Treatment of Multiple Sclerosis with the Pregnancy Hormone Estriol

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Multiple sclerosis patients who become pregnant experience a significant decrease in relapses that may be mediated by a shift in immune responses from T helper 1 to T helper 2. Animal models of multiple sclerosis have shown that the pregnancy hormone, estriol, can ameliorate disease and can cause an immune shift. We treated nonpregnant female multiple sclerosis patients with the pregnancy hormone estriol in an attempt to recapitulate the beneficial effect of pregnancy. As compared with pretreatment baseline, relapsing remitting patients treated with oral estriol (8mg/day) demonstrated significant decreases in delayed type hypersensitivity responses to tetanus, interferon-γ levels in peripheral blood mononuclear cells, and gadolinium enhancing lesion numbers and volumes on monthly cerebral magnetic resonance images. When estriol treatment was stopped, enhancing lesions increased to pretreatment levels. When estriol treatment was reinstituted, enhancing lesions again were significantly decreased. Based on these results, a larger, placebo-controlled trial of estriol is warranted in women with relapsing remitting multiple sclerosis. This novel treatment strategy of using pregnancy doses of estriol in multiple sclerosis has relevance to other autoimmune diseases that also improve during pregnancy.

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that is thought to be mediated by myelin protein–specific CD4+ T lymphocytes secreting T helper 1 (Th1) type cytokines such as interferon-γ (IFN-γ).[1] In murine systems, Th1 and Th2 immune responses are counterregulatory and, in states of health, the two responses exist in a delicate balance.[2] There are some differences in human and murine systems; nevertheless, therapies for MS have aimed to either reduce Th1 responses or increase Th2 responses, thereby causing a therapeutic immune deviation. There are currently three available therapies with proven benefit in relapsing remitting (RR) MS: IFN-β1b, IFN-β1a, and glatiramer acetate. Although these therapies have numerous possible mechanisms of action, several reports indicate that they act at least in part through immune deviation.[3–6] These therapies with proven benefit in RR MS are of questionable benefit in secondary progressive (SP) MS,[7] which may be because of differences in the immunopathogenesis of these two disease phases.

All of the currently available antiinflammatory therapies for MS are injections. The purpose of this pilot trial was to test a noninjectable, oral antiinflammatory hormonal treatment for MS. During pregnancy there is an immune deviation characterized by a decrease in Th1 responses and an increase in Th2 responses that is evolutionarily advantageous because it promotes fetal survival by decreasing Th1 responses involved in rejection of the fetus as an allograft.[8] MS and other putative Th1-mediated autoimmune diseases such as rheumatoid arthritis, improve during pregnancy.[9–13] Specifically, in MS, a significant reduction in relapse rates has been shown in the last trimester.[10]

Pregnancy was shown to have a protective effect in experimental autoimmune encephalomyelitis, an animal model of MS, in numerous species.[9,14] Estriol is an estrogen made by the fetal placental unit that is not present at appreciable amounts in nonpregnant states, but during pregnancy it increases progressively with time. Estriol, administered to nonpregnant female mice at doses to induce pregnancy levels in sera, was shown to ameliorate both encephalomyelitis and collagen-induced arthritis.[15–17] In encephalomyelitis, it was also shown that estriol treatment was associated with a systemic immune deviation consistent with

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that observed during pregnancy.[15,17] These in vivo observations were complementary to in vitro observations of an immune shift from Th1 to Th2 when T-cell lines were cultured with pregnancy levels of estrogens.[18,19] Although progesterone treatment alone did not ameliorate encephalomyelitis or collagen-induced arthritis, it was shown to enhance the protective effect of estrogens in collagen-induced arthritis.[20] Together, observations in animal models of Th1-mediated autoimmune diseases have indicated that estriol is a strong candidate sex hormone for mediating disease protection during pregnancy.

