Side Effects of Minocycline Treatment in Patients With Fragile X Syndrome and Exploration of Outcome Measures

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Abstract

Minocycline can rescue the dendritic spine and synaptic structural abnormalities in the fragile X knock-out mouse. This is a review and preliminary survey to document side effects and potential outcome measures for minocycline use in the treatment of individuals with fragile X syndrome. We surveyed 50 patients with fragile X syndrome who received minocycline for at least 2 weeks and found that the most common reported side effect is gastrointestinal difficulty, including loss of appetite. The families reported an improvement in language and behavioral areas. Outcome measures in the design of future randomized clinical trials should include both behavioral and language measures. As with any other treatments, we emphasize that randomized clinical trials are needed to determine the efficacy of minocycline in fragile X syndrome.

Fragile X syndrome is the most common known inherited form of intellectual disability, with an estimated prevalence of 1 in approximately 2,633 (Fernandez-Carvajal et al., 2009), although the allele frequency of the full mutation may be as high as 1 in about 2,500 (P. Hagerman, 2008). This syndrome is caused by a CGG repeat expansion located in 5' untranslated region of the *FMR1* gene (Verkerk et al., 1991). Normally, the repeat size is 5 to 40 repeats, whereas premutation alleles have 55 to 200 repeats and full mutation alleles, more than 200 CGG repeats (R. Hagerman, 2006). Due to the expansion in the full mutation, the CGG repeats and the surrounding promoter region of the *FMR1* gene is usually methylated, inhibiting *FMR1* transcription and causing absence or deficiency of the fragile X mental retardation 1 protein–FMRP (Oostra & Willemsen, 2003). This disorder is associated with intellectual disability, learning disabilities, and a

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variety of behavioral problems, including attention-deficit/hyperactivity disorder (ADHD), anxiety, social deficits, and autism spectrum disorders (R. Hagerman, Rivera, & Hagerman, 2008).

Advances in understanding the neurobiology of fragile X syndrome have led to new targeted treatments. The most remarkable are the metabotropic glutamate receptor 5 (mGluR5) antagonists. FMRP inhibits the translation of proteins needed to enhance long-term depression. Without FMRP, long-term depression is exaggerated and is associated with several anatomical and neurophysiological parameters, including increased dendritic arborization and weak synaptic connections (Bear, Dolen, Osterweil, & Nagarajan, 2008; Bear, Huber, & Warren, 2004). The mGluR5 antagonists have been studied in animal models of fragile X and have been shown to have a beneficial effect on seizures, cognition, and behavior in the *FMR1* knock-out mice (de Vrij et al., 2008), as well as behavior, life span, and brain structure in Drosophila model of fragile X (McBride et al., 2005). Fenobam, a mGluR5 antagonist shown to be effective in the animal model, has just recently been studied in 12 human subjects with fragile X syndrome. A single dose trial demonstrated improvements in behavior and in frontal gating as measured by prepulse inhibition (Berry-Kravis et al., 2009).

Other aspects of central nervous system function that are dysregulated by the lack of FMRP in fragile X syndrome include the down-regulation of GABA receptors (D'Hulst et al., 2009; D'Hulst & Kooy, 2007; Kooy, 2003), so that $GABA_A$ agonists will likely be targeted treatments for fragile X syndrome. FMRP usually inhibits the translation of many other messages that are important for synaptic plasticity leading to the immature dendritic spines in fragile X syndrome (Bassell & Warren, 2008). There is upregulation of many proteins in the absence of FMRP (Qin, Kang, Burlin, Jiang, & Smith, 2005); one of these is matrix metalloproteinase-9 (MMP-9). First described in cancer research and immunology, matrix metalloproteinases (MMPs) play a role in the extracellular degradation of proteins (Sternlicht & Werb, 2001). It is likely that the increase in MMP-9 levels in fragile X syndrome is triggered by the glutamatergic pathway (Dansie, Bilousova, Ethell, & Ethell, 2009). The high level of MMP-9 activity is hypothesized to be one mechanism for the impaired dendritic spine maturation in fragile X syndrome. Minocycline

inhibits the activity of MMP-9, thus promoting the formation of mature dendritic spines in *Fmr1* knock-out hippocampal neurons in cultures and in vivo in *Fmr1* knock-out mice (Bilousova et al., 2009). Treatment of newborn *Fmr1* knock-out mice with 3 weeks of minocycline rescued the dendritic spine deficits and improved anxiety in the elevated plus maze and enhanced strategic exploratory behavior in the Y maze compared to untreated *Fmr1* knock-out mice (Bilousova et al., 2009).

Minocycline, a second-generation semi-synthetic tetracycline derivative, is one of the most widely used antibiotic treatments for acne vulgaris in adolescence. First introduced in 1967, it is generally well-tolerated (Jonas, 1982; Smith & Leyden, 2005). The second generation agents, including minocycline, are superior compared to first generation tetracyclines because of excellent bioavailability, long half life (allowing once- or twice-daily dosing), high lipid solubility (resulting in excellent tissue penetration), hepatic excretion, low resistance potential, and higher penetration in cerebrospinal fluid (Shetty, 2002).

