Short communication

Oral treatment with desipramine improves breathing and life span in Rett syndrome mouse model

Sébastien Zanella a, Saida Mebarek a, Anne-Marie Lajard a, Nathalie Picard a, Mathias Dutschmann b, Gérard Hilaire a,∗

a MP3-Respiration, UMR CNRS 6153, 280 Boulevard Sainte Marguerite, 13009 Marseille, France
b DFG Research Center Molecular Physiology of the Brain (CMPB), Humboldtalle 23, 37073 Göttingen, Germany

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Abstract

Rett syndrome is a neurodevelopmental disease due to Mecp2 gene mutations that is associated to complex neurological symptoms, with bioaminergic deficits and life-threatening apneas related to sudden and unexpected death. In male mice, Mecp2-deficiency similarly induces medullary bioaminergic deficits, severe apneas and short life span. Here, we show that long-term oral treatment of Mecp2-deficient male mice with desipramine, an old drug of clinical use known to block norepinephrine uptake and to strengthen its synaptic effects, significantly alleviates their breathing symptoms and prolongs their life span. Although these mouse results identify desipramine as the first oral pharmacological treatment potentially able to alleviate breathing symptoms of Rett syndrome, we recommend further studies of desipramine effects in Mecp2-deficient mice before attempting any clinical trials in Rett patients.

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1. Introduction

Rett syndrome (RS) is a severe neurodevelopmental disorder caused by mutations in the X-linked gene that encodes methyl-CpG-binding protein 2 (MeCP2), which is involved in gene silencing (Guy et al., 2001). In RS, the prenatal period and the first postnatal months appear normal but various symptoms develop in infants 6–18 months old, including the regression of acquisitions, various behavioral symptoms and breathing deficits with an erratic respiratory rhythm and life-threatening apneas. No treatments are available for the neurological symptoms in RS and life span is short (Laurvick et al., 2006). Autopsies reveal bioaminergic deficits with reduced norepinephrine (NE) contents (Brucke et al., 1987; Lekman et al., 1989).

In a mouse line of hemizygous Mecp2-deficient mice, males (Mecp2-/y) postnatally develop a stiff, uncoordinated gait, reduced body movements and breathing deficits (Guy et al., 2001; Viemari et al., 2005; Bissonette and Hilaire, 2007; Stettner et al., 2007). Although normal until postnatal day 30 (P30), Mecp2-/y breathing becomes erratic, with frequent and long-lasting apneas, and fatal respiratory distress occurring around P60. Mecp2-/y breathing symptoms are preceded by bioaminergic deficits (Ide et al., 2005; Viemari et al., 2005), with a reduction in the NE medullary concentration and a loss of NE medullary neurons. Because application of NE to medullary slices stabilizes the in vitro respiratory-like activity produced by the isolated respiratory network of Mecp2-/y (Viemari et al., 2005), and because NE released from medullary neurons modulates the respiratory-like activity of wild type (WT) mice (Zanella et al., 2006), we raised the working hypothesis that the NE functional deficits of Mecp2-/y contribute to their pathophysiological breathing patterns. Subsequently, pharmacological treatments aimed at compensating for NE functional deficits could alleviate breathing symptoms of Mecp2-/y and probably RS patients. In this study, we used a treatment with desipramine (DMI), an old drug of clinical use that blocks NE neuronal uptake and potentiates NE effects at the synaptic cleft. However, it has been shown that DMI effects are different in stressed and control conditions (Naitoh and Nomura, 1990).
Thus, we added DMI in drinking water to avoid daily intraperitoneal injections which are stressful, especially in Mecp2-/y that have increased anxiety-like behavior and abnormal stress response (Nuber et al., 2005; McGill et al., 2006). In addition, extreme anxiety and agitation are parts of RS and anything that adds to stress levels such as daily intraperitoneal injections could actually hurt the Rett patients. We compared the breathing pattern, life span and NE medullary concentrations of two groups of Mecp2-/y, a control, untreated group vs. a treated group receiving long-term oral treatment with DMI. Here we show that DMI oral treatment of Mecp2-/y improves breathing, prolongs life span but reduces NE medullary concentrations.

2. Methods

Experiments were carried out in accordance with the European guidelines for the care and use of laboratory animals (Council Directive 86/6009/EEC) in mouse model for Rett Syndrome [strain B6.129P2(C)-Mecp2tm1–1Bird] (The Jackson Laboratory, Bar Harbor, ME). Hemizygous mutant Mecp2 males were generated by crossing heterozygous knock-out females with WT C57BL/6 males (Guy et al., 2001). As reported previously (Viemari et al., 2005), we only studied hemizygous Mecp2 and WT males. Although RS mainly affects female patients, most mouse researches are performed in males because the Mecp2 gene is X-linked, and females will thus have a different amount of normally Mecp2-expressing cells depending on their X-chromosome inactivation profile. Because this produces an unpredictable and heterogeneous phenotype in female mutants, we only used Mecp2-/y. Genotyping was performed by routine PCR technique according to The Jackson Laboratory protocols.