To our knowledge, this is the first time a pregnancy hormone has been given at a pregnancy dose to humans in an attempt to ameliorate a putative Th1-mediated autoimmune disease by using highly sensitive subclinical markers of disease activity as an indicator.

Patients and Methods

Trial Design

A crossover design was used[21] with monthly brain magnetic resonance images (MRIs) during the 6-month pretreatment period, the 6-month treatment period with oral estriol (8mg/day), and the 6-month posttreatment period, with clinical and laboratory evaluations as demonstrated (Fig 1a).

Inclusion Criteria

Women with clinically definite MS, aged 18 to 50 years, with an Expanded Disability Status Scale (EDSS) score of 0 to 6.5 who had not been taking IFN-β or glatiramer acetate for at least 6 months and who had no steroid treatment for at least 3 months were eligible. At least 5cm^3 of lesion burden on a screening T2-weighted brain MRI was required. Subjects who were pregnant or nursing or who were taking oral contraceptives or receiving hormone replacement therapy were excluded. The study was approved by the UCLA Human Subjects Protection Committee, and informed consent was obtained.

Patients

Twelve female patients with clinically definite MS were enrolled. Six had RR disease and six had SP disease. All six RR and four of six SP patients completed the entire 18-month study period. Of the two SP patients who did not complete the study, one was disqualified from the study because of concurrent steroid treatment for tonic spasms by an outside neurologist, and the other did not wish to go untreated in the posttreatment period. Of the 10 patients who completed the entire study, the mean age was 44 years (range, 28–50 years), and the mean EDSS was 3.3 (range, 1.0–6.5). The mean EDSS score for the SP patients was 5.0, whereas the mean EDSS for the RR patients was 2.2. The 18-month trial was extended in RR patients, whereby