Studies in animal models suggest that minocycline is not only useful as an antibiotic but also may have potential as a neuroprotective agent. It is associated with several mechanisms of action in neuroprotection, anti-inflammatory and anti-apoptotic effects, and protease inhibition (Elewa, Hilali, Hess, Machado, & Fagan, 2006). Minocycline has been shown to improve several neuro-degenerative diseases in animal models, including amyotrophic lateral sclerosis—ALS (Kriz, Nguyn, & Julien, 2002), Huntington's disease (Bantubungi et al., 2005), Alzheimer's disease (Choi et al., 2007; Noble et al., 2009), stroke (Liu et al., 2007), traumatic brain injury (Lee et al., 2003), and spinal cord injury (Stirling et al., 2004; Well, Hulbert, Fehling, & Yong, 2003). However, a recent clinical trial of minocycline in patients with ALS failed to show the expected neuroprotective effect (Gordon et al., 2008).

Minocycline affects nonneuronal cells, tissues, and neurons. Studies of minocycline in neurons in-vitro and in-vivo revealed a direct effect on AMPA receptors (Imbesi, Uz, Manev, Sharma, & Manev, 2008), which are part of the glutamatergic pathway. This pathway guides the synaptic plasticity and dendritic arborization (Wang et al., 2005). Excessive glutamatergic activity can cause neurotoxicity in the neurons. Usually, microglia and astrocytes take up excess

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glutamate to protect neurons from toxicity via the excitatory amino acid transporters (Bergles & Jahr, 1998; Danbolt, 2001). Although aspects of the complex mechanisms of action of minocycline are still unclear, recent studies indicate that minocycline interacts with MAP kinases and several other signaling pathways (Nikodemova, Duncan, & Watters, 2006).

Although minocycline is prescribed widely, there are adverse effects, including staining of the teeth and oral cavity (Cascio et al., 2004; LaPorta, Nikitakis, Sindler, & Reynolds, 2005; Sanchez, Rogers, & Sheridan, 2004), a lupus-like syndrome (Akin, Miller, & Tucker, 1998; Lawson, Amos, Bulgen, & Williams, 2001; Porter & Harrison, 2003; Schlienger, Bircher, & Meier 2000; Tournigand et al., 1999), gastrointestinal disturbance (Shetty 2002), autoimmune hepatitis (Abe et al., 2003; Teitelbaum et al., 1998), pigmentation in the skin and other tissue (Eisen & Hakim, 1998), photosensitivity (Smith & Leyden 2005), and pseudotumor cerebri (Ang, Zimmerman, & Malkin, 2002).

The biggest concern of parents and physicians regarding minocycline treatment is staining of the teeth, especially in children because the majority of the mineralization of the permanent teeth is not complete until 8 years old. In a study in children less than 8 years old treated with minocycline in the short term (less then 3 weeks), Cascio et al. (2004) found no significant differences in dental staining and enamel defect between subjects and controls. Nevertheless, in some case reports investigators documented that minocycline might cause staining of adult dentition in 3 to 6% of patients taking long-term minocycline, more than 100 mg daily (Tredwin, Scully, & Bagan-Sebastian, 2005). The mechanism of tooth discoloration caused by minocycline is still controversial. The iron theory is based on the fact that minocycline is well-absorbed in the gastrointestinal tract, and it chelates with hemosiderin, a breakdown product of iron, to form insoluble complexes that lead to tooth staining. The extrinsic theory is related to the excretion of minocycline in high concentration in gingival fluid, where it can stain the enamel by diffusing through the pulp or by affecting odontogenesis. Intrinsic theorists have hypothesized that as minocycline is absorbed, it is bound to plasma proteins and distributed to various tissues in the body (Good & Hussey, 2003; Tredwin et al., 2005).

A rare side effect is minocycline-induced lupus, a serious autoimmune disorder. El-Hallak et al. (2008) reported that .05% of all children referred to the clinic had apparent autoimmunity sequalae related to minocycline use. A retrospective cohort study in the United Kingdom of individuals with acne who were 15 to 35 years of age demonstrated that the hazard ratio for the association of minocycline and lupus erythematosus was 3.11 (1.77 to 5.48) (threefold increased risk to developing lupus erythematosus). The risk increases after 300 days from the first exposure or total dose of more than 50,000 mg of minocycline (Margolis, Hoffstad, & Bilker, 2007). The mechanism of this problem is unclear, but there is a strong relationship between duration of the exposure of minocycline and lupus erythematosus. Several researchers have tried to explain the pathophysiologic mechanism of minocycline-induced lupus, including an immune response to the drug/metabolite, interaction of the drug with nuclear antigens to increase the immunogenicity of nucleic acids, and genetic susceptibility (Elkayam et al., 1998; Shepherd, 2002). Recent data show that minocycline can protect cells from apoptosis by inhibition of caspase-dependent and independent cell death pathways. The protection against apoptosis by minocycline may be incomplete, leading to failed apoptosis of damaged cells. If the apoptosis pathway is not complete, opsonization and subsequent digestion of nuclear material will be insufficient or may not happen at all. In genetically susceptible individuals, exposure of partially digested material may lead to immunization to nucleosomal material, which is highly immunogenic and may be responsible for autoimmunity, particularly with long-term use of minocycline (van Steensel, 2004).

