INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 18, no. 4, 771-778 (2005)

A PILOT, OPEN LABEL, CLINICAL TRIAL USING HYDROXYZINE IN MULTIPLE SCLEROSIS

I. LOGOTHETIS, I. A. MYLONAS, S. BALOYANNIS[1], M. PASHALIDOU, A. OROLOGAS, A. ZAFEIROPOULOS[1], V. KOSTA[1] and T. C. THEOHARIDES[2]

B. Department of Neurology, Aristotle University, AHEPA Hospital, Thessaloniki, Greece;
[1]A. Department of Neurology, Aristotle University, AHEPA Hospital, Thessaloniki, Greece;
[2]Departments of Pharmacology and Experimental Therapeutics, Internal Medicine and Biochemistry, Tufts University School of Medicine, Tufts-New England Medical Center, Boston, MA, USA

Received July 8, 2005 – Accepted September 2, 2005

Multiple sclerosis (MS) is an autoimmune disorder of myelin destruction. Blood-brain-barrier (BBB) disruption precedes pathological or clinical findings and could involve mediators from perivascular brain mast cells, such as histamine and vascular endothelial growth factor (VEGF). Mast cells could be activated by many triggers, including acute stress that has been correlated with MS exacerbations. We considered that the histamine-1 (H_1) receptor antagonist hydroxyzine, which also partially inhibits brain mast cells and has anxiolytic properties, may reduce MS symptoms. This open label, pilot, clinical trial investigated the effect on MS of an oral solution of hydroxyzine (100 mg per day), together with caffeine (200 mg per day) to reduce sedation. Twenty patients (8 males; 12 females) with relapsing-remitting or relapsing-progressive MS completed the study (12 ± 1 months) and were evaluated using disability scales. Most patients on hydroxyzine (75%) remained stable or improved neurologically and all but one showed improved mood. Hydroxyzine could be used as an adjuvant in MS, but the small number of patients enrolled and the short duration of the study precludes any definitive conclusions. A double-blind, placebo-controlled study is warranted.

Multiple sclerosis (MS) is a human demyelinating disease characterized by brain edema and perivascular infiltrates ("cuffing") of mononuclear cells accompanied by various degrees of neurologic disability (1-2). Affected areas fill with fibrotic tissue forming the MS plaques that also contain activated mast cells (3). Blood-brain-barrier (BBB) breakdown precedes any pathological or clinical signs of MS (4-5). Acute stress can disrupt the BBB in rats (6). Most recently, acute stress was shown to increase BBB permeability to ^{99} Technetium-gluceptate through the involvement of brain mast cells (7) and corticotropin-releasing-hormone (CRH) (7). It is, therefore, of interest that MS symptoms can be precipitated by psychological stress (8-9); a meta analysis and two recent publications confirmed a correlation between stress and MS attacks (10).

Mast cells are typically activated by IgE and specific antigen in allergic reactions, as well as in innate and acquired (11) immunity. They have also been increasingly implicated in neuroinflammatory conditions, especially those worsened by stress (12). Mast cells have been associated with brain demyelination (13-14) and are activated in

Key words: blood-brain-barrier, demyelination, hydroxyzine, inflammation, mast cells

Mailing address: T.C. Theoharides, Ph.D., M.D.
Department of Pharmacology and Experimental Therapeutics
Tufts University School of Medicine
136 Harrison Avenue - Boston, MA 02111, USA
Phone: (617) 636-6866 - -Fax: (617) 636-2456
Email: theoharis.theoharides@tufts.edu

0394-6320 (2005)

experimental allergic encephalonyelitis in monkeys (EAE) (15). The involvement of mast cells in MS was recently supported by gene array results from MS plaques that showed increased expression of the high affinity IgE binding protein (FcεRI) and histamine-1 (H_1) receptors, as well as of the unique mast cell protease, tryptase (16). In fact, elevated tryptase had been documented in the CSF of MS patients (17) and can activate peripheral mononuclear cells to secrete pro-inflammatory cytokines (18). We, therefore, hypothesized that a drug capable of inhibiting brain mast cell activation, as well as reducing stress, may be useful in MS.

In this study, we investigated the effect of the heterocyclic piperazine H_1 receptor antagonist hydroxyzine, which penetrates the BBB, on the disability of MS patients. Hydroxyzine had previously been shown to inhibit neurogenic mast cell activation and EAE in rats (19). The present findings suggest that hydroxyzine might be useful in MS.

