SAGE-Hindawi Access to Research
Autoimmune Diseases
Volume 2011, Article ID 134592, 6 pages
doi:10.4061/2011/134592

Research Article

The Effects of Omega-3 Fatty Acids on Matrix Metalloproteinase-9 Production and Cell Migration in Human Immune Cells: Implications for Multiple Sclerosis

Lynne Shinto,[1] Gail Marracci,[2] Lauren Bumgarner,[1] and Vijayshree Yadav[1,2]

[1] *Department of Neurology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, CR 120, Portland, OR 97239, USA*
[2] *Research Division, Department of Veterans Affairs Medical Center, Portland, OR 97239, USA*

Correspondence should be addressed to Lynne Shinto, shintol@ohsu.edu

Received 2 April 2011; Accepted 23 May 2011

Academic Editor: D. N. Bourdette

In multiple sclerosis (MS), compromised blood-brain barrier (BBB) integrity contributes to inflammatory T cell migration into the central nervous system. Matrix metalloproteinase-9 (MMP-9) is associated with BBB disruption and subsequent T cell migration into the CNS. The aim of this paper was to evaluate the effects of omega-3 fatty acids on MMP-9 levels and T cell migration. Peripheral blood mononuclear cells (PBMC) from healthy controls were pretreated with two types of omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Cell supernatants were used to determine MMP-9 protein and activity levels. Jurkat cells were pretreated with EPA and DHA and were added to fibronectin-coated transwells to measure T cell migration. EPA and DHA significantly decreased MMP-9 protein levels, MMP-9 activity, and significantly inhibited human T cell migration. The data suggest that omega-3 fatty acids may benefit patients with multiple sclerosis by modulating immune cell production of MMP-9.

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) in which T-lymphocytes, macrophages, and antibodies are believed to be involved in demyelination and axonal damage [1–3]. Although the cause of MS is unknown, there is general agreement that MS results from an acquired immune-dysregulation and aberrant activation leading to T cell-driven inflammatory processes in the CNS that result in demyelination and axonal damage. MS disease modifying therapies act by altering the T cell-driven inflammatory processes in the CNS [4–10]. Given that these therapies are only partially effective, there is still a need to identify novel therapies that are effective and safe.

Matrix metalloproteinase-9 (MMP-9) is one member in a family of proteases that aid in the remodeling of the extracellular matrix, basement membrane, and other tissues in the body by digesting collagen components in these tissues. In MS, MMP-9 is thought to have a significant role in the transmigration of inflammatory cells into the CNS by aiding in the disruption of the blood brain barrier [11]. Several studies have reported higher MMP-9 levels in MS subjects when compared to control subjects [12–15]. Interferon beta (IFN-beta) has the ability to inhibit MMP-9 levels produced from T-lymphocytes and CD4+ T cells [16–18], which is thought to be one mechanism by which this therapy acts to alter the disease course [17].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two forms of long-chain omega-3 fatty acids (omega-3 FA) that are immune cell modulators and have been reported to decrease proinflammatory cytokine levels secreted from stimulated PBMC from patients with MS [19]. Omega-3 FA are reported to significantly decrease levels of MMP-2, -3, -9, and -13 [20–22]. One in vitro study reports a dose-dependent decrease in MMP-9 protein levels secreted from LPS-activated microglial cells that were incubated with either fish oil or an omega-3 FA mixture (53% EPA and 27% DHA) [23]. Reported data on the ability of EPA and DHA to decrease proinflammatory cytokine and MMP-9 levels suggest a potential therapeutic role for these omega-3 FA in

MS. Our group observed a significant decrease in MMP-9 protein secreted from unstimulated PBMC in an open-label study of ten relapsing remitting MS patients [24].

The objectives of the in vitro study were (1) to evaluate different concentrations of EPA and DHA on MMP-9 protein levels secreted from PBMC, (2) to evaluate different concentrations of EPA and DHA on MMP-9 activity secreted from PBMC, and (3) to evaluate different concentrations of EPA and DHA on the migration of human T cells across a fibronectin barrier.