Minocycline is available by prescription, unlike the mGluR5 antagonists that are experimental medications. Because of the availability of minocycline, there has been an intense interest in and initiation of this treatment in children with fragile X syndrome after publication of Bilousova et al. (2009). In the present study we surveyed 50 patients with fragile X syndrome who were treated with minocycline and are being followed by one of the authors. Our main purpose was to review and document the side effects of minocycline use in patients with fragile X syndrome; our secondary aim was to report on the parent's impression after minocycline treatment. Because this is a pilot survey study, an assessment of the efficacy of minocycline is clearly not possible. Instead, this

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preliminary information is important to guide future controlled treatment studies of minocycline in treatment of individuals with fragile X syndrome.

Method

The subject population was 50 children and adults with fragile X syndrome who had been treated clinically with minocycline by either their primary careprovider or by one of the authors and who are being followed at the Fragile X Research and Treatment Center, Medical Investigation of Neurodevelopmental Disorders (M.I.N.D.) Institute. We surveyed the parents using a questionnaire to assess their child's response to minocycline. We asked them to report any side effects of minocycline treatment using a list of all reported associated problems in addition to an open-ended question about perceived problems. The questionnaire, which was administered either over the phone or in the clinic, utilizes a Likert scale to measure changes (outcomes) in different domains: Language, Academic Abilities, Attention, Behavior, Physical Features, and Side Effects after taking minocycline. The range on the Likert scale is 1 (severely worse), 2 (mildly worse), 3 (no change), 4 (somewhat better), and 5 (very much better). This questionnaire was carried out with the families of patients who have had treatment for at least 2 weeks of minocycline. All medical records were reviewed, including DNA status, autism assessment, duration and dose of minocycline, and concurrent medications. The study was approved by our Institutional Review Board.

Statistical Analysis

Our analysis objective is to describe and summarize drug safety and parent's impression of minocycline's treatment for fragile X syndrome. We used descriptive statistical analysis to summarize each symptom category. Associations between adverse events with age and adverse events with duration on minocycline were based on logistic regression analysis.

Results

Patient Characteristics and Minocyline Treatment

We surveyed 53 patients with fragile X syndrome who received minocycline and summa-

rized results based on all 50 subjects who were on minocyline for at least 2 weeks. Of the 53 patients taking minocycline for only 1 to 4 days, 3 developed intolerable side effects. Fifty patients, 43 males (86%) and 7 females were evaluated. Their mean age was 13.3 years ($SD = 6.2$). Their minimum age was 0.3 years and the maximum was 25 years. The molecular studies demonstrated that 39 patients (78%) had a full mutation allele, 9 patients (18%) had mosaic alleles (premutation and full mutation), and 2 patients (4%) had a premutation allele with features of fragile X syndrome because of lowered FMRP. Autism and pervasive developmental disorder-not otherwise specified were the diagnoses for 12 (24%) and 14 (28%) cases, respectively, after a team evaluation and use of standardized measures, including the Autism Diagnostic Observation Schedule (Lord et al., 2000) and/or the Autism Diagnostic Interview-Revised (Lord et al., 1994) in addition to the *Diagnostic and Statistical Manual–DSM-IV-TR* (American Psychiatric Association, 2000) criteria.

The mean duration of minocycline treatment was 3.5 months ($SD = 4.5$, range = 2 weeks to 20 months), with a dosage range of 25 mg to 200 mg per day. Of the 50 patients, 34 were still continuing minocycline when we conducted our survey. Six patients had discontinued minocycline on their physician's advice due to concerns about tooth graying in the future. Two patients had discontinued the drug because they developed physical side effects while taking it; 4 patients, because their behavioral problems worsened; and 4, because the parents saw no changes. Our survey results indicated that, on average, parents began to notice positive or negative changes after 3.5 weeks ($SD = 2.5$, minimum 2 weeks, maximum 12 weeks) of minocycline treatment.

Side Effects

Of 53 families, we found that 21 patients (39.6%) had a side effect, including 3 subjects who were taking minocycline for only 1 to 4 days and developed intolerable side effects (as mentioned above). Among our defined study patients who were on minocycline for at least 2 weeks ($n = 50$), the most common reported side effects were gastrointestinal problems, including loss of appetite in 8 of 50 (16%) patients; gastrointestinal upset in 6 patients (12%); and diarrhea in 4 patients (8%). Also, there was one teenage boy who had darkening of his nails after being treated

with minocycline for approximately a year (Figure 1). Because he had a good response to minocycline, the family wanted to continue the treatment. Usually the side effects were mild, such as gastrointestinal upset for the first few days on the medication that resolved. There was no report of sun sensitivity, blue gray teeth, hearing loss, fever, swelling skin rash, drowsiness, or dizziness in any patient. In Table 1, we provide a more detailed description of side effects/adverse events by dose (25 to 200 mg/day) among all patients on minocycline for 2 or more weeks. The association between adverse events and age and adverse events and duration on medication were not signifcant.

