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Clinical aspects of neuroAIDS

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Anti-HIV activity of o-(Acetoxyphenyl)Hept-2-ynyl Sulfide (APHS)

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Objectives: Anti-inflammatory molecules are proposed in HIV-1-associated dementia (HAD) therapy. Some anti-inflammatory molecules are also known to possess anti-HIV activity. We found that APHS, a recently synthesized non-steroidal anti-inflammatory molecule can inhibit HIV-1 replication. Our aim is to clarify the mechanism of action of APHS, determine whether APHS can act synergistically with other drugs and assess whether APHS can be neuroprotective.

Methods: HIV-1 replication was quantified by p24 ELISA. Cellular viability was determined by a MTT assay. The IC50 and TC50 and Combination Index (CI) values were determined using the CalcuSyn program. HIV-1 entry efficiency and HIV-1 RT activity in PBMC in the presence of APHS was assessed using real-time PCR to quantify HIV-1 gag RNA or DNA, respectively. The replication of drug-resistant HIV-1 strains was determined with a MTT-based drug susceptibility assay. APHS neuroprotectivity was determined by measuring caspase 3 production by neurons in an in-vitro model of HAD.

Results: When administered during the first steps of the infection APHS is capable of inhibiting the replication of several HIV-1 strains (macrophage-tropic and lymphotropic) in a dose-dependent manner in both peripheral blood mononuclear cells (PBMC), monocyte-derived macrophages (MDM) and peripheral blood lymphocytes (PBL) with IC50 values of about 10 uM. APHS TC50 values were about 100–200 uM. After provirus insertion into the cellular genome, APHS does not affect HIV-1 replication. APHS does not inhibit HIV-1 entry into host cells. APHS inhibits the RT activity in PBMC. APHS inhibits replication of all tested drug-resistant strains in a MT-2 cell line. APHS showed synergistic interactions with the RT inhibitors (RTI) AZT, 3TC and efavirenz ($CI < 1$), while additive interactions were found between APHS and the protease inhibitors (PI) indinavir and ritonavir ($CI = 1$). APHS could inhibit caspase 3 production by neurons.

Conclusions: APHS inhibits HIV-1 RT activity in PBMC. APHS works synergistically with RTI. APHS can inhibit replication of common RTI and PI-resistant HIV-1 strains. APHS seems to be neuroprotective.

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HAART improves neurological functioning

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Objective: The effects of highly active antiretroviral therapy (HAART) on HIV systemic disease have resulted in substantial improvements in health. Less is known about the effect of HAART on HIV related CNS disease and neurological functioning.

Methods: In a prospective longitudinal study of the CNS effects of HAART, we examined 36 subjects at baseline pre-HAART, with 12 having a six-month follow-up on stable HAART. Subjects were seen at baseline prior to start of HAART, and had neurological, psychological and neuropsychological examination with ultrasensitive HIV RNA of both plasma and cerebrospinal fluid (CSF).

Results: A quantitative scoring procedure was utilized for the neurological exam, and summary z scores were calculated for the neuropsychological battery. Using a mixed model, significant improvements in neurological scores [$(F(2,10) = 9.71,\ p < .005$; pre 89.3 (53), HAART 44.4 (52)], and neuropsychological scores [$(F(2,8) = 6.33,\ p < .05$; pre −.77 (.60), HAART −.42 (.60)]. Both plasma [$(F(2,8) = 87.7,\ p < .0001$; pre 4.97 (.56), HAART 3.29 (.52)] and CSF [$(F(2,8) = 33.4,\ p < .0001$; pre 3.07 (.78), HAART 1.84 (.78)].

Conclusions: We previously reported improved neurological functioning in a retrospective study of subjects who started treatment after baseline, and stable neurological functioning in subjects who were on treatment. In the current prospective study, we have found significant improvement in neurological and neuropsychological functioning following treatment with HAART. These improvements in functioning occurred in the context of significant decreases in plasma and CSF viral load. Decreases in viral load with HAART are likely to increase neurological functioning in the short term. However, longer-term improvements in functioning remain to be observed, since failure on HAART regimens occurs in many after 1 year. Further longitudinal studies of the impact of HAART on CNS disease are needed.

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Selective accumulation of AZT, 3TC and Indinavir in the ventricular cerebrospinal fluid

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Background: Information on the levels of antiretroviral drugs in the cerebrospinal fluid (CSF) of humans are exclusively derived from lumbar CSF. However, drug levels in the ventricular CSF are much more relevant to the suppression of HIV replication in the CNS than levels in lumbar CSF, as the chemical composition of lumbar CSF is much different from that of ventricular origin. In addition, HIV replication is highest in periventricular regions such as the basal ganglia that contribute to the formation of ventricular CSF.

Methods: We measured the levels of antiretrovirals in multiple samples of serum and ventricular CSF taken in parallel at multiple time points in two antiretroviral naive HIV-infected patients with a ventricular catheter for obstructive hydrocephalus. Pat. A received AZT, 3TC, RTV and SQV. 14 samples of ventricular CSF were compared with three samples of lumbar CSF. Pat. B received 3TC, d4T, EFV and IDV, and 12 samples were collected. The CSF:serum albumin ratio and the cell count were normal or near normal in each of the samples.

