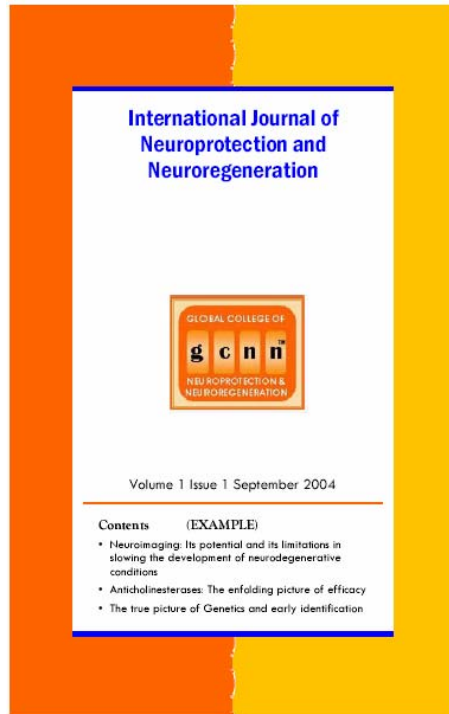


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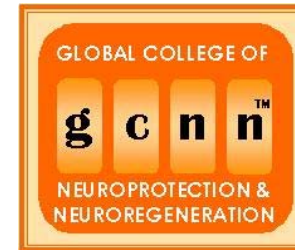
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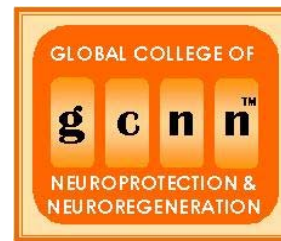
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Muscle power development during the first year of life predicts neuromotor behaviour at 7 years in children born as high-risk preterm infants

Janny Samsom, Laila de Groot.

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Objective:

The aim was to find if neonatal factors and follow-up during the first year of life could predict neuromotor behaviour at 7 years of age in children born as high-risk preterm infants.

Patients and Method:

High-risk was assigned as gestational age (GA) < 32 weeks and/or birthweight (BW) < 1500 g, and categorized according to the medical history in the three highest categories of the Neonatal Medical Index (NMI). Follow-up was performed in 52 children (mean GA: 29 6/7 weeks, mean BW: 1357g). At all ages a full neurological examination was performed, with at 3 and 6 months special emphasis on the relationship between active and passive muscle power and asymmetry and at 12 months on the assessment of postural control, hand function, motility, elicited reactions and asymmetry. At 7 years, the neuromotor behaviour was tested with Touwen's examination of minor neurological dysfunction. Stepwise linear regression was used to find the best predictors on perinatal factors and follow-up during the first year for outcome at 7 years.

Results:

The combination of the NMI and gender could predict neuromotor behaviour at 7 years of age during the neonatal period. However the best predictors were the outcome of muscle power in shoulders and legs at 3 months and postural control at 12 months, taken account of the gender of the child (sensitivity: 95%).

Conclusion:

The described method of follow-up was very sensitive to predict neuromotor behaviour at 7 years of age in high-risk preterm infants.

at one time point only. Allowing for clinical constraints, MR was carried as close to PET studies as possible. PET studies were carried on a GE Medical Advance system. Following transmission, a target dose of 250MBq of ^{11}C -PK11195 was injected prior to imaging in 3D byte mode for four half lives using a total of 38 to 53 frames. To quantify activity derived from blood volume, standardized CBV measurement was carried out using a targeted nasal dose of 750MBq of C^{15}O and venous blood analysis. Later PK frames were summated, co-registered to MR sequences using mutual information software in statistical parametric mapping (SPM) prior to reslicing. Using the Gunn simplified reference tissue [11] (ipsilateral cerebellum) model and a defined region of interest (ROI), parametric binding potential (BP) maps, in addition to parameters R_1 and k_2 (reflecting exchange of ligand between plasma and free compartments in reference and target tissues respectively) can be derived. Ipsilateral cerebellum is used as reference tissue to avoid the confounding factor of crossed cerebellar diaschisis i.e. the physiological phenomenon based on reversible depression of functions anatomically or functionally connected to the damaged area. Following correction of C^{15}O based signal for residual PK activity, corrected CBV maps may be generated. Corrected PK BP maps can thus be derived by subtractive analysis to formulate a corrected PK parametric BP map.

Results, conclusions and future strategy: four patients and four volunteers have been recruited to the protocol. Two of the four patients completed the protocol. Initial studies from the first volunteer demonstrated adequate signal to noise ratios in 3D mode and all imaging sessions thereafter have been in 3D mode. Comparative analysis of voxel based time activity curves in ipsilateral cerebellum of patients and volunteers revealed high levels of correlation (Spearman's rho range =0.92-0.97). Patients and volunteers were injected with high purity ^{11}C (R) -PK11195 with a dose ranging from 178 to 268 MBq. Significant BP, as demonstrated by parametric maps and corrected for CO derived CBV activity, is very low at p00 but increases at p01 before decreasing at p02. Flipped ROI ratios of region vs. contralateral hemispheric BP (matched in volumetric terms), yielded values in the range 0-3. Specific binding is absent in volunteer brain and in contralateral hemispheres of stroke patients. ^{11}C (R) -PK11195 PET and quality controlled methodology can be used to study microglial activation in IS and to establish temporal and spatial characteristics of this phenomenon. In order to establish robust patterns of microglial activation it is planned to image additional patients and volunteers in conjunction with follow up outcome measures using both functional and neurological scales.