2. Methods and Materials

2.1. Subjects. This study was approved by the Oregon Health & Science University's Institutional Review Board and subjects gave consent before having their blood drawn. Thirteen healthy control subjects were enrolled for a single blood draw for PBMC isolation. Inclusion criteria included subjects that were age 18 years or older with no serious health condition (e.g., neurologic disorder, cardiovascular disease, uncontrolled diabetes, and cancer). Exclusion criteria included any one of the following: pregnancy, omega-3 FA supplementation within 4 weeks of blood draw, eating one serving of fish per week within 4 weeks of blood draw, and daily use of prescription or over the counter anti-inflammatory medication within two weeks of blood draw. PBMC were isolated from the heparinized blood of healthy control subjects within 4 hours of blood draw (Lymphocyte Separation media; Mediatech).

2.2. PBMC Incubation with EPA and DHA. PBMC were resuspended in X-Vivo 15 supplemented with 1% L-glutamine, 100 U/mL penicillin, 100 ug/mL streptomycin, 1% sodium pyruvate, and 25 mM HEPES at 2×10^6 cells/mL. Fatty acids to be used for in vitro incubations were prepared based on the method of Curtis et al. [20]. Briefly, fatty acid free albumin (Sigma-Aldrich) was resuspended at a concentration of 3.5 μg/mL in Tyrode-HEPES buffer (20 mM HEPES, 140 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 11 mM glucose, pH 7.4). EPA, DHA, and oleic acid (OA) were obtained from Sigma-Aldrich and were made up in this buffer as a stock solution of 5 mg/mL, incubated for 16 hr at 37°C with rotation, sterilized, and stored for up to 1 week at −20°C.

After separation, PBMC were pretreated for 2 hours with EPA and DHA at the following concentrations: 10 μg/mL, 25 μg/mL, and 50 μg/mL, followed by stimulation with concanavalin A (ConA). Cell supernatants were collected to determine MMP-9 levels and MMP-9 activity. Oleic acid (OA) is a monounsaturated fatty acid and served as an oil control. Control PBMC were incubated with OA for 2 hours at the following concentrations: 10 μg/mL, 25 μg/mL, and 50 μg/mL, followed by stimulation with ConA. Untreated PBMC that were stimulated with ConA served as the reference control (defined as 100% MMP-9 activity).

2.3. Measurement of MMP-9 Levels and MMP-9 Activity. MMP-9 levels were measured by enzyme-linked immunosorbent assay (R&D Systems Inc, Minneapolis, MN). MMP-9 activity from cell supernatants was determined by gelatin substrate zymography [25].

2.4. Jurkat Cell Culture and Transmigration Assay. A human CD4+ T cell line (Jurkat clone E6-1; ATCC) was chosen for the transmigration assays as CD4+ T cells are implicated in MS pathogenesis. These assays were based on those previously described by our laboratory [25]. Jurkat cell cultures were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, penicillin-streptomycin (100 U/mL and 100 μg/mL, resp.), 1 mM sodium bicarbonate, 25 mM HEPES at 37°C and 5% CO_2.

Cells were added to 3 μm fibronectin-coated transwells (VWR International, Bridgeport, NJ) at 2×10^6 cells per mL in 200 μL 1% FBS medium. 600 μL of medium alone was added to the lower chambers. EPA was added at 10, 30, and 100 μg/mL, DHA at 10, 30, and 100 μg/mL, and control wells were incubated with OA at 30 μg/mL. All reagents were added in equal final concentrations to both upper and lower chambers. Migration was carried out for 20 hours at 37°C. Plates were then shaken for 10 minutes to remove adherent cells from the bottom of the transwells. Transwells were removed and Calcein AM was added at a final concentration of 50 mM and cells were incubated at 37°C for 45 minutes. Fluorescence was read on a Cytofluor 4000 (485λ excitation, 535λ emission). Transmigration assays were performed three separate times; values from a representative experiment are expressed as a percentage of cell fluorescence of saline-treated wells.