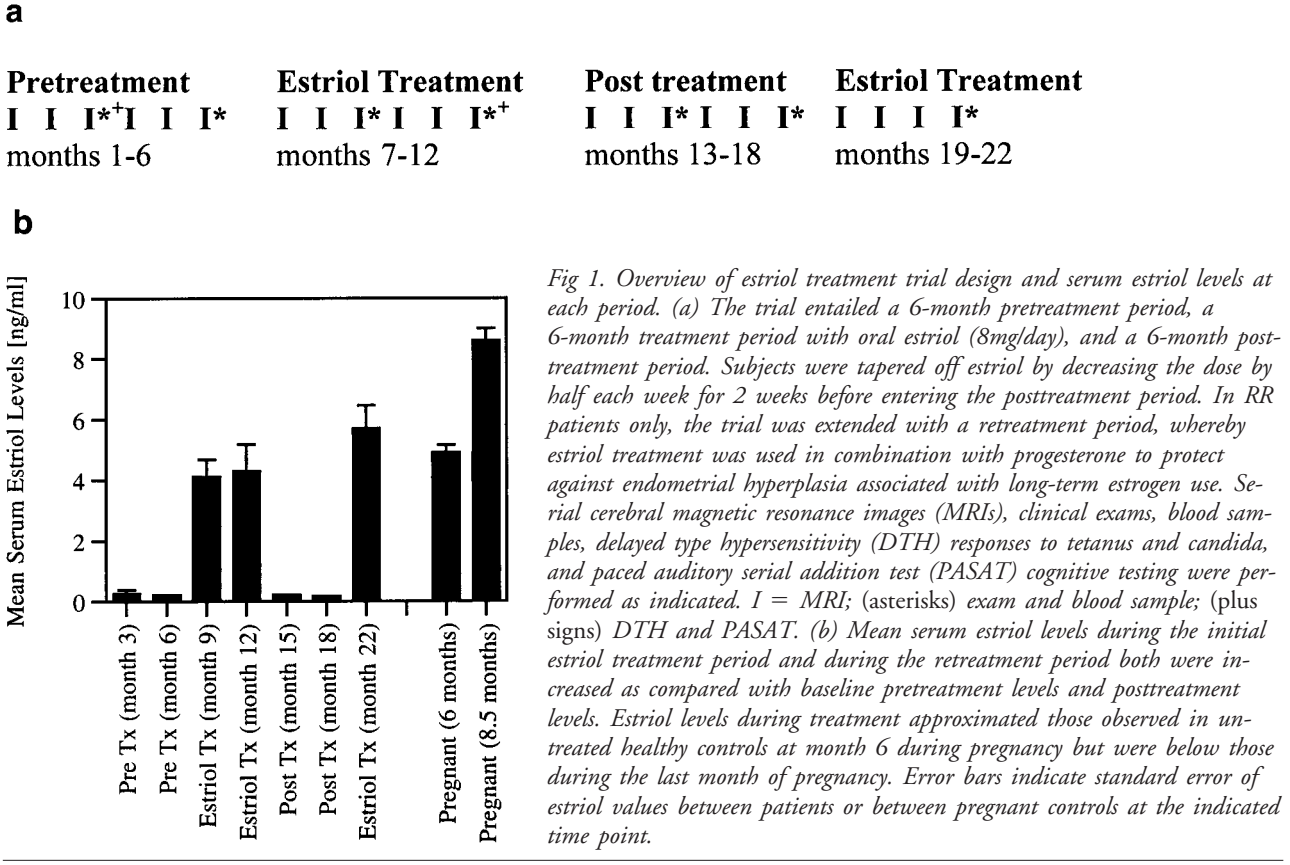


Fig 1. Overview of estriol treatment trial design and serum estriol levels at each period. (a) The trial entailed a 6-month pretreatment period, a 6-month treatment period with oral estriol (8mg/day), and a 6-month posttreatment period. Subjects were tapered off estriol by decreasing the dose by half each week for 2 weeks before entering the posttreatment period. In RR patients only, the trial was extended with a retreatment period, whereby estriol treatment was used in combination with progesterone to protect against endometrial hyperplasia associated with long-term estrogen use. Serial cerebral magnetic resonance images (MRIs), clinical exams, blood samples, delayed type hypersensitivity (DTH) responses to tetanus and candida, and paced auditory serial addition test (PASAT) cognitive testing were performed as indicated. I = MRI; (asterisks) *exam and blood sample;* (plus signs) *DTH and PASAT. (b) Mean serum estriol levels during the initial estriol treatment period and during the retreatment period both were increased as compared with baseline pretreatment levels and posttreatment levels. Estriol levels during treatment approximated those observed in untreated healthy controls at month 6 during pregnancy but were below those during the last month of pregnancy. Error bars indicate standard error of estriol values between patients or between pregnant controls at the indicated time point.*

treatment was reinstituted for 4 months with estriol and progesterone.

Medication

For the initial treatment phase, micronized, United States Pharmacopeia graded estriol powder (Medisca, Plattsburg, NY) was put into capsules by UCLA Pharmaceutical Services. During the extension retreatment phase in the RR patients, all but one received a capsule of estriol (8mg/day) plus progesterone (100mg/day), whereas the single RR patient who had a hysterectomy received only estriol (8mg/day; Women's International Pharmacy, Madison, WI).

Clinical and Safety Measures

Subjects were evaluated using the Kurtzke's EDSS by the same neurologist (R.R.V.) throughout the study. At each visit the study nurse (R.K.) administered the paced auditory serial addition test (PASAT) and the nine-hole peg test. Blood was drawn for SMA12, cholesterol panel, blood counts, and hormone levels (estriol, estradiol, estrone, luteinizing hormone, follicle-stimulating hormone, cortisol, progesterone). Estriol levels in serum were determined by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Oxford Biomedical, Oxford, MI). Gynecological exams and mammograms were performed at the beginning and end of the study. Patients with abnormal menstrual bleeding underwent endometrial biopsy.