Outcome: Language, Attention, and Behavioral Symptoms

The outcome areas with the highest report of improvements according to the parents' impression were language (54%), attention (50%), social communication (44%), and anxiety (30%). Details are provided in Table 2. Although the raw data presented in Table 2 indicate a general skewness towards favoring overall improvement, we emphasize that the degree to which this was due to a placebo effect needs to be assessed with a controlled trial. However, we also note that hyperactivity (14%) and moodiness (12%) were worse on minocycline, and these areas should be carefully monitored in future studies.

Some parents reported that after taking minocycline their child used more language, and

Figure 1. Photograph of the pigmentation of the nail in a 19-year-old male after a year on minocycline. The color change involves only some nails (nonuniform). The worse ones seem to be his index fingers and second toes. The color change appeared after about 2 to 4 months of use of minocycline and has not worsened in the subsequent year. The fungal cultures were negative.

Table 1. Adverse Events Reported for Study Patients

Dose[a]	n[b]	Age[c] (in years)			Duration[c] (months)			Proportion of AE[d]	Description of AE[e]
		Mean	SD	Range	Mean	SD	Range		
25	8	4.23	3.19	0.3–8.5	1.56	1.39	1–5	.13	Loss of appetite (1), diarrhea (1), vomiting (1)
50	17	11.06	3.05	3–17	5.01	6.35	1–20	.41	GI upset (3), loss of appetite (2), increase in appetite (1), itching (1), dry lips (1), more frequent bed wetting (1)
100	18	17.39	4.18	10–25	2	1.68	0.5–6	.50	Loss of appetite (5), GI upset (3), diarrhea (3), headache (1)
200	7	18.86	4.53	11–24	6.28	4.92	1–12	.14	Brown color in the nails (1)

Note. These participants had received minocycline for at least 2 weeks.
[a]Mg per day. [b]Three subjects had intolerable side effects after 2 to 4 days on minocycline. [c]Association between age and adverse event (AE), $p = .114$. [d]Association between age and adverse effect, $p = .268$. [d]Association between duration and adverse event (AE), $p = .114$. [e]An individual may have one or more AEs.

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Table 2. Patients Showing Changes According to Parents' Impression (in %, $N = 50$)

Symptom	Severely worse (1)	Mildly worse (2)	None (3)	Somewhat better (4)	Very much better (5)
Language	-	-	46	42	12
ADHD[a] symptoms					
Hyperactivity	-	14	74	12	-
Attention	-	-	50	38	12
Impulsivity	-	8	72	18	2
Behavior problems					
Tantrums	2	4	80	12	2
Anxiety	-	4	66	20	10
Moodiness/ irritability	-	12	78	4	6
Sleep disturbances	-	12	86	2	-
Social communication	-	-	56	40	4

[a]Attention-deficit/hyperactivity disorder.

the sounds were clearer and more understandable. For older children, parents said that their child improved in expressive language skills, with comments including "becoming more conversational, articulate, and talkative." Some parents reported that their child was more focused and had a longer attention span when playing, doing homework, or participating in another activity (e.g., shopping).

Discussion

This is the first survey of the clinical response to minocyline in patients with fragile X syndrome with the intention to document side effects and determine the parents' impression of treatment benefits or problems. Because this was not a controlled trial, there is likely a significant placebo effect in the family reports, and clearly the efficacy of minocycline in treating individuals with fragile X syndrome cannot be determined. However, the levels and type of side effects and parent reported data provide important information for monitoring and designing outcome measures in future clinical studies, as noted in the *Results* section above. The knock-out mouse data demonstrate that minocycline specifically lowers MMP-9 levels and improves the maturation of dendritic spines in the absence of FMRP (Bilousova et al., 2009). It is possible that this mechanism of action is through the mGluR5 pathway. The downstream components of this

pathway are up-regulated in the absence of FMRP. Minocycline has a neuroprotective effect at the cellular level in the brain. It inhibits MMP-9, one enzyme involved in breaking down and building the extracellular matrix (Kim & Suh, 2009). MMP-9 is also important for synaptic plasticity, although the mechanism for this is not known. Minocycline inhibits several neuroinflammatory processes in the microglia involving cytokines and nitric oxide synthetase (Kim & Suh, 2009). It can also inhibit the MMP-9 at the mitochondrial membrane, preventing the release of apoptosis-inducing factors into the cytoplasm.

There has been significant enthusiasm on the part of families to try this medication, after the knock-out mouse treatment paper demonstrated that minocycline could be a targeted treatment for fragile X syndrome (Bilousova et al., 2009). Our survey suggests a perceived positive response by families, particularly for their child's improvements in language, attention, and social communication.