2.5. Data Analysis. Data from three different healthy control subjects with identical experimental conditions were analyzed. Statistical analysis was performed using SPSS version 14.0. Migratory capacity of untreated control cells, expressed as percentage of Calcein AM-positive cells in the lower chamber, was defined as 100%; migratory capacity of cells incubated with varying concentrations of DHA, EPA, and OA were calculated as a percentage of the control wells. Similarly, the percentage of MMP-9 activity following treatment with varying concentrations of DHA, EPA, and OA were expressed as a percentage relative to the untreated control (defined as 100%). ANOVA was used to compare MMP-9 protein levels and percent activity. Tukey's HSD was used to compare the difference between each concentration of DHA, EPA, and OA to the untreated control. Standard error (SE) was determined from the standard deviation (SD) derived from the means of the three healthy controls for each omega-3 concentration ($SD/\sqrt{3}$). *T*-test was used to compare percent activity and percent migration of each concentration compared to the untreated control. Significance was defined as $P \leq 0.05$.

3. Results

3.1. Omega-3 FA Effects on PBMC Secreted MMP-9 Levels. Figure 1 shows the effects of DHA, EPA, and the oil control OA on MMP-9 protein levels. DHA at 10 μg/mL decreased mean MMP-9 levels to 85.5 ng/mL (SE ± 10.9; $P = 1.0$), at 25 μg/mL decreased mean MMP-9 levels to 41.5 ng/mL

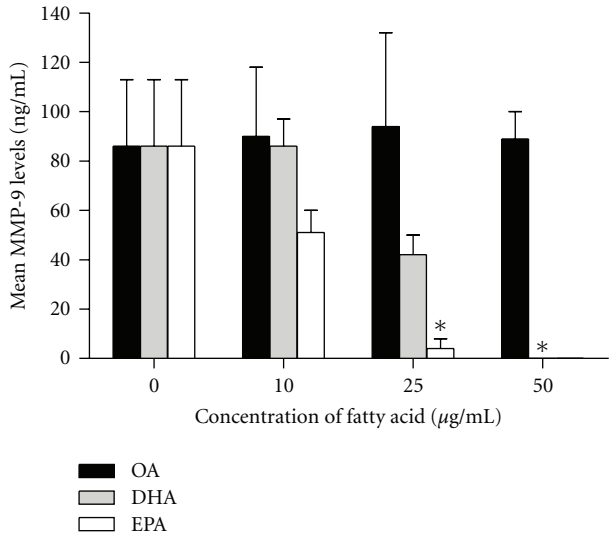


Figure 1: Mean MMP-9 protein levels secreted from PBMC. *PBMC from three different normal controls.* MMP-9 levels secreted from ConA-stimulated PBMC is decreased following treatment with DHA and EPA when compared to an untreated ConA-stimulated PBMC control. OA served as an oil control and showed no significant decrease in MMP-9 levels at any concentration. PBMC were pretreated for 2 hr in the presence of 10, 25, and 50 μg/mL of one of the following, DHA, EPA, or OA. Cells were then stimulated with ConA and supernatants were collected to determine MMP-9 levels by ELISA. *Error bars represent standard error (SE).* *Significant decrease compared to control ($P \leq 0.05$).