After weaning at P21, three or four WT and Mecp2-/y littermates were tagged and housed together in identical cages where they had free access to food and water. From P30, half of the cages had DMI in drinking water (0.25 mg/ml) (Sigma; St. Quentin, France). Every 3 days, mice were weighted, DMI solution was renewed and water consumption was checked. Adding DMI to drinking water did not significantly affect water consumption (3.0 ± 0.1 and 2.8 ± 0.1 ml/day for untreated and treated Mecp2-/y; 4.2 ± 0.1 and 4.0 ± 0.1 ml/day for untreated and treated WT). Forty-one male littermates were studied (8 treated and 8 untreated WT, 14 treated and 11 untreated Mecp2-/y) to compare the evolution of breathing parameters from P30 (start of DMI treatment) until P86. Mice were habituated to stay in the plethysmograph chamber for 2 h prior to P30 to reduce stress effect on breathing.

Methods have been previously reported in details (Viemari et al., 2005). Breathing was recorded every week using a constant flow whole-body plethysmograph (EMKA Technologies, Paris, France), with 200 ml animal chambers maintained 25 ± 0.5 °C. Only periods of quiet breathing were analyzed to measure the respiratory frequency (cycles/min), the number of apneas per minute > two normal respiratory cycles and the irregularity score (variability in duration of respiratory cycles). In four treated and five untreated Mecp2-/y that died at around P70(73 ± 3 days), we achieved the brainstem dissection within a post-mortem delay of about 1 h. The pons and medulla were kept at −80 °C until measurements of NE, serotonin (5HT) and its metabolite 5-hydroxy-indol acid acetic (5HIAA) concentrations (given in nM/mg of tissue) with high pressure liquid chromatography (HPLC) (Waters System: pump P510, electrochemical detector EC2465; Atlantis column DC18; mobile phase: citric acid, 50 mM; orthophosphoric acid, 60 mM; sodium octane sulfonic acid, 0.112 mM; EDTA: 0.06 mM, methanol; pH 3.01).

For statistical analysis, the first step was to determine the normality and the equal variance of the data. The DMI effects on respiratory frequency, irregularity score, number of apneas per minute, body and brain weights and bioamine concentrations were analyzed by a two-way ANOVA for repeated measurements in the same subjects. This was followed by a multiple-comparisons procedure using the Holm–Sidak method. The survival of Mecp2-/y was analyzed using the Kaplan–Meier survival curve estimation (Laurvick et al., 2006). Values are given as mean ± S.E.M.

3. Results

After weaning, three to four WT and Mecp2-/y male littermates were housed in identical cages but from P30, half of the cages received DMI in drinking water (treated mice).

3.1. DMI oral treatment of Mecp2-/y improved breathing and prolonged life span

The breathing of untreated and treated Mecp2-/y was weekly recorded with whole body plethysmography. The evaluation of the variability of the duration of respiratory cycles and the count of apneas per minute revealed that DMI oral treatment significantly delayed the evolution of breathing symptoms and death in 14 treated Mecp2-/y compared to 11 untreated Mecp2-/y littermates (Table 1). Prior to DMI oral treatment at P30, breathing patterns were similar in both groups of Mecp2-/y (Fig. 1a and b) with a respiratory frequency above 200 cycles/min, only few apneas and a stable duration of respiratory cycles. With progressing age, apneas became frequent, the variability of respiratory cycles increased, and respiratory distress developed in both groups of Mecp2-/y: one untreated and one treated Mecp2-/y died at P36 and P45, respectively.

At P51, both untreated and treated Mecp2-/y had marked breathing symptoms, but apneas were significantly less frequent (Fig. 1i) and the duration of respiratory cycles was less variable in treated (Fig. 1c) than untreated Mecp2-/y (Fig. 1d). Until P65, 4/11 (36.4%) untreated Mecp2-/y died while only 2/14 (14.3%) treated Mecp2-/y died. At P65, both groups had an obviously reduced respiratory frequency (Fig. 1h) that probably reflected habituation to the plethysmograph chamber and/or increase in age since a similar reduction occurred in untreated and treated WT (Table 2). Untreated Mecp2-/y showed an erratic breathing, with significantly more frequent apneas and a highly variable duration of respiratory cycles (Fig. 1e) than treated Mecp2-/y (Fig. 1f).

