ATORVASTATIN AND MINOCYCLINE, EFFECTIVE COMBINATION IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental Autoimmune Encephalomyelitis (EAE), the available animal model for Multiple Sclerosis (MS), is a T cell-mediated autoimmune disease. Immunomodulators combined with neuroprotective drugs may represent a useful treatment in MS. We tested the effect of combined treatment of Minocycline, that exhibits multiple anti-inflammatory effects with neuroprotective properties, and Atorvastatin, an immunomodulator. C57Bl6/j female mice were immunized with MOG_{35-55} peptide emulsified in complete Freund's adjuvant. Immunized mice were divided into four groups treated with: Minocycline (50 mg/kg, i.p.), Atorvastatin (1 mg/kg, os), Minocycline (50 mg/kg, i.p.) plus Atorvastatin (1 mg/kg, os), and PBS. The mice were sacrificed 26 and 50 days post immunization (p.i.). Weight loss and clinical signs were examined daily. Anti-MOG antibody response was assessed by solid-phase ELISA (26^{th} day p.i.). Histo- and immunohistochemical studies were carried out on paraffin-embedded spinal cord sections. Inflammation was assessed by the presence of inflammatory infiltrates stained with hematoxylin and eosin an by the glial fibrillary acidic protein (GFAP) immunoreactivity. Axonal and neuronal pathology were assessed by Bielshowsky staining and by the antibody anti NeuN, respectively. Treatment of mice with either atorvastatin or minocycline alone did not alter disease severity until day 36 and day 46 p.i., respectively. Combined treatment, markedly ameliorated the EAE in a statistically significant way by 22 to 30 days p.i. The number per section of inflammatory infiltrates in mice sacrificed at day 26 p.i. was significantly ($p<0.001$) decreased in all treated mice. The demyelination index demonstrated, at day 26 p.i, a beneficial outcome for all three treatments with a median score of 1 for the mice individually treated and 0 for mice treated with the two drugs. We have found by the stereological analysis that activated astrocytes are significantly reduced in mice treated with atorvastatin plus minocycline ($p<0.05$) in comparison to the EAE-PBS mice. In the same group of treated animals neurons are increased in a statistically significant way ($p<0.01$), even if a less marked increase was observed in the group treated with atorvastatin ($p<0.05$). At the onset of clinical signs, we observed a significant reduction of peptide specific proliferation in all pharmacologically treated animals in comparison to the EAE-PBS treated group. Moreover combined treatment resulted in a significant reduction of MOG-antibody response ($p<0.001$). In conclusion these data demonstrate the usefulness of the combined treatment in the therapy of MS. Supported by MIUR 2005 and University of Florence.