MATERIALS AND METHODS

Study design

This is an open label, pilot clinical trial investigating the effect of oral hydroxyzine (mixed together with caffeine to counter sedation) in an oral liquid formulation prepared by Muro Pharmaceuticals, Inc. (Tewksbury, MA) under approval by the US FDA (IND #32,519 issued on Jan. 3, 1989) granted to TCT, as well as the respective Human Investigation Review Boards of Tufts-New England Medical Center (Boston, MA) and AHEPA Hospital (Thessaloniki, Greece). The dosage regimen was 2 teaspoonfuls (10 ml) 4 times per day of 0.25% (12.5 mg/5 ml) hydroxyzine HCl and 0.5% (25 mg/5 ml) caffeine that would yield a total daily dose of 100 mg hydroxyzine and 200 mg caffeine. Hydroxyzine HCl is a well known H_1 receptor antagonist with no adverse effects except for sedation that usually subsides after a few days (20).

Patient Information

Twenty-two patients (9 males, 13 females) entered the study. Two patients dropped out and so twenty patients (8 males and 12 females) completed the study (12±1 months). The patients enrolled fulfilled the following criteria:

Documented MS for at least one year.

Age 18 –40 years.

Relapsing-remitting or relapsing-progressive type of MS. Duration of symptoms for at least one year.

At least two exacerbations during the year prior to enrollment; an exacerbation (attack) was considered by the presence of neurologic or other symptoms for at least 36 hours, especially signs and symptoms not previously experienced by the patient.

Absence of any other medical problems that could potentially complicate MS or make the patient's participation risky.

Patients who could understand the nature of the clinical study, including any potential risks, and could sign the consent form of their own free will.

Pregnant females, or patients enrolled in any other clinical study were excluded.

Of the twenty-two Caucasian patients (mean age 32) enrolled, ten patients were followed by the B. Neurology Clinic of AHEPA Hospital in Thessaloniki, five by the A. Neurology Clinic of AHEPA Hospital in Thessaloniki and seven by the Neurology Clinic of the Egineitio Hospital in Athens; however, the first and last examination of all patients were conducted at the AHEPA Hospital in Thessaloniki.

The patients were allowed to continue on any pre-existing treatment for spasticity or bladder control; they were also allowed symptomatic therapy for any unexpected medical problem. However, no drugs "specific" for MS were used. In the case of any MS exacerbations, the patients were treated with steroids according to established protocols.

Grading of MS

Patient examination was conducted at the beginning and the end of the 12-month study, with bimonthly examinations in between, primarily using disability and functionality scales. The general assessment of the patients included the following:

Recording of any or no changes in patients' neurological status according to the Kurtzkes's Expanded Disability Status Scale (EDSS) and the Functional neurologic systems for EDSS including: (i) pyramidal, (ii) cerebellar, (iii) brainstem, (iv) sensory, (v) bladder and bowel, (vi) visual and (vii) cerebral (or mental) functions (21). In addition, use was made of a walking ability index, time in seconds required to walk 5 meters, and an index for upper extremity functionality, (b) entry of all relapses or new symptoms, (c) listing of any adverse effects.

Presentation of results

This was an open label study and no statistical analysis was carried out. The results are presented in terms of the number of patients who improved or remained unchanged and those who worsened. Because of the short duration of the study and the inclusion of both relapsing-remitting and relapsing-progressive patients, it was not possible to generate data and statistics concerning the number of relapses or their duration.