The results provided here can be used to guide the outcome measures of controlled minocycline trials for future studies. We also suggest that outcome measures that have been documented to be abnormal in fragile X syndrome, and have been shown to be helpful in other targeted treatment trials in fragile X syndrome, should be utilized for controlled trials of minocycline (Berry-Kravis et al., 2009; Farzin, Rivera, & Hessl, 2009; Farzin, Whitney, Hagerman, & Rivera,

2008; Hessl et al., 2009). Such quantitative measures include prepulse inhibition, which is a neurophysiological assessment of frontal gating. Prepulse inhibition is deficient in fragile X syndrome and can be improved with fenobam, an mGluR5 antagonist used in a treatment study in adults with fragile X syndrome (Berry-Kravis et al., 2009; Hessl et al., 2009). In addition, a continuous performance test to document improvement in attention and hyperactivity symptoms (Sullivan, Hooper, & Hatton, 2007), eye tracking measures showing an impaired eye gaze in fragile X syndrome (Farzin et al., 2009), and visual paradigms that demonstrate deficiency in infants and toddlers with fragile X syndrome (Farzin et al., 2008) could be utilized as outcome measures in future treatment trials.

Our preliminary survey documented side effects of minocycline in individuals with fragile X syndrome; this should be followed closely in subsequent treatment studies. Although we did not see photosensitivity, most subjects were on minocycline in the fall and winter of 2008 and early spring of 2009. A treatment in the summer, particularly in hot and sunny parts of the country, could cause this side effect. We recommend the regular use of sunscreen when minocycline treatment is used. A phototoxic reaction that includes edema and a vesicular eruption has been reported in individuals taking tetracycline when exposed to sunlight. However, minocycline rarely causes photosensitivity (Jonas, 1982), which is consistent with our findings. In addition, 3 patients also reported acne clearing, which is one benefit of minocycline for adolescents (El-Hallak et al., 2008).

Gastrointestinal side effects were common; the most frequent problem was a decrease in appetite, perhaps related to a mildly upset stomach. In pediatrics, there is a history of long-term use of minocycline in adolescents who are treated for acne vulgaris (El-Hallak et al., 2008). The side effect of a lupus-like rash is more likely to occur with longer use (El-Hallak et al., 2008; Schlienger et al., 2000). If a rash develops with minocycline use, we recommend checking an ANA (a blood screen for lupus) and, if positive, the minocycline should be discontinued.

The optimal length of time for minocycline treatment of individuals with fragile X syndrome at different ages and the carryover effects after it is stopped are not known. Bilousova et al. (2009) found a positive effect of minocycline treatment on behavior in the fragile X knock-out mouse after 3 weeks. Parents of young children with fragile X syndrome are weighing the possibility of gray teeth versus potential cognitive and behavioral improvement in their decision to use minocycline. This is a difficult decision. The fact that gray teeth can be fixed with dental plating at a later age when cosmetic features may be more important to the child often facilitates the decision to consider minocycline treatment. However, we do not know of other potentially negative effects of prolonged treatment with minocycline in infants or young children, and caution should be used, especially in the treatment of babies. This is why treatment for 1 to 3 months should be considered for a controlled clinical trial before a longer use is considered.

A limited number of patients experienced a worsening of symptoms, such as hyperactivity and moodiness. Parents should be warned of this possibility. If this or other problematic side effects occur, then the minocycline should be discontinued. A worrisome but rare side effect is pseudotumor cerebri, causing increased intracranial pressure and headaches. In case reports of children treated with minocycline, there are a few cases of intracranial hypertension documented. Usually, the patients complained about headache accompanied by dizziness, nausea, vomiting, and visual defects; papilledema was noted on physical examination (Ang et al., 2002; Nagarajan & Lam, 2000; Shiri & Amichai, 1997). Although we did not see these symptoms in our patients with headaches, we recommend discontinuing minocycline if headaches persist. The possibility of drug-induced benign intracranial hypertension should be considered in any patient presenting with unexplained headache on minocycline. Nonverbal patients with fragile X syndrome are not able to tell their parents that they have a headache. However, it is likely that their behavior would get worse if they had a severe headache; therefore, if behaviors become worse, the medication should be stopped. It is not known how many young children treated with minocycline for a month or so will have tooth graying until their permanent teeth come in. Pigmentation can occur elsewhere, and it is a well-recognized adverse effect of minocycline therapy. Various body sites, most notably the skin, nails, bones, thyroid, mouth, and eyes can be affected by the pigmentation.

In general, pigmentation results from long-term administration of minocycline. Nail pigmentation caused by this drug is unusual and less common than skin involvement. Although a number of color changes have been reported, a slate-grey discoloration of the proximal nail bed appears to be the most frequent type, as we saw in our patient (Eisen & Hakim, 1998). Generally, pigmentation develops concomitantly with other sites of involvement and is rarely an isolated finding. Stimulation of nail matrix melanocytes with increased melanin deposition in the nail plate has been described as the cause of pigmentation in longitudinal melanonychia secondary to minocycline. The nail pigmentation may persist for prolonged periods after withdrawal from the drug (Eisen & Hakim, 1998). There is some data from laboratory study in rats that antioxidants, such as vitamin C, will decrease the occurance of pigmentation (Bowles, 1998). This may be worthwhile to consider, because the use of antioxidants, particularly alpha tocopherol and N acetyl L cystine, also appear to improve synaptic connections in the knock-out fragile X mouse related to reversing oxidative stress damage in the brain (de Diego-Otero et al., 2009).

