal and nonverbal communication and stereotyped interests and behaviours (1) as well as limited interest in the surrounding environment associated with stereotyped movements and repetitive plays (2). Research to date indicates that a genetic predisposition may play a role but one or more environmental factors must be in place for symptoms to occur (3,4). It is suggested that genetic and environmental hazards will alter developmental programmes leading to cortical and/or sub-cortical malformations and the formation of misplaced/misconnected neuronal ensembles. The first symptoms occur before 3 years of age with most likely an earlier origin. There is at present no efficient biological/pharmaceutical treatment to IAS.

Brain maturation is associated with a developmental sequential expression of voltage gated, receptor synapse–driven channels and brain patterns (5,6). The developmental shifts of the actions of the inhibitory transmitter GABA is but one example of these changes. Immature neurons have a higher $(Cl^-)_I$ than adults leading to paradoxical excitatory actions of GABA (6). This is because of an early expression of the co-transporter NKCC1 that imports chloride and a late operation of KCC2 that export chloride form neurons (7). In addition, the regulation of $(Cl^-)_I$ is altered by a variety of insults, lesions, seizures and neurological disorders thereby converting the actions of GABA from inhibitory to excitatory (8,9). Consequently, diuretic agents that reduce $(Cl^-)_I$ constitute novel antiepileptic and neuro-protective agents (10,11) and are currently being tested in large clinical trials in infantile epilepsies (Nemo FP7 EU program and Harvard-based trial).

GABA signalling is profoundly altered in IAS (12,13) and see Discussion). This is reflected by the paradoxical effects of GABA acting benzodiazepines on patients with IAS suggesting dysfunction of the GABA signalling and excessive chloride accumulations in neurons. The specific NKCC1 antagonist Bumetanide (Bum) (14) is a classical diuretic that reduces $(Cl^-)_I$ (15) and shifts GABA from excitation to inhibition. Bum has been extensively utilized in adults since 1975 and in children since 1986 and its pharmacokinetic in adults and children and its side effects are well known (16). Bum is used in acute (oedema following head trauma) and long-term conditions including broncho-pulmonary dysplasia, nephritic syndromes or heart congestions (16) and in neonatal rodent hippocampi (11). The use of Bum is safe provided that it is accompanied with clinical and biological surveillance in children.

We have now tested the effects of Bumetanide in five randomly selected autistic infants with ongoing clinical and biological surveillance. The diuretic was administered (1 mg/24 h – 0.5 mg twice a day) and the treatment continued for 3 months, a minimal duration considered to be sufficient for an evaluation of the effects on IAS. We report a significant improvement of the IAS manifestations in the

children. These observations call for wide-range screening of the use of Bum in IAS.

EXPERIMENTAL PROCEDURES

In June 2009, we obtained the authorization from the ad hoc committee of the hospital of Brest to proceed with a large double blind (60 children) randomized trial in IAS using the diuretic bumetanide starting from December 2009. The trial received the agreement of the CPP (4th of June 2009, number CPP west 6-570) and the national ad hoc committee AFFSAPs (A90936-66 the 4th December 2009). With the consent of the parents, we initiated a pilot study in five IAS children. Because of the striking results obtained, we decided to present them, in order to encourage similar large scale tests by other investigators.

MATERIAL AND METHODS

The material and methods are described in detail in supplemental material (*supl. Acta Pediat Scand*). In brief, children were diagnosed by experienced clinical psychiatrist using strict ICD-10 criteria for autistic disorder. These children had no history of neurological disease (normal EEG) Genetic tests systematically performed were negative indicating no identifiable mutation (Caryotype and fragile X). The ADI-R (17) was collected for all participants to confirm the diagnoses. A clinical and biological examinations showed that none of the infants had a counter indication to bum (including blood ionogram, transaminases, alkaline phosphatases, uraemia, creatinemia, creatinine clearance, γGT, glycemia notably). Because hypokalemia can induce wave burst arrhythmia, an ECG was performed to ensure that none of the patients had a lengthening of the QT because they have a higher propensity to generate arrhythmia. A clinical weekly surveillance was performed during the first, second and third month after treatment onset including blood sodium and potassium 1 week and 2 months after treatment onset. None of the infants had associated neurological disorders, and none was under other treatment since at least 3 months.