Results: Median sampling time after the last drug intake was 2,9 h for AZT, and 2,15 h (Pat. A) and 5,9 h (Pat. B) for 3TC. The median ratio of the CSF and serum concentration was 1,4 for AZT, 0,3 (Pat. A) and 0,27 (Pat. B) for 3TC, and 0,18 for IDV. The CSF:serum ratio was much higher for ventricular than for lumbar CSF. d4T was detected in a median of 35 ng/ml in the CSF in only 3 of the 12 samples (limit of detection 25 ng/ml). EFV, RTV and SQV were not detected in any of the samples including lumbar CSF, but were readily detectable in serum.

Conclusions: Our results show the different nature of ventricular and lumbar CSF and thus confirm the need to assess ventricular CSF when evaluating antiretroviral drugs for the suppression of HIV replication in the CNS. AZT, 3TC and IDV, in this order of preference, yield high concentrations in ventricular CSF well above the IC50 levels and may be preferred for the treatment of HIV infection of the CNS.

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A prospective investigation of neurological, neuropsychological, neurophysiological and virological parameters in formerly untreated HIV-infected patients with AZT/3TC/ABC

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Background: HIV-1 associated dementia is a complication attributed primary to the virus. It occurs in up to 20% of untreated HIV-1 infected patients. Only a few of antiretroviral drugs, such as AZT, 3TC and ABC, penetrate into the cerebrospinal fluid (CSF) in sufficient concentrations. The aim of our study was to investigate whether in patients naive to antiretroviral treatment (ART) CSF viral load (VL) can be effectively lowered and neurophysiological and neuropsychological parameters can be improved or stabilised under the combination of AZT/3TC/ABC.

Design: An open prospective cohort trial.

Methods: 6 formerly untreated HIV-infected patients of all stages underwent neurological examination, acoustic event related potentials, a comprehensive neuropsychological assessment and measurement of CSF and plasma VL before, after 6 and 12 months of treatment with AZT/3TC/ABC. There was no patient with confounding neurological illness or drug abuse. A comparison of the results of the neuropsychological performance and the P 300 before ART versus a healthy age and education matched control group took place. In addition, we compared the results of all fields during the infectious process for a period of 6 and 12 months (Friedmann-test).

Results: The initial plasma VL ranged from 22.370 to 260.000 copies/ml and the CSF VL from 361 to 57.400 copies/ml. Before beginning ART the statistical results of the neuropsychological testing and the P300 did not differ to the control group. After 6 months of ART the CSF VL was below 50 copies/ml in 5 cases and in one case 137 copies/ml and after 12 months all patients had a CSF VL of maximum 54 copies/ml. The neuropsychological results showed an improvement in Rey visual learning test ($p = 0,03$) and mostly constant results in all other neuropsychological fields and also in the P 300 over the course of 12 months after medication. We did not observe any deterioration in the neurological examination.

Conclusions: In our small patient group the combination AZT/3TC/ABC was able to lower the CSF VL effectively over a period of 12 months and to stabilise and even partly improve neurophysiological and neuropsychological parameters.

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Involvement of the blood-cerebrospinal fluid barrier in the cerebral bioavailability of anti-retroviral nucleoside analogs

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Effective CNS penetration of anti-HIV drugs is critical in order to decrease the cerebral virus pool and to prevent the development of AIDS dementia complex. The effective barrier between the blood and the cerebrospinal fluid (CSF) is located at the epithelium of the choroid plexuses(CPs). The CPs therefore constitute a direct route for drug delivery into the CSF, subarachnoid spaces and perivascular spaces in which infected macrophages are primarily found, especially during the early phage of the infection. However, CPs being the site of powerful CSF-to-blood efflux pumps, also regulate the cerebral biodisposition of various drugs.

Nucleoside analogs are widely used antiretroviral drugs and are often the only available drugs in developing countries where neuroaids remains an important issue.

Using an in vitro model of the blood-CSF barrier that reproduces the properties of the in vivo epithelium (J. Neurosci., 1999, 19:6275), we established a ranking of the different nucleoside analogs based on their capacity to enter the CSF via the CPs. Their mechanism of transfer across the epithelium was then investigated by comparing permeability coefficients measured in blood-to-CSF and CSF-to-blood directions, and by mean of saturation and competition studies. The results indicate that nucleoside derivative influx is independent of thymidine transport, and is restricted by a

saturable efflux process. After assessing that the three main type of transporters involved in drug efflux at the CP, (SLC21, SLC22 and ABCC families), were expressed and functional in the cellular model, we showed that the main apical transporter involved in nucleoside derivative efflux is a member of the SLC21 (OAT) family. Finally we demonstrated that this efflux can be pharmacologically modulated to increase the efficiency of antiretroviral delivery to the brain.

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Epstein-Barr virus (EBV) DNA load in cerebrospinal fluid (CSF) of patients with AIDS-related lymphomas

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Aims: To quantify EBV DNA in CSF and plasma from HIV-infected patients with brain lymphoma.

Methods and patients: Real-time PCR was performed to quantify EBV DNA in CSF from 32 patients with AIDS-related primary central nervous system lymphoma (PCNSL = 20) and central nervous system involvement of systemic NHL (NHL-CNS = 12). At the time of sampling, 15 patients were receiving anti-herpes drugs for the treatment of citomegalovirus or varicella zoster disease, consisting of ganciclovir (induction treatment n = 1, maintenance treatment n = 12), or aciclovir (induction treatment n = 1, maintenance treatment n = 1). As controls, 16 HIV-infected patients with other CNS disorders were examined.

