dilutions (1:10) of MBX-400. Lastly, infected cells were left untreated for three days, upon addition of drug at 20.0 μM, the proviral load plateaued throughout the remaining seven-day period. Therefore, it is acknowledged that treatment of HHV-6B SUPT-1 infected cells with 20.0 μM of 2,2-bis-hydroxymethyl-cyclopropavir, MBX-400, inhibited proviral load for both the supernatant and cell lysate. Further studies are being conducted using the same model with HHV-6A. The sponsoring organization is Viral Immunology Section, NINDS/NIH, Bethesda, Maryland, United States.

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Testing direct neuroprotective effects of current MS therapeutic compounds with intravital two photon laser scanning microscopy

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Multiple sclerosis (MS) is a chronic, potentially highly disabling autoimmune neurological disease that commonly affects young adults. Neuronal injury is now considered a major part of MS pathogenesis, but few of current therapies are directly targeting neuronal disease mechanisms. It is conceivable, however, that current as well as novel disease modifying drugs derive at least part of their therapeutic action through direct neuroprotective effects. Using intravital two photon laser scanning microscopy, our group previously observed that lymphocyte-mediated long-term calcium up-regulation, leading to dysfunction and eventually neuron death, is partially reversible by NMDA receptor blockade. We presently mimicked lymphocyte-induced excitotoxicity in neurons by applying glutamate to live brain tissue of B6.thy1-TN-XXL mice, and measured live calcium signaling in neurons using two-photon imaging. Following glutamate exposure of 30 min, we applied therapeutic drugs and determined neuro-/axonal calcium changes in vivo. We have found that dimethyl fumerate (DMF), an effective MS treatment that is supposed to exhibit both anti-inflammatory and neuroprotective function, decreases calcium signal intensity, while glutamate increases it. DMF also decreased caspase 3/7 activity when directly applied to primary cortical neurons in vitro. Other therapeutic compounds, including *Fingolimod*, *Laquinimod*, *Modafinil*, *Fasudil* and *Glibenclamide* are currently being tested. These ongoing studies will provide a comprehensive, inclusive overview of the direct neuroprotective potential of current and novel MS therapeutics.

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Fasudil ameliorates disease progression in experimental autoimmune encephalomyelitis, acting possibly through immunomodulation effect

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Background: To study the roles of Fasudil on macrophages, T cells and TLR-4/NF-kappaB signaling pathway for the mechanisms underlying the amelioration of EAE. *Methods:* Splenic encephalomyelitic mononuclear cells (MNCs) from mice immunized with MOG_{35-55} on day 9 post-immunization were obtained and treated with/without Fasudil in vitro for 72 h in the presence or absence of MOG_{35-55}. We analyzed the variances of macrophages phenotypes and T cell subgroups by flow cytometry, the expressions of iNOS, Arg-1, TLR-4 and p-NF-kappaB/p65 by Western blot, the production of NO by Griess reaction, the levels of Arg-1 and cytokines by ELISA. Then MNCs treated with/without Fasudil were resuspended at 5×10^7 cells in 500 μl PBS and transferred by intraperitoneal injection into naïve C57BL/6 recipients. The body weight and clinical scores were recorded every other day after EAE induction.
Results: MNCs treated in vitro with Fasudil declined the expression of CD16/32 and IL-12 on F4/80-macrophages, but elevated the expression of CD206, CD23 and IL-10 on F4/80-macrophages. Using western blot, we found the level of iNOS was decreased and the level of Arg-1 was enhanced in Fasudil-treated MNCs. Simultaneously, Fasudil inhibited the production of NO and increased the concentration of Arg-1 in cultured supernatants. Fasudil-primed MNCs showed the decreased population of $CD4^+$IFN-gamma$^+$, $CD4^+$IL-17^+ and the increased population of $CD4^+$IL-10^+, $CD4^+$TGF-beta$^+$. Moreover, the modulation of Fasudil on T cells and macrophages was MOG-independent. In vitro experiments indicate that p-NF-kappaB/p65 and inflammatory cytokine TNF-alpha were inhibited in Fasudil-treated MNCs. The experiment of adoptive transfer further displayed Fasudil-treated encephalitogenic MNCs did not trigger the development of EAE, and inhibited the expression of TLR-4/p-NF-kappaB/p65 and production of inflammatory cytokine IL-1beta, IL-6 and TNF-alpha in spinal cord.
Conclusions: Fasudil is able to trigger inflammatory M1 macrophages to anti-inflammatory M2 macrophages, converts encephalomyelitic T cells to Tregs, influences TLR-4/p-NF-kappaB/p65 inflammatory pathway, which in part defines that Fasudil has therapeutic potential in EAE through regulating the polarization of macrophage and the balance of immune cells during disease progression of EAE. This study was supported by NSFC (81272163, 81070957) and 2011PY-1.
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Effect of ethyl pyruvate on central nervous system inflammation