In summary, our preliminary results suggest outcome areas in which minocycline may be helpful for the design of future clinical studies involving patients with fragile X syndrome. Our documentation of side effects associated with minocycline treatment in patients with fragile X syndrome should be further assessed in the monitoring of minocycline trials. The design of controlled clinical trials of minocycline should be focused on effects in the areas of language, attention, social communication, and anxiety. We recommend controlled trials in patients with fragile X syndrome in childhood and adulthood, but caution should be used for long duration studies in younger children. Such trials will, we hope, delineate whether minocycline is truly a targeted treatment in patients with fragile X syndrome. It should also be possible to study the effect of minocycline in human neuronal cell cultures to determine whether the effects on synaptic maturity are similar to those in the mouse model (Bilousova et al., 2009). Any improvement in synaptic connections with targeted treatments should be reinforced by concurrent educational and therapy interventions in children and adults with fragile X syndrome, and more information regarding these interventions can be found on the National Fragile X Foundation website (www.fragilex.org).

References

Abe, M., Furukawa, S., Takayama, S., Michitaka, K., Minami, H., & Yamamoto, K., et al. (2003). Drug-induced hepatitis with autoimmune features during minocycline therapy. *Internal Medicine*, *42*, 48–52.

Akin, E., Miller, L. C., & Tucker, L. B. (1998). Minocycline-induced lupus in adolescent. *Pediatrics*, *101*, 926–928.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed., Text Rev.). Washington DC: Author.

Ang, E. R., Zimmerman, J. C., & Malkin, E. (2002). Pseudotumor cerebri secondary to minocycline intake. *Journal of the American Board of Family Practice*, *15*, 229–233.

Bantubungi, K., Jacquard, C., Greco, A., Pintor, A., Chtarto, A., & Tai, K. (2005). Minocycline in phenotypic models of Huntington's disease. *Neurobiology of Disease*, *18*, 206–217.

Bassell, G. J., & Warren, S. T. (2008). Fragile X syndrome: Loss of local mRNA regulation alters synaptic development and function. *Neuron*, *60*, 201–214.

Bear, M. F., Dolen, G., Osterweil, E., & Nagarajan, N. (2008). Fragile X: Translation in action. *Neuropsychopharmacology*, *33*, 84–87.

Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends in Neurosciences*, *27*, 370–377.

Bergles, D. E., & Jahr, C. E. (1998). Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. *Journal of Neuroscience*, *18*, 7709–7716.

Berry-Kravis, E., Hessl, D., Coffey, S., Hervey, C., Schneider, A., Yuhas, J., et al. (2009). A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. *Journal of Medical Genetics*, *46*, 266–271.

Bilousova, T. V., Dansie, L., Ngo, M., Aye, J., Charles, J. R., Ethell, D. W., et al. (2009). Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. *Journal of Medical Genetics*, *46*, 94–102.

Bowles, W. (1998). Protection agaitts minocycline pigment formation by ascorbic acid

(vitamin C). *Journal of Esthetic Dentistry*, *10*, 182–186.

Cascio, A., Di Liberto, C., D'Angelo, M., Iaria, C., Scarlata, F., Titone, L., et al. (2004). No findings of dental defects in children treated with minocycline. *Antimicrobial Agents and Chemotherapy*, *48*, 2739–2741.

Choi, Y., Kim, H., Shin, K., Kim, E., Kim, M., & Kim, H., et al. (2007). Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer's disease models. *Neuropsychopharmacology*, *32*, 2393–2404.

D'Hulst, C., Heulens, I., Brouwer, J. R., Willemsen, R., De Geest, N., Reeve, S. P., et al. (2009). Expression of the GABAergic system in animal models for fragile X syndrome and fragile X associated tremor/ataxia syndrome (FXTAS). *Brain Research*, *1253*, 176–183.

D'Hulst, C., & Kooy, R. F. (2007). The GABAA receptor: A novel target for treatment of fragile X? *Trends in Neurosciences*, *30*, 425–431.

Danbolt, N. C. (2001). Glutamate uptake. *Progress in Neurobiology*, *65*, 1–105.

Dansie L., Bilousova T., Ethell, D., & Ethell, I. (2009). [MMP-9 expression is regulated by mGluR5 activity in hippocampal neurons.] Unpublished raw data.

de Diego-Otero, Y., Romero-Zerbo, Y., el Bekay, R., Decara, J., Sanchez, L., Rodriguez-de Fonseca, F., et al. (2009). α-tocopherol protects against oxidative stress in the fragile X knockout mouse: An experimental therapeutic approach for the *Fmr1* deficiency. *Neuropsychopharmacology*, *34*, 1011–1026.

de Vrij, F. M., Levenga, J., van der Linde, H. C., Koekkoek, S. K., De Zeeuw, C. I., Nelson, D. L., et al. (2008). Rescue of behavioral phenotype and neuronal protrusion morphology in Fmr1 knock-out mice. *Neurobiology of Disease*, *31*, 127–132.