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Microglial activation in acute ischaemic stroke: imaging based insights

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Introduction and objectives: Activated microglia form an important component of the intrinsic CNS response to injury. The peripheral benzodiazepine-binding site (PBBS or ω_3) is located on mitochondria and one specific ligand, the isoquinolone PK11195, binds avidly to cells of mononuclear phagocyte lineage and remains a specific marker for activated microglia [1]. In normal brain such binding remains minimal, whilst *in vivo* binding of its corresponding enantiomer (R) -PK11195, discriminates between astrocytes and microglial binding [2]. When labelled with carbon-11, PK11195 and positron emission tomography (PET) provides an *in vivo* tool for studying anatomical and functional kinetic patterns of activated microglial activity in inflammatory [3], infectious [4] and degenerative lesions [5].

Densities of ω_3 receptors, as assessed by autoradiographic [³H]-PK11195 binding, are significantly increased in experimental focal cerebral ischaemia models [6] where such areas match volumes of infarction. In human post mortem studies such activity is seen within the peri-infarct zone [7]. Such studies have been supported by *in vivo* primate data where ¹¹C-PK11195 binding peaks at 20-40 days in both core and peri-infarct zones before attenuating at later time points [8]. Limited numbers of clinical studies [9],[10] have outlined a pattern of binding but few studies have examined this phenomenon serially early within the natural history of ischaemic stroke (IS) or using the reference tissue method of kinetic modelling to account for non-tissue/specific binding. Furthermore no studies have accounted for variations in cerebral blood volume (CBV) within this context.

Our objectives have been to study the kinetics and spatial distribution of microglial activation in subacute IS using quality controlled methods, (R) -PK11195, PET and C¹⁵O derived cerebral blood volume (CBV) to account for intravascular activity.

Methods: Following informed consent patients with clinically defined, ischaemic cortical middle cerebral artery syndromes were recruited. Healthy male aged matched volunteers were imaged in a single session to form the control group. Each patient met inclusion and exclusion criteria. Target time intervals for paired MR and PET studies were < 72 hours, 7-10 days and one-month post ictus (p00, p01 and p02 respectively). MRI (magnetic resonance imaging) protocols included T₂ inversion recovery sequences in addition to a T₁ based sequence performed for co-registration purposes

Effect of neuroprotective agents against mechanical/ischemic injury *in vitro*.

Doortje C. Engel^{1,2}, Jennifer E. Slemmer¹, Chris I. De Zeeuw¹, Andrew I.R. Maas² and John T. Weber¹

Departments of Neuroscience¹ and Neurosurgery², Erasmus Medical Center, Rotterdam, The Netherlands

Traumatic brain injury (TBI) leads to cell damage by direct mechanical disruption of the brain and by secondary insults such as ischemia.

We utilized an *in vitro* model of stretch-induced injury to investigate the effects of mechanical, ischemic and combined mechanical/ischemic insults to cultured mouse cortical cells. Stretch injury alone increased uptake of the dye, propidium iodide (Prl), 15 min after injury, suggesting cellular membrane damage. Uptake of Prl was dependent on the level of stretch and decreased with time post-injury up to 48 hr. Stretch injury also caused a significant reduction in the amount of MAP2-positive neurons in cultures. Exposure of cultures to ischemic conditions for 24 hr, or a combination of stretch followed by 24 hr of ischemia, caused no change in Prl uptake when compared to stretch injury alone. However, both ischemia and the combined insult paradigm caused a greater reduction in neurons compared to stretch alone.

Next, we tested the effects of the potential neuroprotective agents, 7-nitroindazole (7-NINA), lubeluzole, and superoxide dismutase (SOD) on injured cells. Post-treatment (addition of agents 15 min after stretch-induced injury) by these agents provided no protection against combined insults, as measured by the amount of MAP2-positive neurons. Pre-treatment of cultures by lubeluzole (100 nM) and SOD provided modest protection against stretch injury alone; pre-treatment by SOD provided somewhat protection against ischemia alone; pre-treatment by 7-NINA (1 μ M and 10 μ M) and SOD provided modest protection in the combined insult paradigm, though less than in the stretch injury alone.

These results suggest that degenerative mechanisms caused by secondary ischemic insults may be too devastating to the cortical neurons *in vitro*, which are already compromised from primary mechanical damage following TBI.

Muscle power development in preterm infants with periventricular flaring or leukomalacia in relation to outcome at 18 months

Samsom Janny, Sie Lilian, de Groot Laila.

Department of Paediatrics, VU University Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Objective:

Is it possible to relate muscle power development to periventricular flaring (PVF) or leukomalacia (PVL)?

Patients and Method:

PVF or PVL was diagnosed by brain ultrasound during the neonatal period in 44 infants. They were divided into three groups according to the severity of their condition. At the corrected age of 0, 3 and 6 months an age-adequate neurological examination with special emphasis on the relation between active and passive muscle power and symmetry was performed. Results for the whole body as well as for the shoulders, trunk and legs were classified as optimal, suspect or abnormal. Motor outcome at the corrected age of 18 months was graded the same way.