Delayed Type Hypersensitivity Responses

Delayed type hypersensitivity (DTH) responses to tetanus (Tetanus Toxoid; Wyeth Laboratories, Marietta, PA) and candida (Candin; Allermed Laboratories, San Diego, CA) were tested at two time points: once in the pretreatment period at study month 3 and once at the end of the treatment period at study month 12 (see Fig 1a). A group of six untreated healthy control women were also tested twice, spanning the same time interval (9 months). Each solution (0.1ml) was injected intradermally on the anterior surface of the forearm. Induration at each injection site was read after 48 hours. Each site was measured twice with the average recorded. The same nurse (R.K.) administered all injections and read all responses on all subjects at both time points.

Reverse Transcription Polymerase Chain Reaction

Peripheral blood mononuclear cells were isolated and cryopreserved.[22] Peripheral blood mononuclear cells were thawed in parallel from a given patient during the two pretreatment time points and the two treatment time points. Total RNA was isolated, DNA was removed, and mRNA was reverse-transcribed.[23] Both IFN-γ and actin were amplified from the same cDNA; however, the cDNA was diluted 1 to 9 before amplification for actin. Amplification was conducted in 1mM $MgCl_2$ using IFN-γ and actin primer sequences (Life Technologies, Rockville, MD) as described.[6] Complementary DNA was amplified for 35 cycles: 45 seconds at 95°C, 60 seconds at 54°C, and 45 seconds at 72°C. Polymerase chain eraction products were separated on a 1.5% agarose gel containing ethidium bromide and densitometry was performed.[23]

Magnetic Resonance Images

Scans were performed on a 1.5T G.E. (Waukesha, WI) scanner. The pulse sequences obtained were a T1-weighted scan with and without gadolinium (Omniscan, NYOCOMED, Inc., Princeton, NJ, 0.1mmol/kg) and a PD/T2-weighted scan. Digitized image data were transferred to a SGI workstation (Silicon Graphics, Inc., Mountain View, CA) for further processing. The number and volume of new and total gadolinium-enhancing lesions was determined using a semi-automated threshold based technique (Display; Montreal Neurological Institute) by a single experienced operator (N.L.S.). To calculate T2 volumes, we used a custom semi-automated, threshold based, seed-growing algorithm to determine lesion volume after skull stripping,[24] Rf correction, and spatial normalization. Scans were blinded as to whether patients had RR or SP disease.

Statistical Analysis

Sample paired *t* tests were used to ascertain significance of percentage of changes in DTH responses, IFN-γ levels, and PASAT cognitive testing scores during treatment as compared with pretreatment. The nonparametric, Wilcoxon's signed rank test was used for statistical comparisons in enhancing lesion numbers and volumes on MRI between the 6-month baseline period and each treatment period, post-treatment period, and retreatment period.