Results: EBV DNA was detected in CSF from 16/20 patients with PCNSL (80%, mean 5.13 log copies/ml), from 8/12 patients with NHL-CNS (67%; mean 5.31 log copies/ml) and 2/16 of the controls (13%; mean 1.42 log copies/ml) (PCNSL or NHL-CNS vs. controls; p = 0.01). No association was demonstrated between viral load and the following patient variables: histological type of lymphoma, clinico-radiological presentation of the brain lesion, presence of malignant cells in CSF, CSF white blood cell number, CD4+ count, chemotherapy, HAART administration. EBV DNA load correlated to the administration of anti-herpesvirus therapy. The mean EBV DNA load was 1.79 log copies/mL, SD = 1.59 in CSF samples from 15 patients with PCNSL or NHL-CNS receiving anti-herpesvirus drugs as against the 4.32 log copies/mL, SD = 2.42 in CSF samples from 17 untreated patients (p = 0.003).

Conclusions: High CSF EBV DNA levels are found in HIV-associated brain lymphomas and their measurement can be clinically useful. While high CSF viral load are highly predictive for brain lymphoma, low values should be interpreted cautiously, keeping into account the possible carry over from blood and the effect of anti-herpes drugs.

P201

Association of human neurotropic virus JCV with central nervous system B-cell lymphomas

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The incidence of primary central nervous system (CNS) lymphomas has recently increased markedly world wide up to 6.6% of primary intracranial neoplasms due to the Aids pandemic. Approximately 98% of primary CNS lymphomas are of B cell origin. JC virus is a human neurotropic polyomavirus infecting more than 70% of the human population world wide. Reactivation of JCV in brains of patients with impaired immune systems results in cytolytic destruction of oligodendrocytes, and development of the fatal demyelinating disease, Progressive Multifocal Leukoencephalopath y (PML). JCV has also been detected in B lymphocytes and mononuclear cells in PML patients, suggesting that JCV can also infect and possibly remain at the latent stage in these cells.

Given earlier observations on the presence of JCV in B-lymphocytes, and the oncogenic potential of this virus, we examined the association of JCV with human CNS lymphoma. We obtained 29 CNS lymphomas and examined them for the presence of the JCV genome and the expression of JCV T-antigen by DNA amplification and immunohistochemistry. Results from PCR using a pair of primers that recognize the DNA coding sequence for the viral T-antigen gene followed by hybridization with a JCV specific DNA probe showed that twelve of the samples (41.3%) contain JCV DNA sequences. Expression of T-antigen was detected in the nuclei of cells from six CNS lymphomas (20.7%). No evidence for the production of viral capsid proteins was observed, ruling out productive replication of JCV in these cells. Immunohistological examination of the tumors for the presence of Epstein-Barr virus (EBV), which is commonly associated with B cell malignancies, revealed the presence of viral latent membrane protein (LMP) in eleven samples (37.9%). Five of the LMP positive samples showed positive nuclear staining for T-antigen (17.2%). Altogether, this study shows that nearly 20% of CNS lymphomas contain JCV genomic DNA, and that neoplastic cells contain the viral oncoprotein, T-antigen. The co-presence of EBV and JCV in the tumor cells invites new investigation on the possible collaboration between these two human viruses in the tumorigenesis of CNS lymphomas.

P202

Diagnosis and monitoring of HIV-related progressive multifocal leukoencephalopathy by quantitave real-time PCR for JCV DNA

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Aims: To evaluate the JC virus (JCV) DNA load in cerebrospinal fluid (CSF) as a diagnostic and prognostic marker of HIV-related progressive multifocal leukoencephalopat y (PML).

Patients and methods: JCV DNA was quantified by real time PCR in CSF samples drawn from 26 patients with histological and/or clinico-neurological analysis of PML

observed in the period 1996–2001. CSF samples drawn from 34 patients with other HIV-related central nervous system (CNS) diseases were analysed as controls (5 toxoplasmosis, 5 cryptococcosis, 5 HIV-related dementia, 5 cytomegalovirus encephalitis, 5 herpes simplex virus type-1 encephalitis, 4 primary CNS lymphoma, 5 primary HIV infection,). Following PML diagnosis, all of the patients were treated with HAART with or without cidofovir. Based on the PML clinical outcome patients were divided into responders (n = 14) and non-responders (n = 12). Additional CSF specimens were drawn after a median days of 150 (range 120–450) from 7 responders, and a median of 37.5 days (range 30–75) from 5 non-responders.

Results: At PML onset, JCV DNA was detected in 21/26 patients. None of the controls were JCV DNA positive. The sensitivity and specificity of real-time PCR for diagnosis of PML were 88% and 100%, respectively. JCV DNA was found in CSF from 12/14 responders (86%) with a median load of 4.35 log copies/mL (range <2.00–6.42) and from 9/12 non-responders (75%) with a median load of 3.64 log copies/mL (range <2.00–7.71). Following HAART, JCV DNA decreased significantly in the responders (from a median of 4.35 to <2.00 log copies/mL, p = 0.04) but not in the non-responders (from a median of 3.68 to 6.12 log copies/mL).

Conclusions: JCV DNA load at baseline was not correlated with subsequent response to HAART. On the contrary, good clinical outcome was associated with decrease or clearance of JCV DNA levels in CSF. Quantitative real-time PCR may thus be useful in the clinical management of HIV-related PML.