To determine the possible therapeutic index efficacy, we relied on five classical behavioural determination of IAS severity (details in supplemental material) including:

- *The Childhood Autism Rating Scale* (CARS) is a 15-item rating scale that is used as a screening instrument and to assess the changes in symptoms of autism over time.(18) and can be used to determine alterations produced by a treatment (19) (DiLalla and Rogers, 1994) and see the French version (20). The notation was obtained during a session when the children were placed in a game and animated discussion with the parents concerning the behaviour of the child during the last week.

- *The ABC* (*Aberrant Behaviour Checklist*) is a questionnaire filled by the treating doctor during a discussion with the parents (21).A French version has been used in this study (22).

- *The Clinical Global Impressions (CGI)* is widely used in the majority of clinical trials to examine disease severity and also on novel generation psychotic agents with little side effects (23).

- *RDEG the regulation disorder Evaluation grid* is a French scale of activity (96) that enables to detect the level of dys-regulation, and the slowness of response of the infants (2).

- *The Repetitive and Restricted Behaviour (RRB)* scale (24) is a 35-item standardized checklist that allows item rating on a 5-point scale from 0–the behaviour is never expressed by the person–to –the behaviour is severely expressed and characteristic of the person). Factorial analysis produces four clinical meaningful factors, i.e. sensori-motor stereotypes (F1), reaction to change (F2), restricted behaviours (F3) and modulation insufficiency (F4).

RESULTS

Our patients were selected with no a priori from a large group of IAS children placed in institutions or at home. A summary of the patients is shown in Table 1. Three boys used a functional language, whereas the remaining boy and girl did not. The scores of ADI-R are above the threshold confirming the clinical diagnosis. Childs 1, 3, 5 follow a traditional school accompanied by an auxiliary person. Child 1 and child 2 are followed by a psychologist using the ABA approach (once a week for child 1 and 3 times a week for child 2). Child 2 has also two weekly orthophonic sessions relying on picture exchange communication system. Child 3 has an orthophonic treatment weekly, and child 5 has no treatment. Child 4 is treated in a medical institution specialised in mentally retarded children. The test of bum was made during the summer vacation, when behavioural therapy and school were interrupted.

The scores of the different scales used before and after 3 months treatment are shown in the Supporting Table S1 (Supporting information). Before the treatment, four children (1–4) had a CARS score above 36 indicating a

Table 1 Summary of patients included in the study. Four girls and one boy aged between 3 years and 8 months to 11 years and 5 months with classical autistic signs (F84.0 of ICD 10)

	Age	Sex	Diagno	ADI1	ADI2	ADI3	ADI4
1	8 years 11 months	M	F84.00	18 (10)	13 (8)	5 (3)	5 (1)
2	3 years 8 months	F	F84.00	20 (10)	8 (7)	4 (3)	5 (1)
3	8 years 7 months	M	F84.00	21 (10)	8 (7)	5 (3)	4 (1)
4	11 years 5 months	M	F84.00	24 (10)	14 (7)	4 (3)	5 (1)
5	10 years 1 months	M	F84.00	17 (10)	20 (8)	4 (3)	3 (1)

severe IAS. Child 5 showed a medium degree of autism. Results show an improvement of the total scores of CARS, ABC, RDEG and RRB for all children 3 months after the treatment. CGI1 was not significantly altered, but this test concerns the severity of the disease that at this stage does not reveal significant changes. We also observed a small global amelioration of CGI2 for the five children. Patients 1, 2 and 5 had an index of 3 in CGI3 indicating a moderate action with no side effects. Patients 3 and 4 had an index of 2 indicating a minimal action with no side effects. The number of items equal or above 3 with CARS was reduced by the treatment in the five children. The sub-score of ABC5, was not altered by the treatment. In the five children, ABC2, ABC3, ABC4, RDEG dys-regulation, RDEG slowness, RRBF4, RRB F1 were ameliorated to a variable degree. In contrast, the results of ABC1, RRB F2, RRB F3 are heterogeneous.