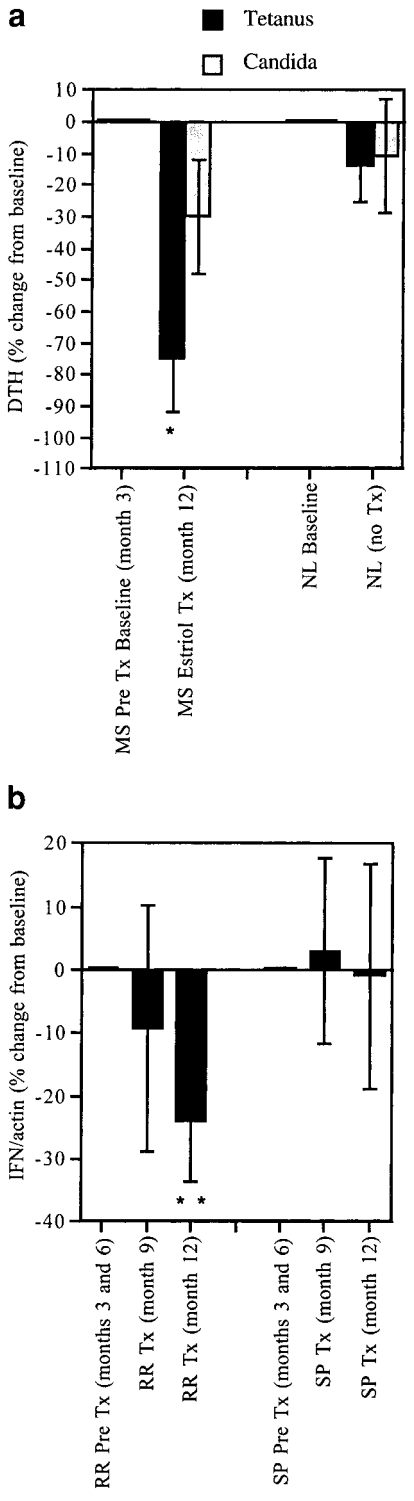
Results

Estriol Levels and Tolerability

Serum estriol levels during treatment and retreatment approximated those observed in women who were 6 months pregnant but were lower than those who were 8.5 months pregnant (see Fig 1b). Consistent with previous reports,[25,26] estriol was well tolerated with only menstrual cycle abnormalities. In three patients, endometrial biopsy was performed to investigate abnormal menstrual bleeding. All biopsies were negative for hyperplasia and treatment with estriol continued. Another patient had transient uterine fibroid enlargement during the treatment period. There were no significant alterations in any laboratory measures including luteinizing hormone, follicle-stimulating hormone, cortisol, progesterone, estradiol, and estrone.

Immune Responses

DTH responses to tetanus were significantly ($p = 0.006$) decreased at study month 12, when patients had been taking estriol for 6 months, as compared with DTH responses at study month 3, the pretreatment baseline (Fig 2a). DTH responses to candida were decreased less drastically and more variably.



Healthy, untreated female controls tested at baseline, then again after 9 months, did not demonstrate a significant decrease in DTH responses compared with their baseline.

In the six RR patients, levels of IFN-γ were variably decreased at study month 9 (after 3 months of estriol treatment) and then significantly decreased (p = 0.003) at study month 12 (after 6 months of estriol treatment) as compared with baseline pretreatment levels (months 3 and 6; see Fig 2b). In contrast, there was no decrease in IFN-γ in the four SP patients.

Magnetic Resonance Images

Compared with the 6-month pretreatment baseline period, the total volume and number of enhancing lesions for all 10 MS patients (6 RR, 4 SP) decreased during the treatment period. This improvement in the group as a whole was driven by the beneficial effect of estriol treatment in the RR, not the SP, group (Fig 3a and b). Within the first 3 months of treatment of RR patients, median total enhancing lesion volumes were decreased by 79% (p = 0.02), and numbers were decreased by 82% (p = 0.09; see Fig 3c and d). They remained decreased during the next 3 months of treatment, with lesion volumes decreased by 82% (p = 0.01), and numbers decreased by 82% (p = 0.02). In

Fig 2. Delayed type hypersensitivity (DTH) responses and interferon-γ (IFN-γ) levels were decreased during treatment as compared with pretreatment baseline. (a) Skin DTH responses to recall antigens (tetanus and candida) were determined during the pretreatment period (month 3) and again at the end of the treatment period (month 12). Seven (5 RR, 2 SP) of the 10 patients had positive DTH responses to tetanus in the pretreatment period, whereas all 10 (6 RR, 4 SP) had positive responses to candida with the definition of positive being at least 10mm induration. Mean percentage changes during the treatment period as compared with the pretreatment baseline were significantly decreased (p = *0.006) for the tetanus response, whereas the decrease in the candida response did not reach significance. There were no significant decreases in the DTH responses tested at the same time interval in untreated healthy controls (NL). (b) Ex vivo peripheral blood mononuclear cells were assessed for levels of IFN-γ and actin message by reverse transcription polymerase chain reaction to establish IFN-γ/actin at months 3 and 6 during pretreatment and at months 9 and 12 during treatment. In the RR group, but not the SP group, IFN-γ/actin was decreased significantly at study month 12 during treatment as compared with baseline pretreatment (months 3 and 6;* p = *0.003) whereas there was a more variable decrease earlier at study month 9. Error bars indicate the standard error of percentage change between patients for indicated test.* (single asterisk) p < *0.05;* (double asterisk) p < *0.005. MS = multiple sclerosis; RR = relapsing remitting; SP = secondary progressive.*