Finally, by P86, all the 11 untreated Mecp2-/y had died while 5 out of the 14 treated Mecp2-/y were still alive (one treated Mecp2-/y survived until P166). Thus, as shown in Fig. 2a, DMI
oral treatment significantly lengthened life span of Mecp2-/y (67 ± 4 days vs. 90 ± 8 days for untreated and treated Mecp2-/y; *p = 0.007). At P86, the five treated survivors had marked breathing symptoms (Fig. 1g), but their number of apneas and variability in duration of respiratory cycles were similar to those seen in treated Mecp2-/y at P65. Furthermore, the number of apneas and variability in duration of respiratory cycles of treated Mecp2-/y at P86 were similar to those of untreated Mecp2-/y at P51 (Fig. 1h and j). Thus, 8 weeks of DMI oral treatment significantly delayed the progression of breathing symptoms in Mecp2-/y. Interestingly in WT, DMI oral treatment had no significant effects on breathing since number of apneas, variability in duration of respiratory cycles and respiratory frequency were similar in untreated and treated WT at a given age (Table 2).

3.2. DMI oral treatment of Mecp2-/y reduced their NE medullary concentration and did not prevent failure to thrive, microcephaly and reduced locomotion

In addition to breathing deficits, several symptoms develop in Mecp2-/y, such as alterations of bioaminergic systems, failure to thrive, microcephaly, reduction of motor activity, etc. (Guy et al., 2001, 2007; Ide et al., 2005; Viemari et al., 2005).

We used HPLC to measure the concentration of bioamines in the brainstem of five untreated and four treated Mecp2-/y that died at around P70 (73 ± 3 days) and where we achieved to dissect the pons and the medulla within a postmortem delay ranging 1h. In the pons, DMI oral treatment did not significantly affect concentrations of NE (19.5 ± 2.5 and 15.9 ± 2.7 nM/mg, for untreated and treated Mecp2-/y, respectively), 5HT (1.8 ± 0.1 and 1.7 ± 0.2 nM/mg) and 5HIAA (3.3 ± 0.4 and 3.6 ± 0.7 nM/mg). In the medulla, however, DMI significantly decreased NE concentrations by around 20% (11.4 ± 0.7 and 9.1 ± 0.6 nM/mg, for untreated and treated Mecp2-/y; *p = 0.04) but did not affect 5HT (3.3 ± 0.3 and 3.6 ± 0.4 nM/mg) and 5HIAA concentrations (4.0 ± 0.3 and 4.2 ± 0.6 nM/mg).

DMI oral treatment did not affect body weight curves of Mecp2-/y and WT (Fig. 2b) and did not prevent microcephaly in Mecp2-/y. At P70, Mecp2-/y showed failure to thrive (body weight: 15.2 ± 0.6 g vs. 25.7 ± 0.5 g for 14 Mecp2-/y and 14 WT; *p < 0.001), microcephaly (total CNS weight: 392 ± 6 mg vs. 521 ± 14 mg, for 14 Mecp2-/y and 14 WT; *p < 0.001; pons: 78 ± 3 mg vs. 108 ± 5 mg; *p < 0.001; medulla: 37 ± 2 mg and 54 ± 5 mg; *p < 0.001; for 9 Mecp2-/y and 14 WT). Although we did not analyze the locomotor activity of Mecp2-/y, the visual observation of mice within their cages revealed a reduced activity in both untreated and treated Mecp2-/y, without any obvious improvement in treated Mecp2-/y.

4. Discussion

5HT neurons from the brainstem, NE neurons from A6 and A5 pontine groups and NE neurons from A2/C2 and A1/C1
**Table 1**
DMI oral treatment from P30 delays the evolution of breathing symptoms in Mecp2-/-

<table>
<thead>
<tr>
<th>Postnatal Age</th>
<th>Number of Mecp2-/- survivors</th>
<th>Respiratory Frequency (cycles/min)</th>
<th>Number of apneas per minute &gt; two respiratory cycles</th>
<th>Variability of duration of respiratory cycles (irregularity score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Mecp2-/-</td>
<td>Treated Mecp2-/-</td>
<td>Untreated vs. Treated</td>
<td>Untreated Mecp2-/-</td>
</tr>
<tr>
<td>P30</td>
<td>11</td>
<td>14</td>
<td>246 ± 7</td>
<td>ns (p = 0.851)</td>
</tr>
<tr>
<td>P30 vs. P51</td>
<td>10</td>
<td>13</td>
<td>196 ± 8</td>
<td>ns (p = 0.814)</td>
</tr>
<tr>
<td>P51 vs. P65</td>
<td>7</td>
<td>12</td>
<td>141 ± 11</td>
<td>ns (p = 0.867)</td>
</tr>
<tr>
<td>P65 vs. P86</td>
<td>0</td>
<td>5</td>
<td>No survivors</td>
<td>–</td>
</tr>
<tr>
<td>P86</td>
<td>0</td>
<td>5</td>
<td>No survivors</td>
<td>–</td>
</tr>
</tbody>
</table>

Breathing parameters of treated and untreated Mecp2-/- at postnatal ages of 30, 51, 65 and 86 days (P30, P51, P65 and P86, respectively). Values are given as mean ± S.E.M. Two-way ANOVA; ns: non significant difference; *, **, ***: significant difference, respectively at p < 0.05, p < 0.01 and p < 0.001.