Results:

An overall optimal muscle power regulation was found in one infant at 0, two at 3 and one at 6 months. Suspect outcome was found at all ages in the three groups. At 0 months muscle power regulation did not differ between the three groups. At 3 and 6 months overall poor muscle power, primarily caused by poor muscle power regulation in the shoulders and trunk, was found in infants with PVL grades III or IV. At 18 months of corrected age 24 infants showed no neurological impairment and 12 infants had severe impairment including all 10 infants categorized as having PVL grades III or IV. The best predictors of impairment at 18 months were the combined results of muscle power in shoulders and trunk at 3 months with those of the shoulders at 6 months.

Conclusion:

Muscle power development during the first year of life can predict outcome in PVF or PVL.

An investigation of Intracellular Calcium Concentration Homeostasis before and during Ischemia

Bijlenga Ph, Bancila M, Bloc A, Nikonenko I, Müller D de Tribolet N

The details of the cellular response to ischemia is still a mystery.

In neurones and glia the intracellular calcium concentration increases very quickly after ischemia is started and seems to act as a trigger of the apoptotic cascade. We therefore were interested in studying how the intracellular calcium concentration homeostasis is maintained and disrupted before and during ischemia.

We propose that in resting neurones and glia the intracellular calcium concentration is maintained by the equilibrium between a constant passive calcium influx and an energy consuming pumping.

T-type voltage gated calcium channels sustain a very small but constant calcium current at membrane potential between -80 and -40 mV. At rest neurones and glia are hyperpolarized close to -70 mV. We expect that in resting neurones and glia calcium continuously flows in through voltage activated T-type calcium channels.

During ischemia the metabolic resources are challenged and we hypothesise that calcium extrusion is affected. Reducing or abolishing the calcium influx would spare energy for other essential house keeping cellular activities.

We show that pharmacological inhibition of the T-type calcium current during or after transient ischemia of rat organotypical hippocampal cultures provides a very significant protection against delayed neuronal death.

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Effects of diethyldithiocarbamate (dedtc) throughout mouse brain development. Alterations in myelination process.

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Different studies “in vivo” reported that the diethyldithiocarbamate (DEDTC) acts as a chelatable agent of endogenous biometals, with a high specificity for copper and zinc. In addition, has been determined that it can induce the inhibition of different enzymes, such as aldehyde deshydrogenase, dopamine beta hidroxylase, certain superoxide dismutase, and other enzymes.

Thus the effects of DEDTC would be similar to the ones determined by Cuprizone with a high chelatable specificity for copper and clearly identified with a demyelination process.

The aim of this work was an “in vivo” study of physiological, cellular and molecular effects of DEDTC in mice brain throughout development.

The animals were injected intraperitoneally by DEDTC daily, from P2 until P15. The animals were perfused at different postnatal days. From the dissected brains, coronal sections were obtained for histochemical and immunohistochemical techniques.

The treatment with DEDTC throughout postnatal development revealed important physiological alterations in the organism, such as a general growth delay. It's important to mention the observed delay in the hair initial growth, and in the eyes opening after birth.

By different histochemical techniques (Nissl, Hematoxilin-eosin) a reduction in different brain areas was clearly observed. In addition, a higher density of cells was detected in specific areas, such as subventricular zone (svz), which is related with cell proliferation, together with alterations in the formation of cortical layers.

The immunohistochemical techniques supported those previous results. Therefore by the GFAP (“Glial Fibrillar Acidic Protein”) antibody, a different pattern of astrocytes was observed, mainly in the cortical areas, together with a higher density of cells in specific zones, such as svz. Observations at high magnification, revealed that GFAP positive cells in treated animals displayed an altered morphology, not correlated with the characteristic migrating aspect detected in untreated animals.

The PCNA (“Proliferating Cell Nuclear Antigen”) antibody reported the existence of changes in rate proliferation, mainly appreciated at the svz where an increase in PCNA-IR cells was determined.

All these results suggested that the sequential DEDTC administration induces a physiological, cellular and molecular delay in development. Moreover, the alterations detected in the pattern and morphology of GFAP-IR cells, together with changes in the migration process, suggested that the myelination process would be affected.

Induction of Zac1 in the neural cells of the limbic system of mice following seizures that provoke extensive cell damage

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Zac1, a new zinc finger protein that regulates both apoptosis and cell cycle arrest, has a strong gene expression in many proliferative/differentiation areas during brain development. In the present work, we study Zac1 gene expression and protein in experimental seizure models after PTZ or KA treatment. In the KA-treated mice, early and intense up-regulation of Zac1 is detected in the limbic system and hypothalamic nuclei. Moreover, in the KA-treated mice previously injected with MK-801, which acts as a block of the NMDA receptors we found a noticeable attenuation of the induction of Zac1 mRNA. In the PTZ-treated mice, slight up-regulation of Zac1 is detected in some limbic areas, except in the hippocampus, which the up-regulation is undetected. Thus, the Zac1 gene seems to be highly induced by the seizure models that generate extensive cell damage (cell death), reinforcing their role in the cell cycle/apoptosis. On the other hand, in the KA-treated mice Zac1 is induced both glía and neurons, which suggest a novel role for the candidate tumor suppressor gene Zac1 in growth regulatory pathways involved in cellular remodelling and in response to injury.