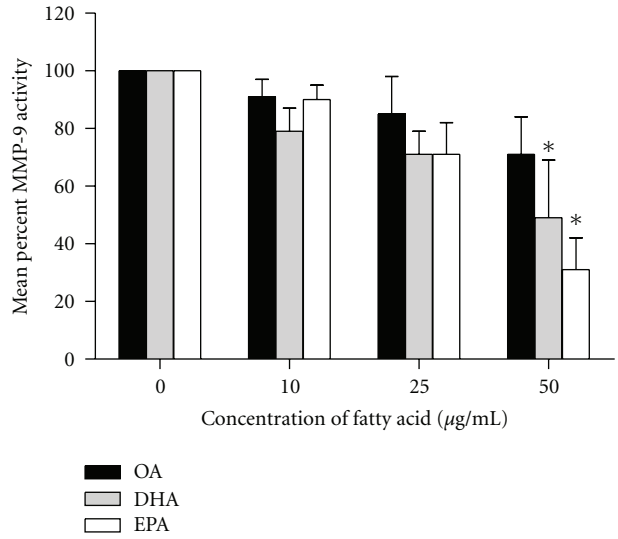


Figure 2: Mean MMP-9 activity from PBMC supernatants. *PBMC from three different normal controls.* MMP-9 activity from supernatants of ConA-stimulated PBMC is decreased following treatment with DHA and EPA when compared to an untreated ConA-stimulated PBMC control (100% MMP-9 activity). OA served as an oil control and showed no significant decrease in MMP-9 levels at any concentration. PBMC were pretreated for 2 hr in the presence of 10, 25, and 50 μg/mL of one of the following, DHA, EPA, or OA. Cells were then stimulated with ConA and supernatants were collected to determine MMP-9 activity by gelatin substrate zymography. *Error bars represent SE.* *Significant decrease compared to control ($P \leq 0.05$).

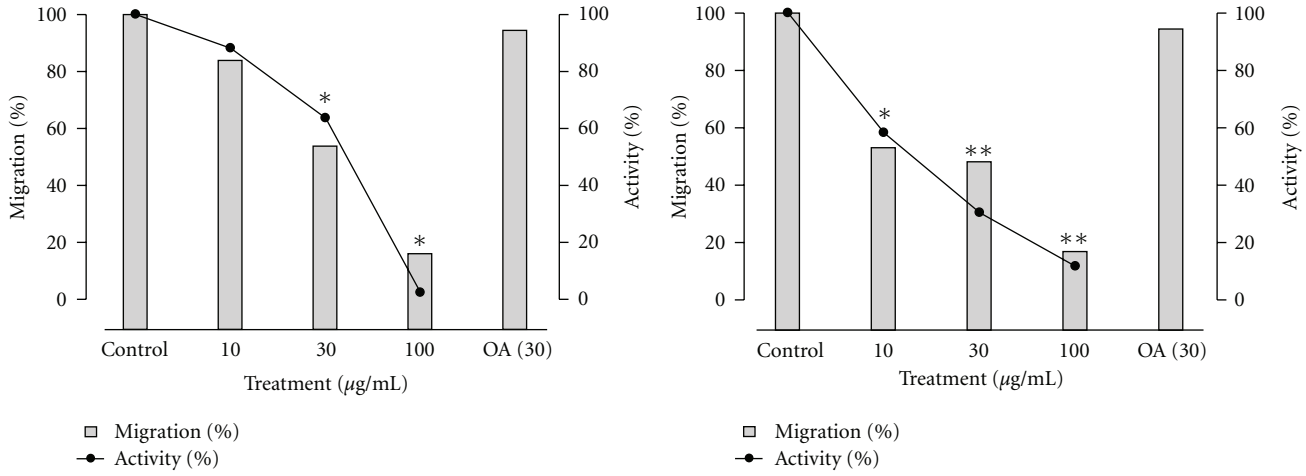
(SE ± 7.5; $P = 0.29$), and at 50 μg/mL deceased MMP-9 levels to non-detectable ($P = 0.05$). EPA at 10 μg/mL decreased mean MMP-9 levels to 50.9 ng/mL (SE ± 9.2; $P = 0.61$), at 25 μg/mL decreased mean MMP-9 levels to 4.4 ng/mL (SE ± 4.4; $P = 0.05$), and at 50 μg/mL decreased MMP-9 levels to non-detectable ($P = 0.04$). There was no significant decrease in MMP-9 levels for any of the OA concentrations ($P = 1.0$ for all concentrations).

