Cerebral biochemical pathways in experimental autoimmune encephalomyelitis and effects of adjuvants: a metabolomic NMR spectroscopy study

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Introduction

Metabolomics, the comprehensive investigation of tissue metabolism, is increasingly being used to characterize disease and identify treatment targets. However, metabolomic studies are still sparse in multiple sclerosis (MS), and are extremely rare in its animal modwel, experimental autoimmune encephalomyelitis (EAE). We have previously demonstrated the feasibility and potential of metabolomic EAE research by NMR spectroscopy of brain extracts [1]. In continuation of this work, we now present the results of an application of this method to the determination of metabolic effects of injecting rats with complete Freund's adjuvant (CFA) and spinal-cord homogenate (SC-H). CFA is a well established mycobacteria-containing immunopotentiator that is employed with SC-H or brain homogenate to facilitate active induction of EAE in laboratory animals. Metabolic fingerprints characteristic of biochemical events caused by these treatments are analyzed. The results of this comprehensive study are interpreted in conjunction with histological results obtained from the same animals. The final objective of this work is to gain detailed insight into the mechanisms involved in this EAE model.

Methods

One gram Guinea pig SC and 1ml saline were homogenized, and the resulting SC-H was then emulsified in 2 ml of incomplete Freund's adjuvant supplemented with 20 mg of *Mycobacteria tuberculosis* (Mt) H37Ra, as described previously with slight modifications [2]. One group of Lewis rats (n=6) received 0.1 ml of the CFA/SC-H emulsion subcutaneously at the basis of the tail. A second group (n=9) received 0.1 ml of the emulsion containing CFA (500 µg Mt) without SC-H, under the same conditions. A third group (n=9) was not injected and served as a control group (Contr). CFA/SC-H rats were anesthetized by isoflurane inhalation and sacrificed by cervical dislocation on the day they displayed clinical signs of EAE (usually 12-15 days after immunization). On the same day CFA and control rats were sacrificed for comparison. The brain was swiftly removed, freeze-clamped and stored at -80°C for metabolomic MR spectroscopy after tissue extraction with methanol/chloroform/water (4:4:4 ml). Several brain hemispheres were stored in formol for histology. The aqueous and organic extract phases were separated and analyzed by ^{1}H and ^{31}P NMR spectroscopy at 400.1 and 162.0 MHz, respectively, on an AVANCE 400 spectrometer (Bruker) as described previously [3]. Subsequently, metabolites [µmol/g tissue] were quantified using Bruker's Topspin software [3]. Both parametric and non-parametric methods were employed for statistical evaluation (Prism 5.0, GraphPad, San Diego, CA, USA), complemented with multivariate analyses (JMP 9.0.0, SAS Institute, Cary, NC, USA).

Results

Selected regions of a high-resolution ^{1}H NMR spectrum of the aqueous phase of a rat brain extract (Fig. 1) show metabolites used in inter-group comparisons (Fig. 2).

Figure 1

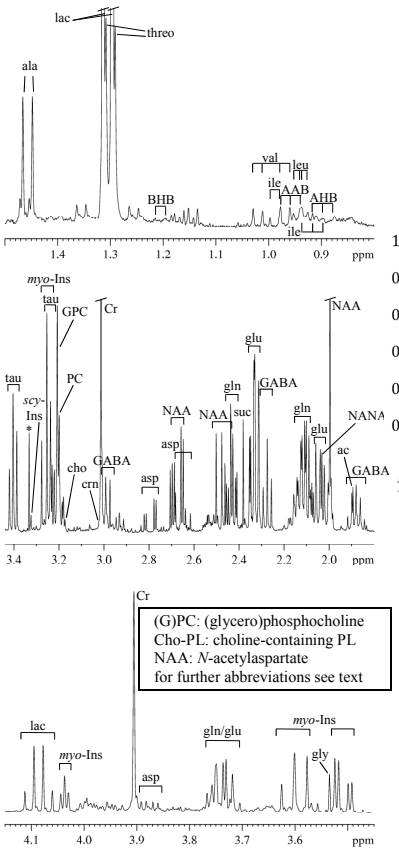
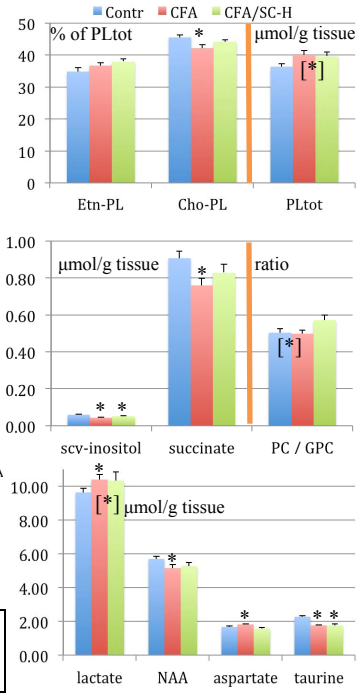


Figure 2



The concentrations of some of these water-soluble metabolites varied significantly when CFA and CFA/SC-H groups were compared individually to the Contr group using multiple-comparison statistics ($p<0.05$, * in Fig.2). Further data evaluation yielded significant linear trends for additional metabolites, notably β-hydroxybutyrate (BHB) and choline (not shown). The PC/GPC ratio was significantly higher in CFA/SC-H than in Contr and CFA combined ($p<0.05$, [*]).Taurine and *scyllo*-inositol were decreased, and lactate was increased in both treated groups vs. Contr. *Myo*-inositol showed a minor trend towards increased values ($p = 0.2$) with increasing treatment. CFA effects on NAA, succinate and aspartate concentrations were larger than CFA/SC-H effects. Finally, all major phospholipid (PL) classes tended to be increased in CFA and CFA/SC-H vs. Contr, in particular ethanolamine-containing PLs (Etn-PL, Fig. 2). Typical ^{31}P NMR spectra of rat brain PLs have been presented elsewhere [3].

Histology revealed major cellular infiltration and mild demyelination in the brain of CFA/SC-H-treated rats, while none of this was observed for the CFA group. Mild gliosis was seen in CFA/SC-H, but only minimal gliosis in the CFA-treated group. However, multivariate analysis showed that CFA and Contr. metabolic profiles were highly discernible (data not shown).

Discussion

This study presents, for the first time, a comprehensive metabolomic brain tissue analysis comparing biochemical processes involved in EAE induced by CFA/SC-H injection and after CFA inoculation. The results obtained for two osmolytes (taurine and NAA reduction) and for PLs (global increase) taken together would be in agreement with a process involving neuron shrinkage (due to loss of osmolytes, notably the neuron marker, NAA) accompanied by a moderate proliferation of glial cells (PLs form the matrix of cell membranes). The PC/GPC ratio, which is enhanced in the CFA/SC-H group only, is known to be a marker of increased cell growth. In contrast to NAA and taurine, the astrocytic osmolyte, *myo*-inositol, was not reduced but rather slightly increased. This observation matches the histological detection of minimal or mild gliosis in treated groups. Moreover, increased levels of lactate and BHB indicate increased anaerobic glycolysis in both treated groups. Major stress [4], e.g. due to adjuvant arthritis (AA) inducible by CFA injection [5], may contribute to an increase in glycolytic metabolites. CFA is also known to increase the blood-brain-barrier (BBB) permeability, with or without SC-H co-injection [6], thus providing another potential mechanism for perturbations of brain metabolic profiles. Additional immunohistochemical studies are underway with the aim to further explain our metabolomic findings in the context of mechanisms involved in EAE induction through the action of both CFA and SC-H.

References

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