Eisen, D., & Hakim, M. D. (1998). Minocycline-induced pigmentation. Incidence, prevention and management. *Drug Safety*, *18*, 431–440.

El-Hallak, M., Giani, T., Yeniay, S. B., Jacobs, K. E., Kim, S., Sundel, R. P., et al. (2008). Chronic minocycline-induced autoimmunity in children. *Journal of Pediatrics*, *153*, 314–319.

Elewa, H., Hilali, H., Hess, D., Machado, L., & Fagan, S. (2006). Minocycline for short-term neuroprotection. *Pharmacotherapy*, *24*, 515–521.

Elkayam, O., Levartovsky, D., Brautbar, C., Yaron, M., Burke, M., Vardinon, N., et al.

(1998). Clinical and immunological study of 7 patients with minocycline-induced autoimmune phenomena. *American Journal of Medicine*, *105*, 484–487.

Ethell, I. M., & Ethell, D. W. (2007). Matrix metalloproteinases in brain development and remodeling: Synaptic functions and targets. *Journal of Neuroscience Research*, *85*, 2813–2823.

Farzin, F., Rivera, S. M., & Hessl, D. (2009). Brief report: Visual processing of faces in individuals with fragile X syndrome: An eye tracking study. *Journal of Autism and Developmental Disorders*, *39*, 946–952.

Farzin, F., Whitney, D., Hagerman, R. J., & Rivera, S. M. (2008). Contrast detection in infants with fragile X syndrome. *Vision Research*, *48*, 1471–1478.

Fernandez-Carvajal, I., Walichiewicz, P., Xiaosen, X., Pan, R., Hagerman, P. J., & Tassone, F. (2009). Screening for expanded alleles of the FMR1 gene in blood spots from newborn males in a Spanish population. *Journal of Molecular Diagnostics*, *11*, 324–329.

Good, M. L., & Hussey, D. L. (2003). Minocycline: Stain devil? *British Journal of Dermatology*, *149*, 237–239.

Gordon, P. H., Cheung, Y. K., Levin, B., Andrews, H., Doorish, C., Macarthur, R. B., et al. (2008). A novel, efficient, randomized selection trial comparing combinations of drug therapy for ALS. *Amyotrophic Lateral Sclerosis*, *9*, 212–222.

Hagerman, P. J. (2008). The fragile X prevalence paradox. *Journal of Medical Genetics*, *45*, 498–499.

Hagerman, R. J. (2006). Lessons from fragile X regarding neurobiology, autism, and neurodegeneration. *Journal of Developmental & Behavioral Pediatrics*, *27*, 63–74.

Hagerman, R. J., Rivera, S. M., & Hagerman, P. J. (2008). The fragile X family of disorders: A model for autism and targeted treatments. *Current Pediatric Reviews*, *4*, 40–52.

Hessl, D., Berry-Kravis, E., Cordeiro, L., Yuhas, J., Ornitz, E. M., Campbell, A., et al. (2009). Prepulse inhibition in fragile X syndrome: Feasibility, reliability, and implications for treatment. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *150B*, 545–553.

Imbesi, M., Uz, T., Manev, R., Sharma, R. P., & Manev, H. (2008). Minocycline increases

phosphorylation and membrane insertion of neuronal GluR1 receptors. *Neuroscience Letters*, *447*(2–3), 134–137.

Jonas, M. C. B. (1982). Minocycline. *Therapeutic Drug Monitoring*, *4*, 137–145.

Kim, H. S., & Suh, Y. H. (2009). Minocycline and neurodegenerative diseases. *Behavioral Brain Research*, *196*, 168–179.

Kooy, R. F. (2003). Of mice and the fragile X syndrome. *Trends in Genetics*, *19*, 148–154.

Kriz, J., Nguyn, M. D., & Julien, J. (2002). Minocycline slows disease progression in a mouse model of amyotropic lateral sclerosis. *Neurobiology of Disease*, *10*, 268–278.

LaPorta, V., Nikitakis, G., Sindler, A., & Reynolds, M. (2005). Minocycline-associated intra-oral soft tissue pigmentation: Clinicopathologic correlation and review. *Journal of Clinical Periodotology*, *32*, 119–122.

Lawson, T., Amos, N., Bulgen, D., & Williams, B. (2001). Minocycline-induced lupus: Clinical features and response to rechallenge. *Rheumatology*, *40*, 329–335.

Lee, S., Yune, T., Kim, S., Park, D., Lee, Y., & Kim, Y. (2003). Minocycline reduces cell death and improves functional recovery after traumatic spinal cord injury in the rat. *Journal of Neurotrauma*, *20*, 1017–1027.