3.2. Omega-3 Effects on PBMC Secreted MMP-9 Activity. Figure 2 shows the effect of DHA, EPA, and OA on mean MMP-9 activity. DHA at 10 μg/mL decreased mean MMP-9 activity by 21.5% (SE ± 8.0; $P = 0.57$), at 25 μg/mL decreased mean MMP-9 activity by 29.0% (SE ± 7.5; $P = 0.34$), and at 50 μg/mL decreased mean MMP-9 activity to by 51.4% (SE ± 20.1; $P = 0.05$). EPA at 10 μg/mL decreased mean MMP-9 activity by 9.6% (SE ± 6.0; $P = 0.91$), at 25 μg/mL decreased mean MMP-9 activity by 29.0% (SE ± 11.0) ($P = 0.28$), and at 50 μg/mL decreased mean MMP-9 activity by 69.0% (SE ± 11.0; $P = 0.007$). OA at 10 μg/mL decreased mean activity to 9.0% (SE ± 6.0; $P = 0.90$), at 25 μg/mL decreased mean activity by 14.6% (SE ± 13.0; $P = 0.71$), and at 50 μg/mL decreased mean activity by 29.0% (SE ± 13.0; $P = 0.21$).

3.3. Jurkat Cell Migration Assay. Coincubation of human Jurkat T cells with DHA and EPA reduced their migratory capacity across a pseudo-blood brain barrier in a dose dependent manner (Figures 3(a) and 3(b)). DHA at 30 μg/mL reduced Jurkat cell migration by 46.2% and at 100 μg/mL by 83.9% ($P \leq 0.05$ for both). Culture supernatants collected at the end of the migration period were subjected to electrophoresis and gelatin substrate zymography to assess MMP-9 activity. Corresponding to the effect on migratory capacity, DHA at 30 μg/mL reduced MMP-9 activity by 36.3% and at 100 μg/mL by 97.6% [$P < 0.05$ for both; Figure 3(a)]. Similarly, EPA treatment of Jurkat cell cultures significantly reduced the ability of Jurkat cells to cross a fibronectin barrier with a corresponding reduction in MMP-9 activity. EPA at 10 μg/mL reduced Jurkat cell migration by 46.9%, at 30 μg/mL by 51.8%, and at 100 μg/mL by 83.1% ($P \leq 0.05$ for all). EPA at 30 μg/mL reduced MMP-9 activity by 69.6% and at 100 μg/mL by 88.3% [$P \leq 0.05$ for both; Figure 3(b)]. OA (oil control) at 30 μg/mL reduced Jurkat cell migration by 5.5% ($P = 0.66$), MMP-9 activity was not assessed in cells treated with OA.

4. Discussion

The study shows that DHA and EPA, individually and at low in vitro concentrations, have the ability to decrease MMP-9 production and activity in PBMC from healthy controls. In addition, both types of omega-3 FA were able to inhibit T cell migration through a fibronectin barrier in a concentration-dependent fashion. Cell viability was checked for all in vitro assays and remained between 68–84% for all concentrations



(a) DHA effect on Jurkat cell migration and MMP-9 Activity

(b) EPA effect on Jurkat cell migration and MMP-9 Activity

Figure 3: Jurkat cell migration is decreased following treatment with DHA or EPA when compared to an untreated control (100% migration). MMP-9 activity from Jurkat cell supernatants is decreased following treatment with DHA or EPA when compared to an untreated control (100% activity). Supernatants were collected from the cultures undergoing migration to determine MMP-9 activity by gelatin substrate zymography. Jurkat cells were incubated for 20 hr in the presence of 10, 30, and 100 μg/mL of DHA or EPA. OA (30 μg/mL) served as an oil control for cell migration. *Migration assays were performed three separate times, representative data is displayed.* *Significant decrease in migration compared to control ($P \leq 0.05$). **Significant decrease in Jurkat cell migration and MMP-9 activity compared to control ($P \leq 0.05$).

tested, indicating that the effects observed were not a result of cell death.