P203

Real-time polymerase reaction (PCR) for diagnosis and treatment follow-up of herpesvirus central nervous system infections in HIV-infected patients

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Background and objectives: Cytomegalovirus (CMV) encephalitis can complicate late stages of HIV infection. In approximately one fifth of CMV encephalitis cases, a concomitant CNS infection by herpes simplex virus type 1 (HSV-1) or 2 (HSV-2) is also observed. Aim of this study was to evaluate a quantitative PCR assay to measure CMV, HSV-1 and HSV-2 DNA load in cerebrospinal fluid (CSF) samples from HIV-infected patients with CMV and/or HSV encephalitis.

Patients and methods: CSF samples drawn from 40 HIV-infected patients with CMV (n = 19), HSV-1 (n = 22) or HSV-2 (n = 2) encephalitis were studied. All of these samples were previously found to be DNA positive for the respective viruses by qualitative nested PCR. Additional CSF samples drawn during antiherpes treatment were available from 18 patients with CMV and 10 with HSV encephalitis. A real-time PCR, based on the TaqMan technology, was used for quantification of CMV, HSV-1 and HSV-2 DNA. CSF samples were run simultaneously on the same plate using a standard curve that allowed DNA quantification of each virus.

Results: At encephalitis onset, CMV DNA was found in CSF samples from 18/19 patients (95%), with a median viral load of 4.38 log copies/mL (range <2–8.03). After a median time of 30 days (range 20–30) of antiviral therapy with ganciclovir, foscarnet or both, CMV DNA was still found in 12/19 patients. The median CMV DNA load change from baseline was −1.18 log copies/mL (range −3.95 −+ 0.75), with no substantial differences with respect to the type of treatment. HSV-1 or 2 DNA was detected in CSF samples from 20/22 patients (91%) with a median viral load of 4.12 log copies/mL (range <2–5.40). After a median time of 19 days (range 13-30) of antiviral therapy with aciclovir, ganciclovir or foscarnet, HSV DNA was still detectable in 9/10 patients. The median viral load change from baseline was −0.63 log copies/mL (range −1.25 −+ 2.75), with no significant differences according to treatment.

Conclusion: Real time PCR is useful for quantification of CMV, HSV-1 and HSV-2 DNA in CSF. This method can be used for both diagnosis and treatment management of herpesvirus complications of the CNS in HIV infected patients.

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A long term survivor with progressive, multifocal leucoencephalopathy (PML) demonstrates a virus specific production of interferon-gamma

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Objective: Progressive multifocal leukoencephalopathy (PML) is an almost fatal, demyelinating disease caused by JC virus (JCV) in patients with severe immunosuppression. Due to the limited availability of viral antigen, studies about the cellular and humoral immune response in patients with PML are rare.

The aim of this study was to investigate the proliferation and cytokine production of peripheral blood mononuclear cells (PBMC) against a JCV related antigen, myelin basic protein (MBP) as a major component of the myelin sheath and tetanus toxoid (TT) as a control antigen in a long term survivor and a rapidly deteriorating PML-patient.

Methods: The major structural protein VP1 of JCV was expressed as virus-like particles (VP1-VLP) in a baculovirus system. PBMC were isolated by ficoll gradient centrifugation and stimulated with VP1-VLP, MBP and TT. Proliferation was determined by 3H-thymidine incorporation. IFN-gamma and IL-10 were measured in the culture supernatant by enzyme linked immunoabsorbent assay (ELISA).

Results: The long term survivor showed a proliferative response and a strong production of interferon-gamma after stimulation with VP1-VLP, which declined after one month. In contrast VP1-VLP specific proliferation and IFN-gamma production was low in a rapidly deteriorating patient. Both patients demonstrated a low or undetectable IFN-gamma response after stimulation with MBP or TT. Production of IL-10 was low or undetectable after stimulation with all three antigens and showed no striking difference between the two patients.

Conclusion: Recovery from PML is associated with a transient production of IFN-gamma in presence of a viral surface antigen, which is absent in the rapidly deteriorating patient. No reactivity against MBP was detectable in the long term survivor and the moribund patient.

P205

Importance of Toxoplasma gondii antibody titres in patients with toxoplasmic encephalitis and AIDS

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Background: Toxoplasmic encephalitis (TE) is the most frequent cause of focal brain lesions in patients with AIDS. The definitive diagnostic criteria needs histologic proof from brain biopsy or at autopsy. The presumptive definition for TE is based on serology, clinical, radiological features and response to anti-Toxoplasma therapy. In AIDS patients, TE is most often caused by reactivation of latent infection, with presence of IgG antibodies. Historically, IgG antibody titres were not used to identify patients with acute reactivation or for following the course of TE. However, there may be some evidence to suggest that high titres may be associated with an increased risk and acute reactivation of TE. Objectives: To describe the importance of Toxoplasma gondii serologic titres in patients with TE and AIDS, and their clinic correlation. Methods: A descriptive study was performed at Emílio Ribas Infectology Institute from june to october 2001. Twenty four HIV patients with a first episode of TE were included. IgG Toxoplasma antibidy titres at entry were determinated by enzime-linked immunosorbent assay in a single laboratory. The patients were treated and prospectively followed for six weeks and classified according to the type of therapeutic clinic response in: complete, partial or null. Results: All patients had IgG titres above 250 UI/ml. Forty two percent (10 of 24) of the subjects had complete clinical response. Fifty four percent (13 of 24) and four percent (1 of 24) had partial and null response, respectivelly. Conclusions: The results of this study indicate that all patients with TE and AIDS had high anti-Toxoplasma gondii antibody titres, regardless of clinic course after therapy. These results suggest the utility of quantitative serologies for diagnostic approach on acute reactivation of TE in patients with AIDS and focal brain lesions.