Liu, Z., Fan, Y., Won, S., Neumann, M., Hu, M. D., Zhou, L., et al. (2007). Chronic treatment with minocycline preserves adult new neurons and reduces functional impairment after focal cerebral ischemia. *Stroke*, *38*(146), 146–152.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., et al. (2000). The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*, *30*, 205–223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: A revised version of a Diagnostic Interview for Caregivers of Individuals With Possible Pervasive Developmental Disorders. *Journal of Autism and Developmental Disorders*, *24*, 659–685.

Margolis, D. J., Hoffstad, O., & Bilker, W. (2007). Association or lack of association between tetracycline class antibiotics used for acne vulgaris and lupus erythematosus. *British Journal of Dermatology*, *157*, 540–546.

McBride, S. M., Choi, C. H., Wang, Y., Liebelt, D., Braunstein, E., Ferreiro, D., et al. (2005). Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a Drosophila model of fragile X syndrome. *Neuron*, *45*, 753–764.

Nagarajan, L., & Lam, G. C. (2000). Tetracycline-induced benign intracranial hypertension. *Journal of Paediatrics Child Health*, *36*(1), 82–83.

Noble, W., Garwood, C., Stephenson, J., Kinsey, A., Hanger, D., & Anderton, B. M. (2009). Minocycline reduces the development of abnormal tau species in models of Alzheimer's disease. *Journal of the Federation of American Societies For Experimental Biology* (*FASEB*), *23*, 739–750.

Oostra, B. A., & Willemsen, R. (2003). A fragile balance: FMR1 expression levels. *Human Molecular Genetics*, *12*(Spec No. 2), R249–257.

Porter, D., & Harrison, A. (2003). Minocycline-induced lupus: A case series. *New Zealand Journal of Medicine*, *116*(1171). Available at http://www.nzma.org.nz/journal/116-1171/384/

Qin, M., Kang, J., Burlin, T. V., Jiang, C., & Smith, C. B. (2005). Postadolescent changes in regional cerebral protein synthesis: An in vivo study in the FMR1 null mouse. *Journal of Neuroscience*, *25*, 5087–5095.

Sanchez, A., Rogers, R., & Sheridan, P. J. (2004). Tetracycline and other tetracycline-derivate staining of the teeth and oral cavity. *International Journal of Dermatology*, *43*, 709–715.

Schlienger, R., Bircher, A., & Meier, C. (2000). Minocycline-induced lupus. *Dermatology*, *200*, 223–231.

Shepherd, J. (2002). Severe complication of a commonly prescribed drug: Minocycline-induced lupus. *Journal of the American Board of Family Medicine*, *15*, 239–241.

Shetty, A. (2002). Tetracyclines in pediatrics revisited. *Clinical Pediatrics*, *41*, 203–209.

Shiri, J., & Amichai, B. (1997). Intracranial hypertension and minocycline. *Annals of Internal Medicine*, *127*, 168.

Smith, K., & Leyden, J. (2005). Safety of doxycycline and minocycline: A systematic review. *Clinical Therapeutics*, *27*, 1329–1342.

Sternlicht, M. D., & Werb, Z. (2001). How matrix metalloproteinases regulate cell behavior. *Annual Review of Cell Developmental Biology*, *17*, 463–516.

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Stirling, D., Khodarahmi, K., Liu, J., McPhail, L., McBride, C., & Steeves, J. (2004). Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. *Journal of Neuroscience*, 24, 2182–2190.

Sullivan, K., Hooper, S., & Hatton, D. (2007). Behavioural equivalents of anxiety in children with fragile X syndrome: Parent and teacher report. *Journal of Intellectual Disability Research*, 51(Pt 1), 54–65.

Teitelbaum, J., Atayde, A., Cohen, M., Bousvaros, A., & Jonas, M. (1998). Minocycline-related autoimmune hepatitis. *Archives of Pediatrics & Adolescent Medicine*, 152, 1132–1136.

Tournigand, C., Genereau, T., Prudent, M., Diemert, M., Herson, S., & Chosidow, O. (1999). Minocycline-induced clinical and biological lupus-like disease. *Lupus*, 8, 773–774.

Tredwin, C. J., Scully, C., & Bagan-Sebastian, J. V. (2005). Drug-induced disorders of teeth. *Journal of Dental Research*, 84, 596–602.

van Steensel, M. A. (2004). Why minocycline can cause systemic lupus—A hypothesis and suggestions for therapeutic interventions based on it. *Medical Hypotheses*, 63, 31–34.

Verkerk, A. J., Pieretti, M., Sutcliffe, J. S., Fu, Y. H., Kuhl, D. P., Pizzuti, A., et al. (1991). Identification of a gene (FMR–1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell*, 65, 905–914.

Wang, J. Q., Arora, A., Yang, L., Parelkar, N. K., Zhang, G., Liu, X., & Mao, L. (2005). Phosphorylation of AMPA receptors: Mechanisms and synaptic plasticity. *Molecular Neurobiology*, 32, 237–249.

Well, J., Hulbert, R., Fehling, M., & Yong, V. (2003). Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. *Brain*, 126, 1628–1637.