MMP-9-mediated disruption of the blood brain barrier is one mechanism in which inflammatory cells enter the CNS in MS. MS patients are reported to have significantly elevated MMP-9 mRNA levels when compared to healthy controls. The increased MMP-9 expression in immune cells (PBMC) may enable these cells to more readily migrate across the blood brain barrier and enter the CNS [26, 27]. Stüve et al. observed that activated T-lymphocytes isolated from untreated MS patients were able to migrate through a fibronectin barrier due to increased MMP-9 activity. T-lymphocytes treated with IFN-beta decreased the migratory ability of these cells [18]. Dressel et al. observed that the migration of CD4+ T cells through a fibronectin barrier, but not CD8+ cells, was enhanced in untreated MS patients compared with controls and that CD4+ cell migration and MMP-9 production were reduced by IFN-beta treatment [16]. Decreasing MMP-9 expression and MMP-9 protein levels in T-lymphocytes is one proposed mechanism for the therapeutic action of IFN-beta therapy in MS [17].

In the present study, we demonstrated that EPA and DHA were able to decrease MMP-9 protein levels and activity at relatively low concentrations. Incubation of PBMC from healthy controls with DHA and EPA at 50 μg/mL reduced MMP-9 levels to non-detectable levels and reduced MMP-9 activity by 51% and 69%, respectively. Oleic acid, a monounsaturated fatty acid, served as an oil control and did not significantly decrease MMP-9 level or activity (Figures 1 and 2). In addition, EPA at as low as 10 μg/famL and DHA at 30 μg/mL significantly inhibited T cell migration through a fibronectin barrier and this inhibition was associated with decreased MMP-9 activity [Figures 3(a) and 3(b)]. Oleic acid did not significantly inhibit T cell migration. These findings indicate that the omega-3 FA, DHA and EPA, have the ability to decrease immune cell secretion of MMP-9 protein and reduce its activity; together, these modulatory effects on MMP-9 may impede cell migration across the basement membrane of the CNS.

Mean C_{max} levels in plasma are reported at 7.7 mg/dL for EPA and 9.8 mg/dL for DHA after supplementation with fish oil at a daily oral dose of 3.0 grams (containing 0.57 grams EPA and 0.45 grams DHA) [28]. In the present study, the in vitro concentration of EPA and DHA at 5 mg/dL (50 μg/mL) showed a decrease in MMP-9 levels, activity, and a decrease in T cell migration. The results presented suggest that it may be possible to decrease MMP-9 levels at a dose of omega-3 FA that is one-third of the dose reported to decrease MMP-9 levels in MS subjects [24]. There are limited studies evaluating the effects of omega-3 FA supplementation in MS and only one study evaluating omega-3 FA effects on MS disease activity. One double-blind placebo-controlled trial that randomized MS patients ($n = 312$) to 20 fish oil capsules/day or an olive oil placebo reported a trend in improvement in the fish-oil-treated subjects compared to controls in disease severity (Expanded Disability Status Scale) over 2 years ($P = 0.07$) [29]. The olive oil placebo contained 72% oleic acid, and the fish oil contained EPA 1.71 grams/day and DHA 1.41 grams/day. One study reported a significant decrease from baseline in the levels of the proinflammatory cytokines Interleukin-1beta (IL-1 β) ($P < 0.03$), tumor necrosis factor-alpha (TNF-α) ($P < 0.02$), IL-2 ($P < 0.002$), and IFN-γ ($P < 0.01$), produced from unstimulated and stimulated PBMC of MS subjects and healthy controls supplemented with fish oil [19]. Our group has reported a decrease in MMP-9 levels from baseline in an open label

study of ten relapsing remitting MS patients receiving fish oil concentrate at 8 grams/day (2.9 grams EPA and 1.9 grams DHA) over 3 months. The reduced MMP-9 levels were observed in all ten subjects whether or not they were taking MS disease modifying medication [24]. Drawing from the limited clinical trial reports, there is some suggestion that omega-3 fatty acids have immunomodulatory properties that may be beneficial in MS. Further exploration on the optimal dosing of omega-3 FA in MS is needed.