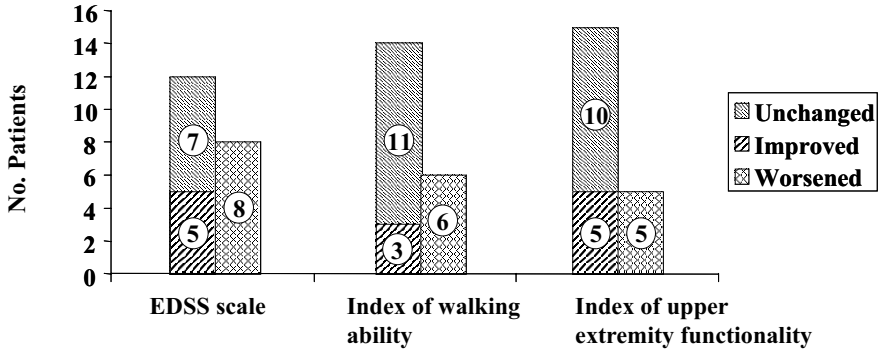

Fig. 1. *Summary of the results of the quantitative assessment of patient disability according to three different different scales. The number of patients who improved remained unchanged or worsened are shown in circles in the respective columns.*
Kurtzke's EDDS;
The Walking Ability Index. Patients were evaluated and assigned a grade from 0 to 9.
0. Asymptomatic, fully active
1.Walks normally but easily getting tired
2. At times unsteady, some gait problem
3. Needs no help to walk but gait abnormal
4. Needs one sided support >80% of the time
5. Constant unilateral or bilateral support
6. Bilateral support, periodic use of wheelchair
7. Several steps with bilateral support, in wheelchair
8. Restricted to wheelchair, able to self-transfer
9. Restricted to wheelchair, unable to self-transfer
Upper Extremity Functionality Index:
0. Essentially normal
1. Mild weakness or unsteadiness of arm, fully capable with some delay
2. Arm moderately disturbed, feeds him/herself, poor in writing, movements unsteady
3. Arm severely disturbed with only limited use, cannot feed him/herself 21
4. The arm is useless but still moving.

RESULTS

Objective finding according to Kurtzke

All patients for this and subsequent evaluations were separated in 3 groups. Those who improved, those who remained stable (unchanged) and those who worsened (Figs. 1 & 2). On the disability status according to Kurtzke's EDSS five patients improved, seven remained stable or had partial improvement and eight patients worsened by one point (Fig. 1).

On the walking ability index, three patients improved, eleven remained unchanged and six worsened (Fig. 1).

The upper extremity functionality index showed that five patients improved, nine were unchanged and six worsened (Fig. 1).

With respect to the individual functional systems for EDSS, the results are presented in Fig. 2: For pyramidal functions, four patients improved, ten remained unchanged and six worsened; for cerebellar functions, five patients improved, ten were unchanged and five worsened; for brainstem functions, five patients again improved, ten were unchanged and five worsened; for sensory functions, five patients improved, eleven remained unchanged and four worsened; for bowel and bladder functions, four improved, thirteen remained stable and three worsened; for visual functions one patient improved, fifteen remained unchanged and four worsened. Finally, for cerebral (or mental) functions three patients improved, fourteen remained unchanged and three worsened.