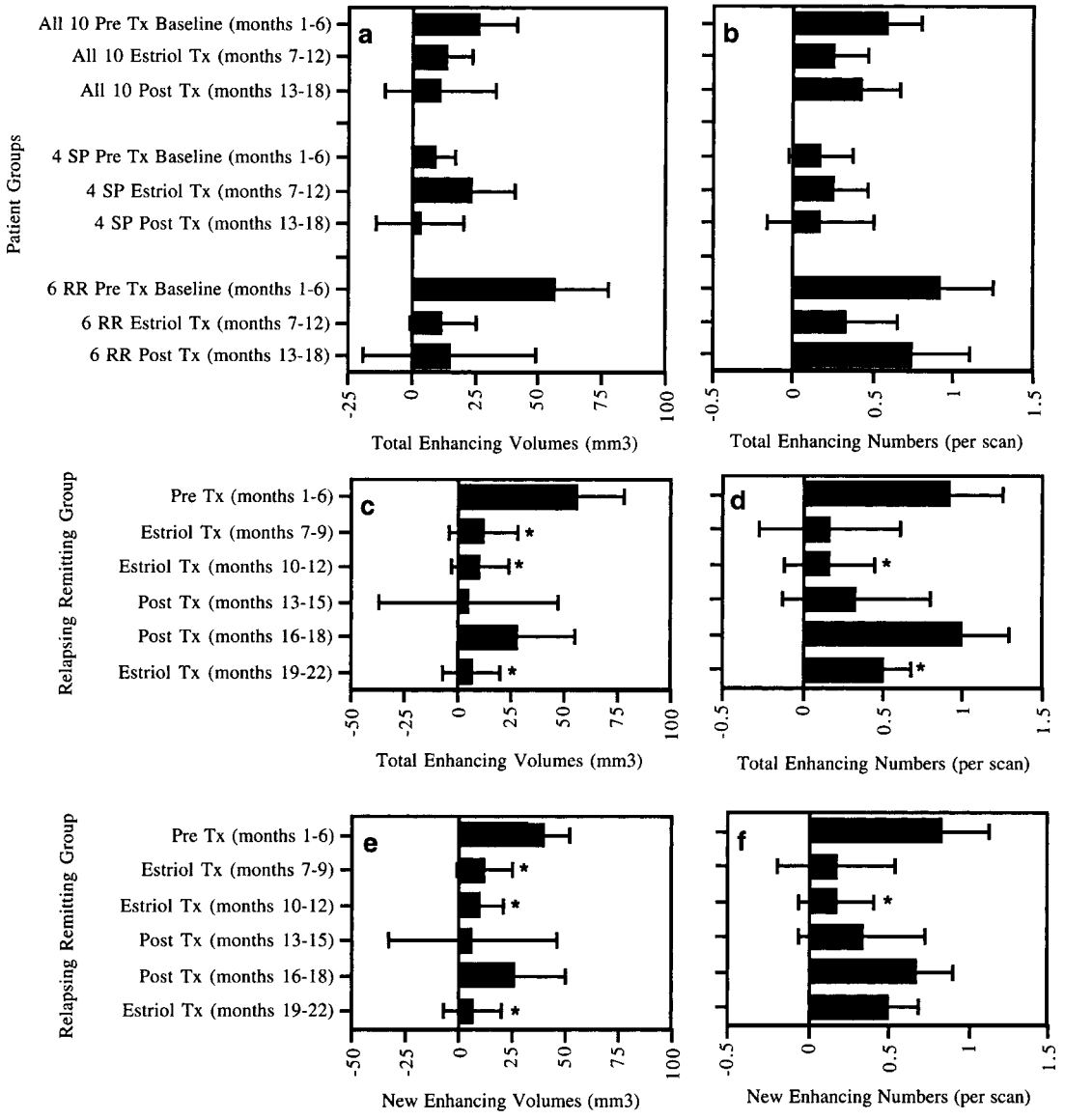


Fig 3. Enhancing lesion volumes and numbers on serial magnetic resonance images were significantly decreased with treatment as compared with pretreatment baseline, increased back to baseline after treatment, and then were again significantly decreased with reinstitution of treatment in relapsing remitting (RR), but not secondary progressive (SP), patients. (a) Median total volumes of gadolinium-enhancing lesions were determined in the 6-month pretreatment period as a baseline, during the 6-month treatment period, and during the 6-month posttreatment period in all multiple sclerosis patients (all 10), in SP patients (4 SP), and in RR patients (6 RR). (b) Median total lesion numbers as in panel a. (c) In RR patients, the median total volumes of gadolinium enhancing lesions were determined in the 6-month pretreatment period as a baseline, during study months 7 to 9 and 10 to 12 during treatment, during study months 13 to 15 and 16 to 18 after treatment, and during months 19 to 22 in the retreatment period. (d) Median total lesion numbers as in panel c. (e) Median new lesion volumes as in panel c. (f) Median new lesion numbers as in panel d. Error bars indicate standard errors between patients for each median volume or number at each time. (asterisks) $p < 0.05$.

the posttreatment period, median total enhancing lesion volumes and numbers became variable in the first 3 months off treatment, before returning to near baseline levels in the last 3 months of the posttreatment period. During the 4-month retreatment extension phase, enhancing lesion volumes decreased again by 88% ($p = 0.008$), and numbers decreased again, this time by 48% ($p = 0.04$) as compared with original baseline (see Fig 3c and d). Changes in median new enhancing lesion volumes and numbers followed similar patterns as median total lesion numbers and volumes (see Fig 3e and 3f).