GCNN gratefully acknowledges the support of Eisai/Pfizer.



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| 17.00 | Welcome and Introduction | Dr Relkin (USA) |
| 17.05 | Targeting Cell Death in Dementia | Dr Francis (UK) |
| 17.30 | Donepezil Protects Neuronal Cells Against Hypoxic & Excitotoxic Damage | Dr Akaike (J) |
| 18.00 | Evidence for a Neuroprotective Action of Cholinesterase Inhibitors in Alzheimer’s Disease | Dr Nordberg (Sw) |
| 18.25 | Clinical Evidence for a Neuroprotective Effect of Donepezil | Dr Krishnan (USA) |
| 18.50 | Panel Discussion | Dr Relkin (USA) |
| 19:00 | <i>Coffee & Posters</i> | |

Focal cerebral ischemia was induced by intra-luminal suture method described by Zea longa. We occluded middle cerebral artery for 120 min and measured infarct volume after 24 h of MCAo by TTC staining. *Oresu120* was orally administered at the dose of 80, 400 and 2,000 mg/kg (p.o.) twice at 0 and 120 min after ischemia. We also investigated the effects on motor function by rota-rod, balance beam test, foot fault test, and prehensile tractile test after 20 h of ischemia.

Global cerebral ischemia was induced by the method of Pulsinelli. We occluded both common carotid artery and vertebral artery for 10 min. Neuronal damage was measured by counting intact pyramidal neuron in CA1 region of hippocampus after 7 d of occlusion. *Oresu120* was administered at 0 and 90 min after occlusion.

Result

Body temperature the drug treated groups did not showed significant differences compared with vehicle treated group. 2 h of MCAO and 22 h of reperfusion resulted in the infarct volume of $33.3 \pm 1.17\%$. Administration of *Oresu120* 80, 400 and 2000 mg/kg, p.o. at 0 and 120 min after MCAo produced $34.7 \pm 4.19\%$, $25.6 \pm 1.86\%$ ($p < 0.01$) and $23.4 \pm 2.40\%$ ($p < 0.01$) in infarct volume, respectively. Ischemia induced cerebral edema was reduced in a dose dependent manner (Fig. 1.). The *Oresu120* 2000 mg/kg treated rats showed about 76% of the neuroprotective effect compared with minocycline (45 mg/kg).

In neurobehavioral test, *Oresu120* 400 and 2000 mg/kg showed a reduced deficit in balance beam test ($p < 0.05$, 0.01), a improved fall latency in rotarod test ($p < 0.01$), a reduced foot slip in foot fault test ($p < 0.01$) and a improved fall latency in prehensile traction test ($p < 0.01$) as compared with vehicle treated rats.

In 4-VO rat model, it did not show the significant protective effect on delayed neuronal damage in CA1 induced by ischemia.

Natural sesquiterpen alcohol a-bisabolol strongly induces apoptosis in glioma cell lines without affecting normal glial cell viability

Elisabetta Cavaliere¹, Sofia Mariotto¹, Cinzia Fabrizi², Alessandra Carcereri de Prati¹, Rossella Gottardo³, Stefano Leone², Luigi Valentino Berra¹, Giuliana Maria Lauro², Anna Rosa Ciampa¹ Hisanori Suzuki^{1*}

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Among human tumours, glioma is one of the most malignant ones and despite aggressive surgical resection and radiotherapy, the median survival in these patients does not normally exceed 1 year (1,4) The use of systemic chemotherapy may improve the efficacy of treatment, but its use is associated with significant toxicity and the long-term prognosis remains poor (2). Carmustine, one of the effective anti-glioma drug in the clinic, is, at concentration corresponding to LD₁₀ (13 mg/kg), not able to kill completely glioma cells *in vitro*(5). Numerous compounds from plants have been reported to be potential anti-glioma agents, although major parts of these compounds sank into oblivion.. In the course of our research attempting to identify new natural compounds modulating inflammatory processes, we observed that a-bisabolol killed quickly a number of human transformed cell lines, including highly malignant glioma cell lines . a-bisabolol is a small oily sesquiterpene alcohol with molecular mass of 222.37 Daltons isolated from the essential oil of a variety of plants, shrubs and trees. Due to its no or very low toxicity in animals (LD₅₀ = 13-14 g/kg, Merck Index), it is widely used in cosmetic preparations. However, only few scientific reports describing the biological effects of a-bisabolol are so far available in the literature (6,7). In the present study, we wanted to envisage in detail the cytotoxic effect and the type of death induced by a-bisabolol in glioma cells. For this purpose, we examined, as a human glioma cell model, T67 and U87 cell lines. As an animal model, we tested the rat glioma cell line C6.