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Contribution for the study of the prevalence in Portugal of JC virus infection among Human Immunodeficiency Virus infected patients with neurological disorders

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Progressive multifocal leukoencephalopath y (PML) is a fatal central nervous system (CNS) demyelinating disease, caused by the human polyomavirus JC (JCV), which affects predominantly immunosuppressed patients. Originally, PML was a rare disease, but in recent years became a common opportunistic viral infection of the CNS among HIV infected patients, along with other viral infections, specially those associated to herpesvirus, such as CMV (most frequently), HSV-1 and 2, VZV, EBV and HHV-6.

The aim of our study was to contribute for the evaluation of the prevalence of JCV infection among Portuguese HIV infected patients with CNS disorders suggestive of PML. Cerebrospinal fluid (CSF) from 31 HIV infected patients with clinical signs and brain MRI scans consistent with PML was examined for the presence of JCV DNA. After DNA extraction from clinical samples, JCV DNA was detected by nested PCR, performed with specific primers from the T-antigen coding region of the JCV genome. This specific JCV target sequence was amplified in 10 out of 31 the patients studied.

In spite of the lack of confirmatory diagnosis by brain biopsy, we found that 32% of Portuguese HIV infected patients with neurological disorders were infected by JC Virus.

This study suggests that the prevalence of JC Virus in Portuguese HIV infected patients with neurological disorders is among the highest in the world published to date.

P207

Alteration of blood-CSF barrier functions by retrovirus-infected lymphocytes and pro-inflammatory cytokines°

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The choroid plexuses (CP) forming the blood-cerebrospinal fluid barrier (BCSFB), are constituted by a tight epithelium, a conjonctive stroma embedding large capillaries and cells from the myeloid and lymphoid lineage. In addition to tight junction (TJ) complexes that seal epithelial cells, the neuroprotection functions of CP are reflected by their high antioxidant capacity and multiple efflux systems that pump deleterious compounds, e.g. several organic anions (OA), out of the CSF. Several Matrix metalloproteinases (MMPs), extracellular matrix degrading zinc-endopeptidases that can also release bioactive forms of latent receptors and cytokines, are constitutively expressed by CP.

Retrovirus infected leukocytes are found in the CP stroma where they may affect CP functions. Moreover, this location points out CP as a potential route of entry into the CNS.

We studied the alteration of BCSFB functions upon stimulation by HTLV-1-infected lymphocytes and by two pro-inflammatory cytokines TNF-a and IL1-a known to be produced by these activated cells. We used a cellular model of the barrier, which allows separate access to the basolateral (blood) and apical (CSF) compartments.

Basolateral exposure to HTLV1-activated lymphocytes induced an alteration of TJ and an increase of paracellular permeability while the antioxidant capacity was preserved. MMP-2 and -9 were also detected in both compartments. Treatment with TNF-a and IL1-a led to an increased and polarized secretion of proMMP-9, proMMP-2 and MMP-2 and a decreased OA efflux, but did not alter the paracellular permeability and antioxidant capacity of the choroidal epithelium. Thus, activated lymphocytes but not TNF-a and IL1-a 'per se,' altered the BCSFB integrity suggesting that the mechanisms underlying the barrier 'opening' involve the action of other soluble factors. The pathophysiological relevance of the increased MMP secretion and altered OA transport remains to be clarified.

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Vascular endothelial growth factor (VEGF) is increased in serum, but not cerebrospinal fluid in HIV-associated CNS diseases

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Vascular endothelial growth factor (VEGF) is a potent angiogenic and mitogenic peptide. Activated by different stimuli, such as cytokines, oxidative stress, and hypoxia, VEGF also induces several mediators e.g., plasminogen activators, matrix metalloproteinases and adhesion molecules, which are crucial in inflammation and have been suggested to play a role in HIV-neuropathogenesis. To investigate whether VEGF may be involved in HIV-associated CNS damage, we determined the levels of this mediator in cerebrospinal fluid (CSF) and serum samples of HIV-infected patients with (n = 18, HIV-CNS) or without (n = 19, HIV-controls) central nervous system (CNS) complications and HIV-negative control patients (n = 18, controls) by ELISA. VEGF CSF concentrations were not different between the three groups (HIV-CNS: mean 41.6 ± 7.4 pg/ml; HIV-controls: mean 42.2 ± 7.8 pg/ml; controls: mean 52.7 ± 7.9 pg/ml). However, serum VEGF levels were significantly increased in HIV-CNS patients compared to HIV-controls and controls (HIV-CNS: mean 268.8 ± 40.7 pg/ml; HIV-controls: mean 117.0 ± 12.5 pg/ml; controls: mean 133.1 ± 14.8 pg/ml). Serum samples of patients with untreated HIV-associated encephalopath y (n = 3) contained the highest levels. In two of these patients "follow-up" samples were available and VEGF concentrations decreased upon initiation of antiretroviral therapy and clinical improvement (patient 1: 670 pg/ml to 304 pg/ml; patient 2: 441 pg/ml to 210 pg/ml). Correlation analysis revealed that VEGF serum concentration correlates with both, HIV viral load in CSF and plasma. No correlations were found with the CD4 cell count and CSF parameters. Taken together systemic VEGF release is (1) increased in HIV-infection with high virus turn-over and (2) in disorders involving the CNS. These preliminary results hint at a possible involvement of VEGF in HIV-induced CNS damage. Further studies e.g., on VEGF brain tissue expression in HIV-infection are warranted.