Median T2 lesion volumes for the whole group were 15.3cm^3 (range, 6.1–33.8), with no significant differences in median T2 volumes between RR and SP groups. In the RR group, median T2 lesion volumes remained stable during the 6-month treatment period (0% change), increased during the 6-month posttreatment period (7.4% higher), and then declined in the 4-month retreatment extension period (2.0% lower).

Clinical Measures

Relapses were few and showed no significant changes during the study. EDSS and Nine-Hole Peg Test scores showed no significant changes during the study (Table). PASAT cognitive testing scores were significantly improved in the RR group ($p = 0.04$) but not in the SP group (Fig 4).

Discussion

This is the first time to our knowledge that a pregnancy hormone has been given at a pregnancy dose to nonpregnant women with a putative Th1-mediated autoimmune disease and resulted in a decrease in the Th1 response and an improvement in an inflammatory marker of disease activity. The response in the RR patients but not the SP patients reached statistical significance. The efficacy of estriol in RR but not SP MS is consistent with the response to other approved MS therapies with potent antiinflammatory effects[7] but also could be because of the small sample sizes. Although this is a small trial on a very limited number of RR patients, it is noteworthy that the degree of improvement in enhancing lesions in this study was within the realm of what has been observed previously for the four approved treatments in much larger trials.[27,28]

If larger studies confirm a beneficial effect of estriol treatment on MRI, further studies of estriol treatment for longer periods of time will be needed to determine whether estriol treatment can result in a decrease in relapse rates and disability scores. If estriol is to be given for long periods of time, it should be given in combination with progesterone to protect against uterine endometrial hyperplasia. Data from our patients who reinstituted treatment with estriol and progesterone demonstrated no evidence that progesterone antagonized the beneficial effect of estriol, at least in the short term.