At 2.5-3.5 mM the viability of these cells was reduced to 50 % with respect to untreated cells in 24 hours. Furthermore the same concentrations failed to affect the viability of normal rat astroglial cells, in line with its reported non-toxicity in rats (). At higher concentrations (10 μM) a-bisabolol killed completely the cells. Judging from caspase 3 activation, poly(ADP-ribose) polymerase cleavage, DNA ladder formation and hypo-G1 accumulation, the cytotoxicity triggered by a-bisabolol results from the induction of apoptosis. It is widely accepted that apoptosis is preferred to necrosis as a mechanism of tumour cell killing, since it does not induce inflammatory processes. Apoptosis is a physiological process which is characterised by the formation of apoptotic bodies inside cells and seems to be genetically programmed. Tumour cells may be resistant to apoptosis, presumably due to defects in apoptosis pathways. Two major routes, extrinsic and intrinsic, have been identified through which cytotoxic drugs induce apoptosis. The first one is mediated by death receptors. In the second pathway, mitochondria play essential roles.

The dissipation of mitochondrial-inner transmembrane potential and the release of cytochrome c from mitochondria indicate that apoptosis occurs through the intrinsic pathway. Taken together, these results point out that α -bisabolol may be considered a novel compound able to inhibit glioma cell growth and survival.

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In Vitro Antioxidant Neuroprotective Activity of BN 80933, a Dual Inhibitor of Neuronal Nitric Oxide Synthase and Lipid Peroxidation

Eltagi Abdalla

BN 80933, a dual inhibitor of neuronal nitric oxide synthase and lipid peroxidation, prevents in vivo brain ischemic/reperfusion injury. In the present study, BN 80933 was shown to protect neurons from hypoxia-induced cell death in primary cultures of cortical neurons. BN 80933 prevented lactate dehydrogenase activity elevation induced by hypoxia, displaying an IC_{50} value of $0.15 \pm 0.05 \mu M$. This effect was likely due to the antioxidant properties of BN 80933 because Trolox, but not N^G -nitro-L-arginine, also elicited protection. The antioxidant property of BN 80933 was then further investigated on HT-22 cells subjected to buthionine sulfoximine- or glutamate-induced glutathione depletion. The relative order of potency of the various compounds to inhibit oxidative stress-induced neuronal death (BN 80933 > U104067 > butylated hydroxytoluene > 17β -estradiol > Trolox > vitamin E) correlated with their ability to inhibit brain membrane lipid peroxidation (correlation coefficient = 0.939). BN 80933 afforded protection even when added 6 h after glutamate exposure. BN 80933 did not reverse intracellular glutathione depletion but prevented elevation of the level of 8-epiprostaglandin $F_2 \alpha$ (8-isoprostane), which appeared to be a delayed phenomenon. In conclusion, BN 80933 induces a potent cytoprotection that may be mediated by inhibition of delayed lipid peroxidation.

Neuroprotective effect of Oresu120[®], herbal prescription from traditional Korean medicine, on brain ischemia in rats *Hocheol Kim, Youngmin Bu, Minjung Kong*

Graduated school of East-West medical science, Kyung Hee university; Korea Institute of Science and Technology for Eastern Medicine (KISTEM) attached to NeuMed Co., Seoul 130-701, Korea

Introduction

Stroke is the most common cause of death. In westernized countries, stroke is the third leading cause of death after heart disease and cancer. While pharmacological therapy to reduce ischemic damage is being pursued, effective neuroprotective agents are to be developed. Even though many compounds were reported to be effective in various animal models, replication of the experiments with the neuroprotective agents in humans has regularly failed. Stroke has been treated by traditional Korean medicine, which is originated from China, over thousands of years. The abundant clinical data and extensive experience of stroke treatment have been accumulated in FEM. In this study, we developed new neuroprotective agent (Oresu120) from FEM prescriptions by adding two kinds of herbs to *Kyungok-go*, which had been described in FEM Classic as anti-aging effect. The major components of Oresu120 are the roots of Ginseng, Siberian ginseng, *Rehmannia glutinosa*, *Poria cocos* and honey. We investigated the neuroprotective effect of Oresu120 on the brain ischemia models, 4-vessel occlusion (4-VO) and middle cerebral artery occlusion (MCAo) model.

pared with vehicle treated group. 2 h of MCAO and 22 h of reperfusion resulted in the infarct volume of $33.3 \pm 1.17\%$. Administration of *Oresu120* 80, 400 and 2000 mg/kg, p.o. at 0 and 120 min after MCAo produced $34.7 \pm 4.19\%$, $25.6 \pm 1.86\%$ ($p < 0.01$) and $23.4 \pm 2.40\%$ ($p < 0.01$) in infarct volume, respectively. Ischemia induced cerebral edema was reduced in a dose dependent manner (Fig. 1.). The *Oresu120* 2000 mg/kg treated rats showed about 76% of the neuroprotective effect compared with minocycline (45 mg/kg).

In neurobehavioral test, *Oresu120* 400 and 2000 mg/kg showed a reduced deficit in balance beam test ($p < 0.05$, 0.01), a improved fall latency in rotarod test ($p < 0.01$), a reduced foot slip in foot fault test ($p < 0.01$) and a improved fall latency in prehensile traction test ($p < 0.01$) as compared with vehicle treated rats.

In 4-VO rat model, it did not show the significant protective effect on delayed neuronal damage in CA1 induced by ischemia.