5. Conclusions

The presented combined results from the in vitro study and the open-label study in MS suggest that DHA and EPA omega-3 FA may have a role in inhibiting the migration of activated immune cells into the central nervous system and that further investigation of omega-3 FA in MS is warranted.

Acknowledgments

This paper was funded by National Institutes of Health Grant P50 AT00066-01, Biomedical Laboratory R&D Service, Department of Veterans Affairs, and the Nancy Davis Center Without Walls. L. Shinto and G. Marracci contributed equally to this paper.

References

[1] D. A. Hafler and H. L. Weiner, "Immunologic mechanisms and therapy in multiple sclerosis," *Immunological Reviews*, no. 144, pp. 75–107, 1995.

[2] R. Martin, H. F. McFarland, and D. E. McFarlin, "Immunological aspects of demyelinating diseases," *Annual Review of Immunology*, vol. 10, pp. 153–187, 1992.

[3] Y. Morales, J. E. Parisi, and C. F. Lucchinetti, "The pathology of multiple sclerosis: evidence for heterogeneity," *Advances in Neurology*, vol. 98, pp. 27–45, 2006.

[4] L. Kappos, E. W. Radue, P. O'Connor et al., "A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis," *New England Journal of Medicine*, vol. 362, no. 5, pp. 387–401, 2010.

[5] The IFNB Multiple Sclerosis Study Group, "Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial," *Neurology*, vol. 43, no. 4 I, pp. 655–661, 1993.

[6] L. D. Jacobs, D. L. Cookfair, R. A. Rudick et al., "Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis," *Annals of Neurology*, vol. 39, no. 3, pp. 285–294, 1996.

[7] K. P. Johnson, B. R. Brooks, J. A. Cohen et al., "Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double- blind, placebo-controlled trial," *Neurology*, vol. 45, no. 7, pp. 1268–1276, 1995.

[8] PRISMS and S. Group, "Randomised double-blind placebo-controlled study of interferon β-1a in relapsing/remitting multiple sclerosis," *Lancet*, vol. 352, no. 9139, pp. 1498–1504, 1998.

[9] M. Niino, C. Bodner, M. L. Simard et al., "Natalizumab effects on immune cell responses in multiple sclerosis," *Annals of Neurology*, vol. 59, no. 5, pp. 748–754, 2006.

[10] R. A. Rudick, J. C. Lee, J. Simon, and E. Fisher, "Significance of T2 lesions in multiple sclerosis: a 13-year longitudinal study," *Annals of Neurology*, vol. 60, no. 2, pp. 236–242, 2006.

[11] D. Leppert, R. L. P. Lindberg, L. Kappos, and S. L. Leib, "Matrix metalloproteinases: multifunctional effectors of inflammation in multiple sclerosis and bacterial meningitis," *Brain Research Reviews*, vol. 36, no. 2-3, pp. 249–257, 2001.

[12] M. A. Lee, J. Palace, G. Stabler, J. Ford, A. Gearing, and K. Miller, "Serum gelatinase B, TIMP-1 and TIMP-2 levels in multiple sclerosis. A longitudinal clinical and MRI study," *Brain*, vol. 122, no. 2, pp. 191–197, 1999.

[13] M. Trojano, C. Avolio, G. M. Liuzzi et al., "Changes of serum sICAM-1 and MMP-9 induced by rIFNβ-1b treatment in relapsing-remitting MS," *Neurology*, vol. 53, no. 7, pp. 1402–1408, 1999.

[14] E. Waubant, D. E. Goodkin, L. Gee et al., "Serum MMP-9 and TIMP-1 levels are related to MRI activity in relapsing multiple sclerosis," *Neurology*, vol. 53, no. 7, pp. 1397–1401, 1999.