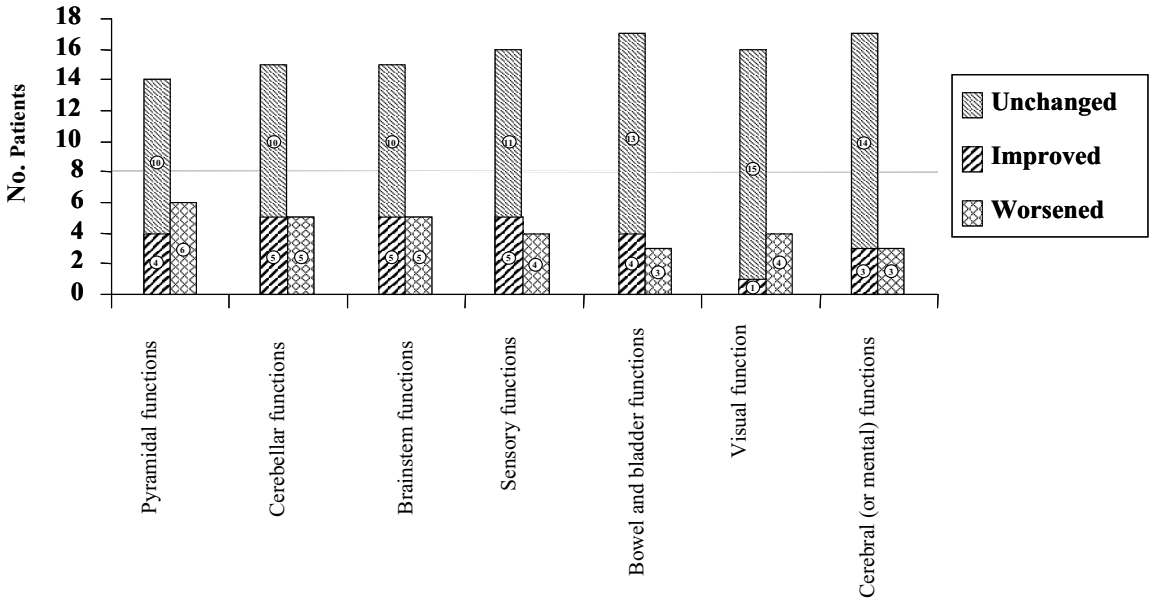

Fig. 2. *Summary of the results of the patient neurologic evaluation using the specific functional systems for EDSS. The number of patients who either improved, remained unchanged or worsened is shown in circles in the corresponding columns.*

If one were to group together the patients who improved and those who remained unchanged, it is apparent from Figures 1 and 2 that about 75% improved or remained stable and 25% worsened.

Observations on patients who improved

Three of the five patients who improved included two who were university students and were able to graduate. Of the seventeen patients presenting with sensory symptoms, five improved significantly and in one the Lhermitte sign disappeared for the first time after six years of MS. Four patients with severe bladder symptoms improved significantly and their improvement lasted throughout the duration of the study. One patient who was cortisone-dependent did not require cortisone any more. Two patients had noticeable improvement in sexual activity. One patient with recurring retrobulbar neuritis especially during her menstrual cycle had no episodes during the course of the clinical study.

In general, all patients but one experienced improvement in their psychological profiles, in self-care and interest in hobbies or work.

Observations on patients who worsened/adverse effects

Four patients with sensory symptomatology had worsening paresthesias and some required use of carbamazepine. Ten patients experienced worsening of their symptoms during the last two months of the study and two of them required hospitalization. The two patients who dropped out of the study did so because of apparent symptom worsening two months after entering the study.

Adverse effects

During the first 20 days of therapy, 7 patients experienced somnolence, as well as increased spasticity and tremor that subsided over time. Three patients reported weight gain.

DISCUSSION

The outcome of this pilot study indicates that while on hydroxyzine, the majority (75%) of the MS patients improved or stabilized. These results are important because, given the nature of MS where most patients are expected to worsen, even maintaining a large number of patients stable is an advantage. This apparent benefit of hydroxyzine

Table I. *Hydroxyzine's properties relevant to MS*

- Anxiolytic, but not muscle relaxant
- Partial mast cell secretion inhibitor
- Partial BBB permeability blocker
- Penetrates into the brain

Unlike other histamine-1 receptor antagonists, hydroxyzine has additional properties that could be useful in the treatment of MS. In particular, it penetrates into the brain and it has mild anxiolytic properties, without causing muscle relaxation such as that caused by benzodiazepines that may be misconstrued as an MS exacerbation.

could not be due simply to its H_1-receptor antagonism because other typical H_1-receptor antagonists, such as diphenhydramine, do not appear to have any effect on MS. Hydroxyzine is a heterocyclic, piperazine H_1-receptor antagonist (Table I) that penetrates the BBB. Hydroxyzine has additional properties relevant to MS; these include anxiolytic actions, without muscle relaxation, and partial inhibition of BBB permeability. Hydroxyzine inhibits secretion from mast cells and rat basophil leukemia cells; interestingly, hydroxyzine's non-sedating metabolite cetirizine (Zyrtec) does not inhibit mast cell activation (22). Hydroxyzine has also been shown to inhibit neurogenic mast cell activation and EAE in rats (19). Administering hydroxyzine in the evening reduces its sedative effect, while permitting its beneficial actions (20).

Increased permeability of the BBB is recognized as a key early event that precedes demyelination or clinical signs in MS (4). We had hypothesized that mast cells might regulate BBB permeability and leukocyte infiltration into the brain (23). Increased BBB permeability due to stress was documented in Gulf war soldiers. Acute stress by immobilization increased BBB permeability that was dependent on mast cells (7) and CRH (24), typically released under stress. Acute stress was also shown to shorten the time of onset of experimental allergic encephalomyelitis (EAE) in mice (25), These findings are relevant in view of the fact that acute stress has been correlated to new episodes and brain lesions in MS patients (8). In fact, recent findings (9) including a meta analysis (10) have correlated stress and MS attacks. The molecular basis of BBB alterations in MS is unknown, but inflammatory mediators have been implicated (26). The involvement of mast cells in BBB permeability (7) is supported by reports that the mast cell secretagogue, compound 48/80, increased BBB permeability in pigeons (27). Mast cell derived molecules are known to increase permeability of the BBB (27) and include histamine (28), bradykinin and vascular endothelial growth factor (VEGF) (29).