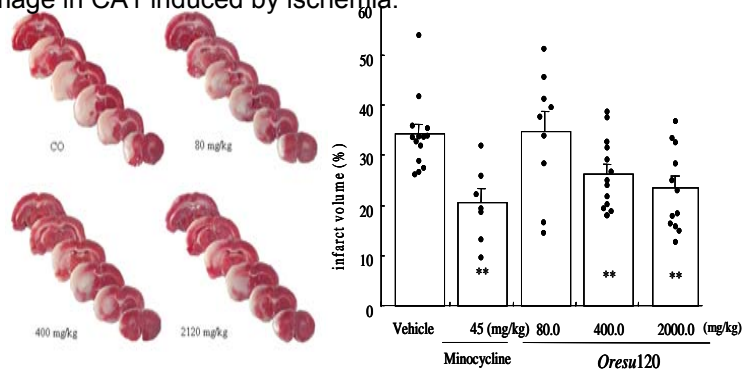


Fig. 1. The left photographs shows the 2,3,7-Triphenyltetrazolium chloride (TTC) stained brain sections taken from rats at 24 h after ischemia induction. The right graph shows the neuroprotective effects of *Oresu120*. The values are mean \pm SE (** : $P < 0.01$).

Conclusion

Oral administration of *Oresu120* reduced infarct size as well as improved sensory motor function following MCAo in rats. *Oresu120* (2000 mg/kg) showed 76% of the neuroprotective effect compared with minocycline (45 mg/kg) without any side effect. *Oresu120* can be used as a neuroprotective drug and a potential sources for drug development.

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Preservation of Cortical Metabolic Function by Donepezil in Patients with Mild to Moderate Alzheimer's Dementia: A 24-Week Clinical Trial With Placebo Control

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Objective: To evaluate the brain response of patients with Alzheimer's disease (AD) to donepezil therapy using positron emission tomography with [¹⁸F]-fluorodeoxyglucose (FDG/PET) and a multivariate analysis with high regional statistical sensitivity.

Background: Donepezil hydrochloride, a piperidine-based selective acetylcholinesterase inhibitor, has consistently demonstrated clinical benefits for AD patients.

Methods: In this double-blind, 24-week study, patients with mild to moderate AD (mean MMSE 21.0 ± 3.9) were randomized to donepezil (10 mg/day) or placebo (n=14 per group). Resting-state FDG/PET scans were acquired to quantify grey matter glucose metabolism in 27 regions-of-interest (ROI) per hemisphere at baseline, Week 12, and Week 24. ROI metabolism was corrected for patient differences in regional brain atrophy—based on individual structural MRIs. A brain-wide, multivariate statistical analysis (Canonical Variates Analysis) was performed on corrected regional metabolic activity to quantify the ROI responsiveness to donepezil therapy compared with placebo. The primary outcome measure was the change in regional metabolic activity from baseline to Week 24. Cognitive function was assessed at 6-week intervals using the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog).

Results: The donepezil and placebo groups did not significantly differ on demographic factors; nor did they differ at baseline on cognitive function or in atrophy-corrected regional metabolic activity. Post baseline, corrected metabolic activity revealed significant differences between treatment groups in the following ROI: the temporo-parieto-occipital, visual associative, and calcarine areas in the posterior cortex; and Broca's area, superior and middle frontal, and premotor areas in prefrontal cortex. Metabolic activity in these ROI was maintained in donepezil-treated patients, whereas patients receiving placebo showed a marked post-baseline metabolic decline in these ROI at Weeks 12 and 24 ($P < 0.005$). ADAS-cog scores improved in both groups, albeit to a greater extent in donepezil-treated patients. In the donepezil group, the changes in the most responsive regions at Week 24 were correlated with patient improvement in ADAS-cog scores ($r = 0.84$; $P < 0.0005$). In the placebo group, metabolic decline in these regions was not correlated with change in clinical status.

Conclusion: Donepezil treatment appeared to preserve metabolic function in temporo-parietal and prefrontal regions of AD patients with mild to moderate AD, where the

Orexin-1 receptor expression after transient global ischemia in mice.

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Introduction

Orexins (orexin-A and orexin-B) are originally identified as appetite stimulating neuropeptides (1). Orexin-containing perikarya are localized in the lateral hypothalamus and its immunopositive fibers are widely distributed throughout the brain including cerebral cortex, hippocampus, limbic system and brain stem. Orexin-A and -B act through two types of G-protein coupled, orexin-1 (OX1R) and orexin-2 (OX2R) receptors and they are also widely distributed throughout the brain (2). It is reported that orexin might be involved in neuronal disease because prepro-orexin gene was located at chromosome 17 which have some genes relevant to neurodegeneration disease collectively (1). It is also reported that orexin deficient mice and OX2R mutant dogs have occurred narcolepsy and the narcolepsy dogs have given rise to neurodegeneration in the amygdala and basal forebrain before this symptom onset. In addition, various acute or subacute neurological disorders, such as narcolepsy, Guillain-Barre syndrome, head trauma and encephalitis patients are reported to decrease orexin-1 level in the cerebrospinal fluid. Therefore orexin may play an important role for integrity of neurons. More recently, Irving et al have reported that expression of gene and protein for OX1R is increased in ischemic hemisphere after focal ischemia in rat (3). However, little is known on the morphological evidences of OX1R immunoreactivity after brain ischemia.