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The ongoing success of dimethyl fumarate (DMF; Tecfidera) opens a perspective for further development of the redox strategy in multiple

sclerosis (MS) treatment. The mechanisms of anti-inflammatory and neuroprotective effects of DMF involve the formation of adducts with redox-sensitive thiol switches on the surface of T cell resulting in suppressed activity, proliferation and pro-inflammatory cytokine release, and the activation of antioxidative defense in the central nervous system (CNS) cells. Ethyl pyruvate (EP) appears to be a safer non-toxic redox analogue of DMF, as they share common molecular targets. We examined the effects of EP on CNS resident cells (astrocytes, microglia) and encephalitogenic T cells, as well as its influence on the clinical course of actively induced experimental autoimmune encephalomyelitis (EAE). Astrocytes and microglia were stimulated with pro-inflammatory cytokines and treated with EP in vitro. Encephalitogenic immune cells were isolated from draining lymph nodes of rats that were immunized with myelin basic protein (MBP) and complete Freund's adjuvant (CFA) and treated with EP in vivo. EP modulated cytokine generation in the examined cells in an anti-inflammatory manner: (i) the production of interleukin (IL)-6, but not IL-10, was down-regulated in astrocytes; (ii) the release of IL-6, tumor necrosis factor and nitric oxide from microglial cells was reduced; (iii) the production of interferon-gamma and IL-17, but not IL-10, was suppressed in lymph node cells isolated from MBP + CFA-immunized rats. In addition, EP alleviated EAE course in rats, delaying the onset, shortening the relapse, and lowering clinical scores. These results imply that EP has a potency to inhibit inflammation in the CNS. While further studies on the effect of EP on the CNS inflammation are warranted, we believe that EP might be applicable and beneficial in MS treatment.

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Mental health problems treated with medicinal plants by the Khyang of Chittagong Hill Tracts within Bangladesh: From bench to bedside

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Bangladesh has a number of aboriginal groups distributed throughout the various hilly and forested regions of the country. Although some degree of modernization is creeping in, the various aboriginals still rely on their aboriginal medicinal practitioners (AMPs) for treatment of various ailments. The AMPs usually administer various formulations of plants for treatment of diseases. Because this practice has continued for centuries, and because of the forested regions of the aboriginal habitats, the AMPs have an extensive knowledge of plants and their specific uses. The studies were conducted among the aboriginal medicinal practitioners of a large Khyang group of people residing on the Chittagong Hill Tracts within Bangladesh to gather information on plants used to treat various types of mental health problems — a debilitating disease affecting millions of people throughout the world. Aboriginal medicinal practitioners were interviewed with the help of a semi-structured questionnaire and plant specimens collected from the AMPs through guided field-walks in which the AMPs pointed out various plants and mentioned the uses for these plants. The various plants used by the Khyang AMPs for treatment of mental health problems included *Abrus precatorius* L., *Achyranthes aspera* L., *Agaricus campestris* L., *Aloe vera* (L.) Burm.f., *Asparagus racemosus* Willd., *Bacopa monnieri* (L.) Wettst., *Calotropis gigantea* (L.) Dryand., *Cannabis sativa* L., *Cinnamomum camphora* (L.) J.Presl, *Citrus aurantiifolia* (Christm.) Swingle, *Cocos nucifera* L., *Geodorum densiflorum* (Lam.) Schltr., *Helichrysum luteoalbum* (L.) Rchb., *Ipomoea aquatica* Forssk., *Lawsonia inermis* L., *Nicotiana tabacum* L., *Nigella sativa* L., *Ocimum gratissimum* L., *Oroxylum indicum* (L.) Kurz, *Plumbago indica* L., *Rauvolfia serpentina* (L.) Benth. ex Kurz, *Santalum album* L., *Trichosanthes kirilowii* Maxim., and *Zingiber officinale* Roscoe. It is concluded that the afore-mentioned plants should be more thoroughly studied scientifically towards discovery of anti-mental health problems principles, because modern medicine does not have any absolute cure for mental health problems.

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Dimethyl fumarate alters microglia phenotype and protects neurons against proinflammatory toxic microenvironments

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Pathological activation of central nervous system (CNS) resident microglia is considered to be a key factor in the progression of multiple sclerosis (MS) and other neurodegenerative disorders. Activated microglia can drive and propagate oxidative stress and proinflammatory responses within the CNS, damaging sensitive cells such as neurons and oligodendrocytes. Altering the phenotype of microglia, and subsequent response to inflammatory stress, is a viable therapeutic approach toward the reduction of neuroinflammation and an increase in the population of potentially protective cells. Protection of CNS cells against toxic stress by these means could ameliorate disease pathology.

Delayed-release dimethyl fumarate (DMF) is approved for the treatment of MS in the United States, Canada, Australia and within the European Union. The immunomodulatory and cytoprotective properties of DMF have been described, however direct effects on microglia and microglia-associated pathological actions have not been well characterized.

Here we demonstrate that DMF can alter microglia inflammatory responses by significantly reducing proinflammatory cytokine and chemokine production (IL-6, IL-12, TNF-alpha, RANTES and CCL5) in primary cultures of rodent microglia after an inflammatory challenge. These cytokines/chemokines are the same as those expressed at elevated levels in the inflamed CNS of MS patients, suggesting that DMF could exert a beneficial anti-inflammatory effect. To assess the effect of DMF-treated microglia on neuronal survival, conditioned media from inflammatory-stimulated (lipopolysaccharide plus interferon gamma: LPS + IFN-gamma) primary microglia cultures were treated with, or without DMF then transferred onto naive primary cortical neurons. The oxygen consumption rate (OCR) in the neurons was measured at 6 h with an XF-96 analyzer (Seahorse Biosciences). Conditioned media from microglia stimulated with LPS caused a reduction in basal OCR, ATP production and maximal respiration in the recipient cortical neurons. In contrast, mitochondria respiration was restored in neurons that were incubated with conditioned media from DMF + LPS stimulated microglia. These data demonstrate an indirect protective effect of DMF on neurons via alteration of microglia inflammatory responses.

These data provide evidence that DMF can modulate the inflammatory response in stimulated microglia and subsequently reduce the toxicity of inflamed microglia on the neurons.

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