Mast cells derive from a distinct precursor in the bone marrow and migrate from the circulation into tissues, including the brain, where they mature and acquire their perivascular localization, in close association to neurons. Mast cells are critical for allergic, autoimmune and neuroinflammatory reactions, especially those exacerbated by stress (12). Mast cells are also a rich source of most known cytokines including TNF−α and could release it upon immunologic stimulation (30). TNF−α (31) and soluble TNF receptor (31) were detected in CSF of MS patients and could participate in BBB impairment (32). The unique mast cell protease tryptase was also increased in the CSF of MS patients (17). Tryptase induces microvascular leakage (33), activates mononuclear cells to secrete pro-inflammatory cytokines (18) and stimulates protease activated receptors (PARs) inducing widespread inflammation.

Mast cells had previously been identified in

brain lesions of MS (3, 13, 34), especially around edematous capillaries (35), including non-human primates (15). Mast cells can be activated by myelin basic protein (MBP) (13), leading to syngeneic brain demyelination and myelin degradation *in vitro*. Moreover, MBP and estradiol showed a synergistic action in triggering mast cell secretion and brain demyelination, both of which are more prominent in EAE susceptible rats (14). Mast cells were also shown to be essential for early onset and severity of EAE in mice, as W/W^V mast cell deficient mice had a delayed and attenuated EAE course (36).

The diagnosis, pathophysiology (1) and therapeutic options in MS were reviewed recently, especially with respect to the involvement of both allergic and autoimmune mechanisms and mast cells in particular (12, 37). Recent findings from gene array analysis of MS plaques (16) and EAE lesions (38) showed upregulation of genes for FcεRI, H_1-receptor and the mast cell marker tryptase. The mixed serotonin and H_1-receptor antagonist cyproheptadine could inhibit brain vascular permeability and reduced EAE, possibly due to its ability (similar to that of hydroxyzine) to inhibit mast cell secretion (39). Inhibition of EAE was also reported by the experimental mast cell "stabilizer" picroximil.

Our present data suggest that hydroxyzine may be useful in MS. Similar findings were obtained from a pilot double-blind, placebo-controlled, clinical trial using the same formulation of hydroxyzine and caffeine on relapsing-remitting MS (40). A randomized, double-blind clinical trial with sufficient number of patients should be in order.

ACKNOWLEDGEMENTS

We thank Ms. Jessica Christian for her patience and her word processing skills. The use of hydroxyzine together with flavonoids for the treatment of MS is covered by US patents No 6,645,482, 6,689,748 and US patent application 10/811,826 awarded to TCT. Aspects of this work were supported in part by grant RG-1961-A-1 from the US National Multiple Sclerosis Society (New York, NY) and a grant from Muro Pharmaceutical, Inc. (Tewksbury, MA) to TCT.

REFERENCES

1. **Smith K.J. and W.I. McDonald.** 1999. The pathophysiology of multiple sclerosis: The mechanisms underlying the production of symptoms and the natural history of the disease. *Philos. Trans. R. Soc. Lond. B. Biol. Sci. 1390:1649*.
2. **Confavreux C., S. Vukusic, T. Moreau and P. Adeleine.** 2000. Relapses and progression of disability in multiple sclerosis. *N. Engl. J. Med. 343:1430*.
3. **Ibrahim M.Z.M., A.T. Reder, R. Lawand, W. Takash and S. Sallouh-Khatib.** 1996. The mast cells of the multiple sclerosis brain. *J. Neuroimmunol. 70:131*.
4. **Kermode A.G., A.J. Thompson, P. Tofts, D.G. MacManus, B.E. Kendall, D.P.E. Kingsley, I.F. Moseley, P. Rudge and W.I. McDonald.** 1990. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. *Brain 113:1477*.
5. **Kwon E.E. and J.W. Prineas.** 1994. Blood-brain barrier abnormalities in longstanding multiple sclerosis lesions. An immunohistochemical study. *J. Neuropathol. Exp. Neurol. 53:625*.
6. **Sharma H.S., J. Cervos-Navarro and P.K. Dey.** 1991. Increased blood-brain barrier permeability following acute short-term swimming exercise in conscious normotensive young rats. *Neuroscience Res. 10:211*.
7. **Esposito P., D. Gheorghe, K. Kandere, X. Pang, R. Conally, S. Jacobson and T.C. Theoharides.** 2001. Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. *Brain. Res. 888:117*.
8. **Mohr D.C., D.E. Goodkin, P. Bacchetti, A.C. Boudewyn, L. Huang, P. Marrietta, W. Cheuk and B. Dee.** 2000. Psychological stress and the subsequent appearances of new brain MRI lesions in MS. *Neurology 55:55*.
9. **Li J., C. Johansen, H. Bronnum-Hansen, E. Stenager, N. Koch-Henriksen and J. Olsen.** 2004. The risk of multiple sclerosis in bereaved parents: A nationwide cohort study in Denmark. *Neurology 62:726*.
10. **Mohr D.C., S.L. Hart, L. Julian, D. Cox and D.**