To estimate the effect of orexins on ischemic insult, we investigated the localization of OX1R after transient global ischemia in mice, induced by bilateral common carotid artery occlusion. Additionally, we demonstrated cellular localization of OX1R after global ischemia in mice using double immunofluorescence technique.

Materials and Methods

Adult male C57/BL6 mice (20 ~ 25 g) were anesthetized with 2.0% sevoflurane and the both carotid arteries were occluded with clips for 25 min followed by reperfusion upon removal of the clips (transient common carotid arteries occlusion; tCCAO). The mice were anesthetized with sodium pentobarbital (50 mg/kg ip) at 0, 8 hours, 1, 2, 4 and 7 days after tCCAO, and fixed by the perfusion with saline followed by 2% paraformaldehyde in 50 mM phosphate buffer (PB). The brain within bregma -1.6 mm to -2.2 mm area were embedded in paraffin or were cryoprotected in 20% sucrose and embedded in OCT compound on the liquid nitrogen. Four micrometer paraffin sections were used for the toluidine blue and terminal deoxynucleotidyl transferase-mediated dUTP end-labeling (TUNEL) staining to evaluate neuronal death and eight micrometer cryostat sections were used for

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Introduction

Stroke is the most common cause of death. In westernized countries, stroke is the third leading cause of death after heart disease and cancer. While pharmacological therapy to reduce ischemic damage is being pursued, effective neuroprotective agents are to be developed. Even though many compounds were reported to be effective in various animal models, replication of the experiments with the neuroprotective agents in humans has regularly failed. Stroke has been treated by traditional Korean medicine, which is originated from China, over thousands of years. The abundant clinical data and extensive experience of stroke treatment have been accumulated in FEM. In this study, we developed new neuroprotective agent (*Oresu120*) from FEM prescriptions by adding two kinds of herbs to *Kyungok-go*, which had been described in FEM Classic as anti-aging effect. The major components of *Oresu120* are the roots of Ginseng, Siberian ginseng, *Rehmannia glutinosa*, *Poria cocos* and honey. We investigated the neuroprotective effect of *Oresu120* on the brain ischemia models, 4-vessel occlusion (4-VO) and middle cerebral artery occlusion (MCAo) model.

Method

We made the mixture with the powder of the roots of *Panax ginseng* and *Poria cocos*, the juice of *Rehmannia glutinosa*, and honey together, and heated the mixture in the pot with the water boiling for 72 h and cooled for 24 h in cold water bath. After cooling, we added another extracts of *Acanthopanax senticosus* and *Scutellaria baicalensis* to the mixture, and heated again for 24 h. We used this mixture (*Oresu120*) as sample material after standardizing with HPLC.

Focal cerebral ischemia was induced by intra-luminal suture method described by Zea Longa. We occluded middle cerebral artery for 120 min and measured infarct volume after 24 h of MCAo by TTC staining. *Oresu120* was orally administered at the dose of 80, 400 and 2,000 mg/kg (p.o.) twice at 0 and 120 min after ischemia. We also investigated the effects on motor function by rota-rod, balance beam test, foot fault test, and prehensile tractile test after 20 h of ischemia.

Global cerebral ischemia was induced by the method of Pulsinelli. We occluded both common carotid artery and vertebral artery for 10 min. Neuronal damage was measured by counting intact pyramidal neuron in CA1 region of hippocampus after 7 d of occlusion. *Oresu120* was administered at 0 and 90 min after occlusion.

Result

Body temperature the drug treated groups did not show significant differences com-

Forty eight hours after tMACO, the mice of IL-1 KO and wild-type mice were sacrificed and infarct volumes of them were determined by triphenyl tetrazolium chloride staining. Moreover, the brains were removed from sham-operated controls (0 h) and at 3, 6, 12, 24, and 48 h after tMCAO in time dependent manner. In some mice, the brain were used to make cryosection for immunohistological detection of IL-1 α , type I IL-1 receptor (IL-1RI), and 3-nitrotyrosine (3-NT) following by fixation by perfusion of 2 % paraformaldehyde. The cortical regions of brain in other mice were snap-frozen by liquid nitrogen and extracted total RNA to determine reverse transcriptase polymerase chain reaction (RT-PCR) for IL-1 α , and three types of nitric oxide synthase (NOS) subtypes.

Results and Discussion

RT-PCR and Immunostaining for IL-1 α was determined that IL-1 α increased at 6 hours (mRNA) and 24 hours (protein) after tMCAO in the ipsilateral hemisphere. Immunostaining for IL-1 α was colocalized in the microglia and macrophage not in the astrocytes and neurons. The immunoreactivities of IL-1RI were also increased progressively in the microvasculature and neuron-like cells of the ipsilateral hemisphere.

Then, we compared the infarct volume in IL-1 KO and wild-type mice 48 hours after tMCAO. Infarct volumes were significantly lower in IL-1 KO mice compared with wild-type mice 48 hours after tMCAO ($p < 0.01$). To estimate increasing of oxidative stress by IL-1 α dependent pathway, the immunoreactivities of 3-nitro-L-tyrosine were determined in the neurons and microvasculature 24 h after tMCAO and were significantly decreased in the IL-1 KO mice compared to wild-type mice. In addition, gene expression of NOS subtypes in IL-1 KO mice were lower than that measured in wild-type mice.