[15] A. Bar-Or, R. K. Nuttall, M. Duddy et al., "Analyses of all matrix metalloproteinase members in leukocytes emphasize monocytes as major inflammatory mediators in multiple sclerosis," *Brain*, vol. 126, no. 12, pp. 2738–2749, 2003.

[16] A. Dressel, D. Mirowska-Guzel, C. Gerlach, and F. Weber, "Migration of T-cell subsets in multiple sclerosis and the effect of interferon-β1a," *Acta Neurologica Scandinavica*, vol. 116, no. 3, pp. 164–168, 2007.

[17] D. Leppert, E. Waubant, M. R. Bürk, J. R. Oksenberg, and S. L. Hauser, "Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis," *Annals of Neurology*, vol. 40, no. 6, pp. 846–852, 1996.

[18] O. Stüve, N. P. Dooley, J. H. Uhm et al., "Interferon β-1b decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9," *Annals of Neurology*, vol. 40, no. 6, pp. 853–863, 1996.

[19] V. Gallai, P. Sarchielli, A. Trequattrini et al., "Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids," *Journal of Neuroimmunology*, vol. 56, no. 2, pp. 143–153, 1995.

[20] C. L. Curtis, S. G. Rees, J. Cramp et al., "Effects of n-3 fatty acids on cartilage metabolism," *Proceedings of the Nutrition Society*, vol. 61, no. 3, pp. 381–389, 2002.

[21] M. A. Harris, R. A. Hansen, P. Vidsudhiphan et al., "Effects of conjugated linoleic acids and docosahexaenoic acid on rat liver and reproductive tissue fatty acids, prostaglandins and matrix metalloproteinase production," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 65, no. 1, pp. 23–29, 2001.

[22] A. J. McCabe, J. Wallace, W. S. Gilmore, J. J. Strain, and H. McGlynn, "The effect of eicosapentanoic acid on matrix metalloproteinase gene expression," *Lipids*, vol. 34, no. 6, pp. S217–S218, 1999.

[23] G. M. Liuzzi, T. Latronico, R. Rossano, S. Viggiani, A. Fasano, and P. Riccio, "Inhibitory effect of polyunsaturated fatty acids on MMP-9 release from microglial cells—implications for complementary multiple sclerosis treatment," *Neurochemical Research*, vol. 32, no. 12, pp. 2184–2193, 2007.

[24] L. Shinto, G. Marracci, S. Baldauf-Wagner et al., "Omega-3 fatty acid supplementation decreases matrix metalloproteinase-9 production in relapsing-remitting multiple sclerosis," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 80, no. 2-3, pp. 131–136, 2009.

[25] G. H. Marracci, R. E. Jones, G. P. McKeon, and D. N. Bourdette, "Alpha lipoic acid inhibits T cell migration into

the spinal cord and suppresses and treats experimental autoimmune encephalomyelitis," *Journal of Neuroimmunology*, vol. 131, no. 1-2, pp. 104–114, 2002.

[26] R. Lichtinghagen, T. Seifert, A. Kracke, S. Marckmann, U. Wurster, and F. Heidenreich, "Expression of matrix metalloproteinase-9 and its inhibitors in mononuclear blood cells of patients with multiple sclerosis," *Journal of Neuroimmunology*, vol. 99, no. 1, pp. 19–26, 1999.

[27] V. Özenci, L. Rinaldi, N. Teleshova et al., "Metalloproteinases and their tissue inhibitors in multiple sclerosis," *Journal of Autoimmunity*, vol. 12, no. 4, pp. 297–303, 1999.

[28] T. A. Marsen, M. Pollok, K. Oette, and C. A. Baldamus, "Pharmacokinetics of Omega-3-fatty acids during ingestion of fish oil preparations," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 46, no. 3, pp. 191–196, 1992.

[29] D. Bates, N. E. F. Cartlidge, J. M. French et al., "A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis," *Journal of Neurology Neurosurgery and Psychiatry*, vol. 52, no. 1, pp. 18–22, 1989.