Pelletier. 2004. Association between stressful life events and exacerbation in multiple sclerosis: a meta-analysis. *B.M.J. 328:731*.

11. **Galli S.J., J. Kalesnikoff, M.A. Grimbaldeston, A.M. Piliponsky, C.M. Williams and M. Tsai.** 2005. Mast cells as "tunable" effector and immunoregulatory cells: recent advances. *Annu. Rev. Immunol. 23:749*.
12. **Theoharides T.C. and D.E. Cochrane.** 2004. Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J. Neuroimmunol. 146:1*.
13. **Johnson D., P.A. Seeldrayers and H.L. Weiner.** 1988. The role of mast cells in demyelination. 1. Myelin proteins are degraded by mast cell proteases and myelin basic protein and P_2 can stimulate mast cell degranulation. *Brain Res. 444:195*.
14. **Theoharides T.C., V. Dimitriadou, R.J. Letourneau, J.J. Rozniecki, H. Vliagoftis and W.S. Boucher.** 1993. Synergistic action of estradiol and myelin basic protein on mast cell secretion and brain demyelination: changes resembling early stages of demyelination. *Neuroscience 57:861*.
15. **Letourneau R., J.J. Rozniecki, V. Dimitriadou and T.C. Theoharides.** 2003. Ultrastructural evidence of brain mast cell activation without degranulation in monkey experimental allergic encephalomyelitis. *J. Neuroimmunol. 145:18*.
16. **Lock C., G. Hermans, R. Pedotti, A. Brendolan, E. Schadt, H. Garren, A. Langer-Gould, S. Strober, B. Cannella, J. Allard, P. Klonowski, A. Austin, N. Lad, N. Kaminski, S.J. Galli, J.R. Oksenberg, C.S. Raine, R. Heller and L. Steinman.** 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat. Med. 8:500*.
17. **Rozniecki J.J., S.L. Hauser, M. Stein, R. Lincoln and T.C. Theoharides.** 1995. Elevated mast cell tryptase in cerebrospinal fluid of multiple sclerosis patients. *Ann. Neurol. 37:63*.
18. **Malamud V., A. Vaaknin, O. Abramsky, M. Mor, L.E. Burgess, A. Ben-Yehudah and H. Lorberboum-Galski.** 2003. Tryptase activates peripheral blood mononuclear cells causing the synthesis and release of TNF-alpha, IL-6 and IL-1 beta: possible relevance to multiple sclerosis. *J. Neuoimmunol. 138:115*.
19. **Dimitriadou V., X. Pang and T.C. Theoharides.** 2000. Hydroxyzine inhibits experimental allergic encephalomyelitis (EAE) and associated brain mast cell activation. *Int. J. Immunopharmacol. 22:673*.
20. **Goetz D.W., J.M. Jacobson, S.J. Apaliski, D.W. Repperger and M.E. Martin.** 1991. Objective antihistamine side effects are mitigated by evening dosing of hydroxyzine. *Ann. Allergy 67:448*.
21. **Kurtzke J.F.** 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology 33:1444*.
22. **Nielsen P.N., P.S. Skov, L.K. Poulsen, M. Schmelz and L.J. Petersen.** 2001. Cetirizine inhibits skin reactions but not mediator release in immediate and developing late-phase allergic cutaneous reactions. A double -blind, placebo-controlled study. *Clin. Exp. Allergy. 31:1378*.
23. **Theoharides T.C.** 1990. Mast cells: the immune gate to the brain. *Life Sci. 46:607*.
24. **Esposito P., N. Chandler, K. Kandere-Grzybowska, S. Basu, S. Jacobson, R. Connolly, D. Tutor and T.C. Theoharides.** 2002. Corticotropin-releasing hormone (CRH) and brain mast cells regulate blood-brain-barrier permeability induced by acute stress. *J. Pharmacol. Exp. Ther. 303:1061*.
25. **Chandler N., S. Jacobson, R. Connolly, P. Esposito and T.C. Theoharides.** 2002. Acute stress shortens the time of onset of experimental allergic encephalomyelitis (EAE) in SJL/J mice. *Brain Behav. Immun. 16:757*.
26. **Abbott N.J.** 2000. Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell Mol. Neurobiol. 20:131*.
27. **Zhuang X., A.-J. Silverman and R. Silver.** 1996. Brain mast cell degranulation regulates blood-brain barrier. *J. Neurobiol. 31:393*.
28. **Boertje S.R., D. Le Beau and C. Williams.** 1989. Blockade of histamine-stimulated alterations in cerebrovascular permeability by the H2-receptor antagonist cimetidine. *Neuropharmacology 28:749*.
29. **Grutzkau A., S. Kruger-Krasagakes, H. Baumeister, C. Schwarz, H. Kogel, P. Welker, U. Lippert, B.M. Henz and A. Moller.** 1998. Synthesis, storage and release of vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) by