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A broader window for CNS-HSV-2 infections

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Our aim was to compare the diagnostic potential of PCR and CSF antibody measurements at the onset of CNS disease. Forty-one patients with CNS symptoms of suspected viral etiology had HSV-2 -specific nucleic acid in their CSF samples. These patients formed the study group. HSV-specific IgM was studied from the CSF by using a commercial kit for testing indirect IgM immunofluorescence (Meridian Diagnostics Merifluor HSV IgM IFA/IFT test for IgM antibodies, Gull Laboratories, Salt Lake City, UT) (dilution 1:2) and HSV-1 and HSV-2-specific IgG antibodies in an enzyme immunoassay (Focus Technologies Herpeselect^{TM} HSV-1/HSV-2 EIA tests) (dilution 1:10).

HSV-2 PCR was positive during the first week after the onset of CNS symptoms and only occasionally after that (Fig). At the follow-up the specific nucleic acid disappeared from the CSF. By using new sensitive tests, HSV-2-specific IgG and IgM antibodies were detected occasionally already at the onset of CNS symptoms and could be detected for two to three weeks, almost a week after the disappearance of the specific nucleic acid, sometimes even later.

In conclusion, HSV-specific antibodies can be detected early in the course of the disease, when studied using sensitive methods, and may persist longer in the CSF than specific nucleic acid. The two methods complement each other and increase the diagnostic yield considerably. Already three weeks after the onset of symptoms the specific diagnosis is too late to achieve.

P210

Advanced MR-techniques in HIV-1 associated minor motor disorders (HIV-1 MMD)

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Objective: The aim of this study was to investigate, if advanced MR-techniques such as diffusion- and perfusion-weighted imaging and MR-Spectroscopy can illuminate the time course and metabolic correlates of HIV-1 MMD.

Materials and methods: In a total of 32 HIV-1 seropositive, clinically not yet compromised patients with electrophysiologically determined different degrees of HIV-1 MMD, routine MR-examination of the brain, diffusion (DWI)- and perfusion-weighted (PWI) images and a 1H-MR-Spectrocopy of the basal ganglia were performed on a 1.5 T MR-Scanner.

Results: None of the patients showed any abnormality in the routine MRI or DWI.

PWI revealed a significantly elevated regional cerebral blood flow (rCBF) in patients with incipient HIV-1 MMD (n = 8). Patients with sustained HIV-1 MMD (n = 14) showed significantly higher ratios of myo-inositol/creatine compared to those with no or incipient (n = 18) HIV-1 MMD.

Conclusion: PWI and 1H-Spectroscopy show pathologic changes in patients with HIV-1 MMD when clinical examination and conventional MRI are still normal. Therefore PWI and 1H-Spectroscopy seem to be beneficial in patients with HIV-1 associated brain disease and in clarifying the neuropathogenesis of HIV-1 MMD.

P211

Does MRI give an earlier prognostic information than CMAP in patients with Bell's palsy?

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Purpose: We wanted to assess, whether quantitative analysed MRI has a prognostic information at an earlier stage as electrophysiological methods.

Material and methods: 35 patients suffering from Bell's palsy less than 6 days were examined by MRI on the first day of inpatient treatment. A T1 Gradient Echo Sequence was performed native and after intravenous application of GdDTPA using a surface coil placed on the ear of the affected side. The signal intensity of the native and contrast enhanced sequence was analysed by region of interest drawn in the five

segments of the intratemporal facial nerve. The measurement results were added together to perform an index, which was compared with the electrophysiology data and the clinical outcome.

Results: 3 patients developed a chronic facial paralysis, 32 patients had a good clinical course. The patients with a bad course showed an MRI index of higher than 5.8, the patients with a good outcome of lower than 4.5. All patients with an MRI index higher than 5 showed a CMAP lower than 20% compared to the healthy side on the 7th day of illness. No patient with a CMAP higher than 20% presented an MRI index higher than 4.5.

Conclusion: MRI gives prognostic information at an early stage of illness, if it is performed as a region of interest measurement including a quantitative analysis. MRI presents this information at a time, when the Wallerian degeneration has not taken place and causal therapy is possible. It gives this information at a time, when CMAP is not possible to do at an early stage until the 6th day of illness.

P212

Identification of target tissue for Glioma gene therapy by multi-tracer PET imaging

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Objective: To identify viable target tissue in heterogenous gliomas amenable for biological treatment strategies such as gene therapy.

Background: Multi-tracer positron emission tomography (PET) imaging has been successfully used to identify target tissue for gene therapy of gliomas in the clinical application (1). To further develop experimental gene therapy protocols, multi-tracer PET imaging was used to characterize the DNA-, protein- and glucose-metabolism of various rat and human glioma models. Methods: Rat F98 glioma, human Gli36dEGFR and U87dEGFR glioma cells were grown as s.c. tumors in nude rats (n = 8) and nude mice (n = 8). After tumors had grown to at least 200 mm^3, DNA-, protein- and glucose-metabolism was determined by means of 3′-deoxy-3′-[18F]-fluoro-L-thymidine (FLT), [11C]-methionine (MET) and [18F]-2′-fluoro-2′-deoxy-D-glucose (FDG) PET after i.v. administration of FLT (250 μCi/rat; 50 μCi/mouse), MET (600 μCi/rat; 200 μCi/mouse) and FDG (250 μCi/rat; 50 μCi/mouse) using new generation ECAT HRRT (Siemens, CTI) and microPET scanners (Concord).