**Table 2**
DMI oral treatment from P30 did not affect breathing of WT mice

<table>
<thead>
<tr>
<th>Postnatal Age</th>
<th>Number of WT</th>
<th>Respiratory Frequency (cycles/min)</th>
<th>Number of apneas per minute &gt; two respiratory cycles</th>
<th>Variability of duration of respiratory cycles (irregularity score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated WT</td>
<td>Treated WT</td>
<td>Untreated vs. Treated</td>
<td>Treated WT</td>
</tr>
<tr>
<td>P30</td>
<td>8</td>
<td>8</td>
<td>234 ± 6</td>
<td>ns (p = 0.601)</td>
</tr>
<tr>
<td>P30 vs. P65</td>
<td>8</td>
<td>8</td>
<td>177 ± 6</td>
<td>ns (p = 0.999)</td>
</tr>
<tr>
<td>P65 vs. P86</td>
<td>8</td>
<td>8</td>
<td>180 ± 6</td>
<td>ns (p = 0.333)</td>
</tr>
<tr>
<td>P86</td>
<td>8</td>
<td>8</td>
<td>No survivors</td>
<td>–</td>
</tr>
</tbody>
</table>

Breathing parameters of treated and untreated WT at P30, P51, P65 and P86. Values are given as mean ± S.E.M. Two-way ANOVA; ns: non significant difference; *, **, ***: significant difference, respectively at p < 0.05, p < 0.01 and p < 0.001.
medullary groups exert a complex modulation on the respiratory network activity (Bou-Flores et al., 2000; Burnet et al., 2001; Hilaire, 2006; Zanella et al., 2006; Bissonette and Hilaire, 2007). We hypothesized that Mecp2-/- breathing deficits originate from bioaminergic deficits that develop from P30 to death and that compensating for the latter could alleviate the former (Ide et al., 2005; Viemari et al., 2005; Roux et al., 2007). DMI is an old and cheap tricyclic antidepressant of clinical use that potentiates NE effects at the synaptic cleft. Here we show that DMI oral treatment of Mecp2-/- improves their breathing and life span. In this view, adding NE agents to DMI might involve α1 (Subhash et al., 2003), α2 (Invernizzi and Garattini, 2004) and β adrenoceptors density (Goodnough and Baker, 1994), the expression of NE transporter, NE biosynthesis enzyme and mRNA (Zhu et al., 2002, 2005). DMI also affects non-NE systems, such as the 5HT (Goodnough and Baker, 1994) and BDNF systems (Khundakar 2002, 2005). DMI also affects non-NE systems, such as the 5HT receptors density (Goodnough and Baker, 1994), the expression of MECP2 in the A6 pontine group, the main source of NE to the brain, and its upper targets. However, little is known about DMI effects on the medulla and, to the best of our knowledge, this may be the first report of a reduction of NE medullary concentration after DMI treatment. In pons and brain, DMI affects α2 adrenoceptors to DMI treatment could be more efficient than treatment with DMI alone. We hypothesized that Mecp2-/- breathing symptoms in Mecp2-/- are due to alteration of NE mechanisms modulating the respiratory rhythmogenesis. However, the pulmonary regulation of breathing is altered in Mecp2-/- (Stettner et al., 2007) and probably the hypoxic regulation (Bissonette and Hilaire, 2007). Whether DMI compensates for NE functional deficits affecting the intrinsic respiratory rhythmogenic mechanisms and/or those affecting respiratory regulations by peripheral inputs is an open question.

Finally, safety of DMI treatment in RS patients must be thoroughly questioned. Even though DMI has been extensively used as tricyclic antidepressant, DMI at therapeutic doses may increase the risk of sudden death (Amitai and Frischer, 2006) and DMI effects depend on genetic backgrounds (Lahmame and Armario, 1996) and stress conditions (Naitoh and Nomura, 1990). This has to be taken into account in RS infants with genetic anomalies and extreme anxiety.

To conclude, although our results identify DMI as the first oral pharmacological treatment able to alleviate breathing symptoms in RS infants, further mouse experiments are required to analyze, understand and improve DMI efficiency prior to attempting any clinical trials in RS patients.
in MeCP2-γ, and possibly RS patients, we do recommend further mouse experiments before attempting any clinical trials in RS patients because of the complexity of DMI effects, the poorly-known interactions between the different brainstem bioaminergic groups and the complex modulations the brainstem bioaminergic groups exert on respiratory and non-respiratory systems (cardiovascular regulation, sleep, mood, etc.).

Acknowledgments

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References