It did not appear that discontinuing estriol treatment caused an increase in MRI disease activity to levels higher than baseline, and there were no significant increases in clinical relapses in the posttreatment period. However, in this study, estriol was tapered over 2 weeks to avoid a precipitous decrease in levels. Interestingly, treatment with pregnancy levels of estrogens during a short period immediately after par-

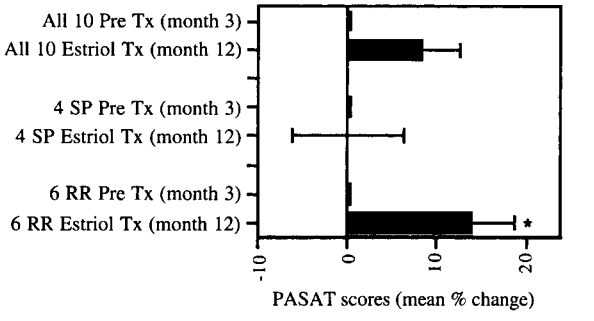


Fig 4. Clinical measures of disease activity. Paced auditory serial addition (PASAT) cognitive testing was performed at study month 3 in the pretreatment period and again at study month 12 at the end of the treatment period. Note that in this test higher scores are better. PASAT scores, expressed as mean percentage change from baseline, were significantly improved in the relapsing remitting (RR) group (p = *0.04), whereas they were unchanged in the secondary progressive (SP) group. Error bars indicate standard errors between patients within each group.* (asterisk) p < *0.05.*

Table. EDSS and Nine-Hole Peg Test Scores, Mean (SE)

	Pretreatment (mo)		Estriol Treatment (mo)		Posttreatment (mo)	
Test	3	6	9	12	15	18
EDSS						
6 RR	2.2 (0.6)	2.0 (0.5)	1.5 (0.7)	1.7 (0.6)	1.8 (0.6)	1.8 (0.5)
4 SP	5.0 (0.9)	5.0 (0.9)	4.9 (1.0)	5.0 (0.9)	5.1 (1.1)	5.0 (0.8)
Nine-Hole peg Test						
6 RR						
R	22.2 (2.4)	21.8 (1.6)	22.5 (2.3)	21.5 (1.9)	21.0 (1.7)	21.4 (2.4)
L	24.8 (3.2)	22.9 (1.6)	24.3 (2.5)	23.3 (2.1)	23.0 (2.1)	22.7 (2.3)
4 SP						
R	26.8 (0.4)	29.9 (2.4)	30.2 (1.4)	31.7 (4.8)	29.4 (5.2)	34.0 (8.7)
L	23.5 (1.4)	25.6 (2.5)	22.7 (1.7)	24.8 (2.6)	26.7 (0.7)	25.0 (1.8)

EDSS = Expanded Disability Status Scale; SE = standard error; RR = relapsing remitting; SP = secondary progressive; R = right; L = left.

turition has been shown to protect mice with collagen-induced arthritis from postpartum flares of the disease.[29] These data suggest that a trial should be designed to test the use of pregnancy doses of estrogens in the postpartum period to determine whether postpartum exacerbations in MS and rheumatoid arthritis might be decreased.

In summary, further study of oral estriol treatment is warranted in RR MS, either as a monotherapy or in combination with other established therapies that rely on immune deviation, such as glatiramer acetate[4] or T-cell receptor vaccination.[30,31] In addition to significantly decreased Th1 responses, other actions of estriol may be possible, such as other immune mechanisms,[32] more direct actions on the blood–brain barrier,[33] or effects on cells in the target organ such as microglia[34] and neurons.[35–38] Finally, if pregnancy doses of oral estriol prove to be of benefit in MS in larger trials, then its use in other putative Th11mediated autoimmune diseases with known improvement during pregnancy should be considered.

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