Results: In small tumors (<500 mm^3), homogenous uptake of all three tracers indicated actively proliferating tumor tissue as potential target tissue for gene therapy. In larger tumors (>500 mm^3), heterogenous tracer uptake was observed with a rim of high FLT-, MET- and FDG-uptake immediately adjacent to metabolically inactive or necrotic tumor, where no specific tracer accumulation occurred. The metabolically active tumor tissue presented as a comparatively narrow band indicating that application and targeting of gene therapy vectors into the active proliferating tissue compartment in large tumors might be difficult.

Conclusions: Identification of target tissue for gene therapy is possible by multi-tracer PET in experimental animals at high spatial resolution. Multi-tracer PET imaging of tumor metabolism and gene expression shall contribute to the development of standardized gene therapy protocols and of efficient and safe vector applications in humans.

References: (1) Jacobs *et al.* (2001). *The Lancet* **358:** 727–729. Supported in part by MSWF 516-400 002 99, ZMMK-TV46 and DFG-Ja 981/1-1.

P213

Magnetic resonance imaging of Modoc virus-induced encephalitis in a hamster model for Flavivirus infections

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Introduction: Flaviviruses are a major cause of severe encephalitis in man. Infections with Japanese encephalitis, Murray valley encephalitis, Saint Louis encephalitis or tick-borne encephalitis virus are characterized by mortality rates ranging from 20–50%. About one third of the patients recover without neurological sequelae, whereas another third survives with long-lasting neurological sequelae that often resemble a poliomyelitis-like illness.

Results: Modoc virus (MODV) infections in hamsters are characterized by 50% mortality, 35% of the hamsters recover without apparent neurological sequelae, and another 15% survive with obvious long-lasting sequelae. Cranial magnetic resonance imaging (MRI), performed on hamsters with severe acute MODV encephalitis, showed unambiguously hyperintense lesions in the temporal lobes. Also, the olfactory lobes and amygdalohippocampal area are frequently involved, and widening of the lateral ventricles was often noted. Furthermore, MRI showed residual hyperintense lesions in the temporal lobes of all hamsters that survived the acute phase of infection with, or without, neurological sequelae. Other areas of the brain appeared to be less affected. Upon histological examination (at 40 positions along the anterior-posterior axis of the brain), severe and diffuse leptomeningitis with bilateral and nearly symmetrical necrosis was observed, as was inflammation of the amygdalohippocampal and hypothalamic area. The olfactory bulbs were almost completely necrotic in all hamsters. In addition, at the margins of the affected areas, a classic gliomesodermal reaction with microglial nodules and satellitosis was observed. Accumulation of inflammatory cells in the Virshow Robin spaces (perivascular cuffing) was diffusely present throughout the parenchyma of the cerebrum. The ventricles presented with a thickened plexus choroideus infiltrated by mononuclear cells, and an increased cellularity of the liquor was noted.

Conclusion: The MODV/hamster model permits the study of the MRI features of an acute flavivirus encephalitis, and, in particular, the neurological sequelae in animals that survived the acute phase of flavivirus encephalitis.

P214

In vivo detection of reversible neurochemical changes during acute SIV infection by Magnetic Resonance Spectroscopy

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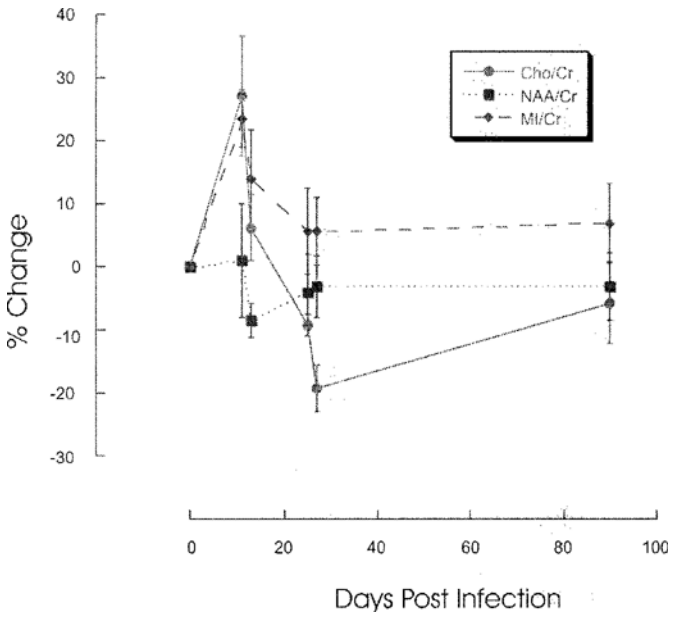
Magnetic Resonance Spectroscopy (MRS) is used as a non-invasive imaging technique for the detection of neurological injury associated with HIV infection. We have applied MRS to the SIV-infected macaque model of AIDS. The use of this animal model allows us to study the changes that occur during the acute phase of the disease, and to determine the natural course of the disease. It also permits elucidation of the neuropathological basis of the MR changes.

We have examined 14 rhesus macaques by MRS before infection with SIVmac251, and then at 2 weeks (11 days-4 animals, 13 days-10 animals), 4 weeks (25 days-6 animals, 27 days-5 animals) and 3 months (10 animals). MRS was performed at 1.5 T using the GE Probe-P spectroscopy package (TE = 35, TR = 3000 ms). Axial images were used for voxel placement in frontal gray, basal ganglia, and white matter. SAGE-GE was used for metabolite quantification: N-Acetyl-Aspartate (NAA), Choline (Cho), Creatine (Cr) and Myo-inositol (MI).