human mast cells: Implications for the biological significance of $VEGF_{206}$. *Mol. Biol. Cell 9:875*.

30. **Cocchiara R., A. Bongiovanni, G. Albeggiani, A. Azzolina and D. Geraci.** 1998. Evidence that brain mast cells can modulate neuroinflammatory responses by tumor necrosis factor-α production. *Neuroreport 9:95*.
31. **Hartung H.-P., K. Reiners, J.J. Archelos, M. Michels, P. Seeldrayers, F. Heidenreich, K.W. Pflughaupt and K.V. Toyka.** 1995. Circulating adhesion molecules and tumor necrosis factor receptor in multiple sclerosis: Correlation with magnetic resonance imaging. *Ann. Neurol. 38:186*.
32. **Kim K.S., C.A. Wass, A.S. Cross and S.M. Opal.** 1992. Modulation of blood-brain barrier permeability by tumor necrosis factor and antibody to tumor necrosis factor in the rat. *Lymphokine Cytokine Res. 11:293*.
33. **He S. and A.F. Walls.** 1997. Human mast cell tryptase: a stimulus of microvascular leakage and mast cell activation. *Eur. J. Pharmacol. 328:89*.
34. **Toms R., H.L. Weiner and D. Johnson.** 1990. Identification of IgE-positive cells and mast cells in frozen sections of multiple sclerosis brains. *J. Neuroimmunol. 30:169*.
35. **Krüger P.G.** 2001. Mast cells and multiple sclerosis: a quantitative analysis. *Neuropathol. Appl. Neurobiol. 27:275*.
36. **Secor V.H., W.E. Secor, C.-A. Gutekunst and M.A. Brown.** 2000. Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. *J. Exp. Med. 191:813*.
37. **Zappulla J.P., M. Arock, L.T. Mars and R.S. Liblau.** 2002. Mast cells: new targets for multiple sclerosis therapy? *J. Neuroimmunol. 131:5*.
38. **Pedotti R., J.J. DeVoss, S. Youssef, D. Mitchell, J. Wedemeyer, R. Madanat, H. Garren, P. Fontoura, M. Tsai, S.J. Galli, R.A. Sobel and L. Steinman.** 2003. Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination. *Proc. Natl. Acad. Sci. USA 100:1867*.
39. **Theoharides T.C., S.K. Kops, P.K. Bondy and P.W. Askenase.** 1985. Differential release of serotonin without comparable histamine under diverse conditions in the rat mast cell. *Biochem. Pharmacol. 34:1389*.
40. **Theoharides T.C., M. Stein, K. Spear and S. Hauser.** 2002. A pilot, double-blind, placebo-controlled clinical trial using hydroxyzine in remitting-relapsing multiple sclerosis (RR-MS). *The Pharmacologist 44: A165.*