The average scores from grouped data for the four major tests (total RRB, total ABC, total CARS and total RDEG) are statistically significant only in evolution score of total RRB (55 ± 4.7 in control vs 33.4 ± 2.5 in bum treatment, $p < 0.05$). However, all these scores are statistically significant in children 1, 2 and 3 [total CARS: 39 ± 0.8 in control and 31.3 ± 2.4 in bum ($p < 0.05$); total ABC: 84 ± 2.9 in control vs 56 ± 3.8 in bum ($p < 0.05$); total RRB: 61 ± 5.3 in control vs 29.7 ± 1.9 in bum ($p < 0.01$); total RDEG: 49.3 ± 6.1 in control vs 37.7 ± 5.8 in bum ($p < 0.05$)]. Therefore, bumetanide may be more effective in younger patients (also see Discussion).

Starting 1 week after the treatment and once monthly, a clinical surveillance was made including research of deshydratation, orthostatic hypotension, hyper-sensitivity, cramps, asthenia, diarrhoeas, myalgia, arthralgia, nausea, dizziness. The levels of sodium and potassium remained stable (tests made a week and 2 months after the beginning of the treatment). No adverse effect was found.

DISCUSSION

Present results suggest that bumetanide ameliorates behavioural aspects of IAS, suggesting that the diuretic has a global action. To the best of our knowledge, this is the first report raising the possibility of chloride alterations in autism.

Extensive observations suggest that GABA signalling play a role in IAS including specific reduction of GABAergic receptor systems, reduction of ^{3}[H] flunitrazepam and muscimol biding (ibid), reduced levels of glutamic acid decarboxylase (reviewed in 25). Genetic forms of autism include deletions of the proximal long arm of chromosome 15 where 3 GABA receptor subunit genes are located (26). The dynamic alterations of $(Cl^{-})_I$ and shifts of GABA from inhibitory to excitatory (10,11) after seizures and other insults provide the conceptual basis for this study. Also, patients treated with oxytocin–that alters $(Cl^{-})_I$ (27)–exhibit more appropriate social behaviour and affect (28).

The conclusions derived form our observations are hampered by the lack of randomized double blind and placebo investigations that are more prevalent in children than adults (29). Clearly, wide-scale investigations are needed to confirm or infirm the observations. Nevertheless, we were encouraged to present our observations because bumetanide has no side effects, the dramatic behavioural amelioration and the insistence of the parents that their children are more present and their wish to pursue the treatment. Interestingly, the same term of presence was used by all parents.

The patients included in this study are quite heterogeneous in age, in the severity of the disease and age of detection. It is interesting that patients 4–5 were the least responder to the treatment. These are older children, and patient 5 goes to conventional school with clearly less severe IAS, whereas patient 4 is severely affected and hospitalized in a specialized institution. Therefore, positive effects of the diuretic may prevail in certain types of IAS but this will require large trails such as the one presently being performed.

It is not possible at present to determine whether bumetanide exerts a preferential action on one aspect of the symptomatology. The lack of effects of bum on ABC5–inappropriate, excessive speech out of context–is expected because amelioration in 3 months of speech is unlikely to occur. In contrast, almost all scores were ameliorated to variable degrees stressing the general action of the diuretic. Bumetanide provided a better cognitive regulation in keeping with the improved presence reported by the parents. The results of the subscales of ABC suggest an amelioration of states of vigilance and social interactions, stereotypic movements and hyperactivity again in keeping with the notion of cognitive regulation. A wide range of experimental investigations suggest that bum reduces seizure severity (11). Bumetanide is currently being investigated as a novel treatment for neonatal seizures (EU FP7 Nemo project).

Our observations are compatible with the concept that neurons who fail to respect the developmental programme keep immature features, including possibly high $(Cl^{-})_I$ and other electrical and architectural properties (30).

In conclusion, an emerging series of studies suggest that chloride accumulates during brain maturation in relation to various developmental malformations. Present observations suggest that a conventional diuretic that reduces this accumulation and acts to reinstate the inhibitory actions of GABA exerts beneficial actions in autism calling for more detailed experimental and clinical studies on the links between GABA/$(Cl^{-})_I$ and IAS.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1 Summary scores of the effects of bumetanide in the five patients (C = before and Bum = 3 months after bumetanide).

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