The observed evolution of metabolite values in the frontal cortex as a function of time of infection is shown in Figure 1. The values are plotted as the percentage change from the preinfection scan. Both the Cho/Cr (27%) and the MI/Cr (24%) are significantly elevated at 11 days post infection (dpi), a time of high viremia in this model. The MI/Cr then drops to values that are not significantly elevated. However, the Cho/Cr ratio drops below preinfection values (−19%) at 27 dpi before a return to near-normal values at 3 months. Decreased Cho/Cr is a rare spectroscopic finding and may represent a cerebral repair mechanism. Decreases in Cho/Cr in the frontal cortex has been observed in human studies after initiation of antiretroviral therapy (Chang, Neurology, 1999, 53, 782), suggesting that the systematic control of viral replication by the macaque in the acute phase may resemble pharmaceutical control of viral replication in patients.



Much of the focus in human MRS is on changes in NAA/Cr, which is a marker for neuronal injury. We have detected an 8.6% decrease in NAA/Cr at 13 dpi. This demonstrates the *in vivo* detection of neuronal injury during the acute phase of SIV infection.

P215

Detection of HSV in experimental encephalitis by means of [18F]FHPG and PET

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Objectives: The diagnosis of herpes simplex encephalitis (HSE) in humans is difficult: The initial clinical course is highly variable and the infection may be rapidly fatal. A non-invasive and rapid diagnostic test is not available. Thus we investigated the potential of a non-invasive PET imaging technique utilizing a known "virus tracer."

9-[(1-[18F]Fluoro-3-hydroxy-2-propoxy)methyl]guanine ([18F]FHPG) is derived from ganciclovir and is phosphorylated by the HSV-tk enzyme. PET scanning with this tracer has been proven successful in the detection of HSV-tk transformed C6 glioma cells outside the brain (Hospers *et al*, 2000; Cancer Res 60:1488–1491). It is unknown whether the tracer is sufficiently transported over the Blood Brain Barrier, and consecutively selectively trapped in HSV infected neurons *in vivo*. The aim of this study is to investigate whether HSV can be detected with [18F]FHPG PET scanning in a rat model of herpes simplex encephalitis.

Methods: Rats were infected with HSV-1 by application of 10E7 pfu to the olfactory epithelium. Controls received PBS. Seven days after inoculation a dynamic PET scan was acquired for 1 hour after a bolus injection of 15-20 MBq [18F]FHPG. The brain was dissected, frozen and 80 micron slices were cut for consecutive phosphor imaging. Regional FHPG uptake was calculated from manually drawn regions of interest.

Results: Dynamic PET images showed enhanced trapping of [18F]FHPG in the encephalitic group as compared to the control group. After 1 hour tracer uptake was $4.7 \times 10E\text{-}2$ percent of the injected dose per gram tissue in the infected group versus $0.04 \times 10E\text{-}2$ in controls. Phosphor images showed enhanced accumulation of [18F]FHPG in regions known to be infected after olfactory infection with HSV: Bilateral bulbi olfactorii, cortical regions, and thalamus.

Conclusion: This study shows, that HSV in the "encephalitic" brain can be detected non-invasively with PET. We showed that the [18F]FHPG uptake was sufficient to be detected by PET and that the region specific distribution can be revealed by phosphor imaging. Thus, this technique may prove to be a promising tool in the early diagnosis of HSE in humans.

P216

HIV-1 Tat protein and methamphetamine synergize to impair dopaminergic function

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Basal ganglia structures are highly susceptible to infection with the human immunodeficiency virus (HIV). The HIV transactivating protein Tat may be pathogenically relevant in HIV-induced neuronal injury. Patients with HIV infection often abuse drugs such as methamphetamine (MA), which may cause long-term structural and functional changes of the basal ganglia. In light of the mounting evidence that Tat and MA share common mechanisms of injury, we tested the hypothesis that co-exposure to Tat and MA would lead to enhanced dopaminergic toxicity. Four groups of rats (n = 8/group) were used. Group 1 received i.p. saline injections, group 2 received a threshold dose of MA (5mg/kg i.p. every 2 hours × 4), group 3 was injected with a threshold concentration of Tat (20 μg Tat1-72) into the striatum and group 4 received striatal injections of Tat and 24 hours later was exposed to MA. One week later the animals were euthanatized and striatal biogenic amines measured by HPLC. Group 2 demonstrated a 6% decline in striatal dopamine levels while group 3 showed an 8% reduction compared to controls (group 1). Group 4, on the other hand, showed a profound enhancement of toxicity with an almost 65% reduction in striatal dopamine and 40% reduction in DOPAC. In another group of animals, we quantified the number of dopamine transporters as an index of dopamine terminal integrity. Using the ligand [125I]-RTI-121, we observed a 60% reduction in the binding capacity in the striatum of animals exposed to both Tat and MA compared to saline-injected controls. In parallel studies using human fetal neurons, we observed a similar (~300%) synergy in cell death when cells were exposed to both Tat and MA. In these cells, mitochondrial function, determined by measuring the membrane potential, was disrupted and could be prevented by treatment with several different antioxidants. This study demonstrates that the HIV "virotoxin" Tat enhances MA-induced striatal damage and suggests that HIV-infected individuals who abuse MA may be at increased risk for basal ganglia dysfunction. Supported by DA13144 to WFM, NS39253 to AN. and DA10115 to WAC.