In conclusion, IL-1 KO mice have significantly reduced brain injury after tMCAO as compared with wild-type mice. One of the mechanisms underlying this is the induced oxidative stress brought about by the release of an oxidative substance, ONOO $^-$, perhaps via the regulation of NOS.

Acknowledge

This study was supported in part by grants from the Ministry of Education, Science, Sports and Culture (S.S.), and a High-Technology Research Center Project from the Ministry of Education, Science, Sports and Culture of Japan (H.O. and S.S.).

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OX1R immunostaining.

Cryostat sections were incubated in 1% hydrogen peroxide following the incubation in phosphate-buffered saline (PBS) containing 10% normal goat serum. For single staining, sections were incubated in the rabbit anti-OX1R antibody (CHEMICON International Inc., Temecula, CA) at 4°C overnight. Antibody detection was carried out using the biotinylated goat anti rabbit IgG as secondary antibody for 2 hours, followed with the Vectastain ABC kit for 1 hour, and then reacted with stable 3,3'-diaminobenzidine complex.

For double immunofluorescent staining, we used the primary antibodies to detect neurons and glial cells as shown follows: mouse anti-NeuN antibody as neuronal marker, the mouse anti-glial fibrillary acidic protein (GFAP) antibody, the mouse anti-2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) antibody, the rat anti-CD11b antibody as the astrocytic, oligodendrocytic and microglial markers respectively. Sections were incubated overnight at 4 °C in the anti-OX1R antibody and cellular markers antibody simultaneously. The immunoreactivities of OX1R was detected using Alexa 594 labeled goat anti-rabbit IgG, and that of NeuN, GFAP, CNP, CD11b were detected using Alexa 488-labeled goat anti-mouse or anti-rat IgG antibodies for 90 minutes incubation.

Results and Discussion

We evaluated the neuronal death in the hippocampus CA1 region using toluidine blue and TUNEL staining after tCCAO. Toluidine blue staining provided a morphological approach for counting intact cells and TUNEL staining was used to detect apoptotic-like damaged cells. The number of intact cells was decreased significantly after 4 days tCCAO compared with sham operated control. The number of TUNEL-positive damaged cells was noted at 2 days and peaked at 4 days after tCCAO. Although the sum of numbers from intact cells and TUNEL-positive cells showed similar levels up to 4 days after tCCAO, but decreased at 7 days after tCCAO.

Next, we examined the time course change of localization for OX1R immunoreactivity after tCCAO. In sham-operated control (0 hours after ischemia) group, OX1R immunoreactivity was detectable through the mouse brain, including the neocortex, hypothalamus hippocampus, and amygdale. The intensity of OX1R immunoreactivity was slightly decreased at 8 hours, increased again at 1 day and peaked at 2 days after tCCAO in hippocampus and neocortex. Four to 7 days after ischemia, the immunoreactivity was decreased again. Interestingly, OX1R immunoreactivity increased prior to the increase of TUNEL positive cells in the hippocampus and neocortex. On the contrary, OX1R immunoreactivity did not change in the hypothalamus following tCCAO. Although the hypothalamus is a feeding regulating center, these results give rise to the view that

The brain ischemia induced up-regulation of OX1R in the hippocampus and neocortex but not in the hypothalamus. We then carried out cell identification of OX1R expression cells using double immunohistochemical technique in the sham-operated control mice and in the mice at 2 days after ischemia. These observations were focused on the hippocampus and cortex that were increased OX1R immunoreactivity after tCCAO. The majority of OX1R immunoreactivity was co-localized with the NeuN immunopositive

cells in the hippocampus, cerebral cortex before and after ischemia. OX1R immunoreactivity was not merged with GFAP-, CNPase- and CD11b- immunopositive cells in the sham operated control group. However, OX1R immunoreactivities were co-localized with GFAP- (astrocyte) and CNPase- (oligodendrocyte) positive cells, not with CD11b (microglia / macrophage) positive cells at 2 days after tCCAO. OX1R positive astrocytes were observed around the CA1 region and OX1R positive oligodendrocytes were observed in the CA1 region and neocortex. These results suggest that the effect of orexin by ischemia mediates glial pathway.

In conclusion, since OX1R immunoreactivity was increased prior to the delayed neuronal death, orexin might play an important role in regulating delayed neuronal death after ischemia. In addition, we identified that OX1R immunoreactivities were co-localized with GFAP or CNPase immunoreactivities after global ischemia. It suggests that effect of orexin for ischemia mediate astrocyte and oligodendrocyte.

Acknowledge

This study was supported in part by grants from the Ministry of Education, Science, Sports and Culture (H.O. and S.S.), and a High-Technology Research Center Project from the Ministry of Education, Science, Sports and Culture of Japan (H.O. and S.S.).

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Suppression of Oxidative Neuronal Cell Death after Transient Middle Cerebral Artery Occlusion in Interleukin-1 Gene Deficient Mice

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Introduction

Interleukin-1 (IL-1) is a proinflammatory cytokine, which plays a crucial role in the host's response to inflammation, infection, injury, and immunological challenge. IL-1 consists of two molecular species, IL-1 α and IL-1 β , which are derived from two distinct genes located 50 kb apart on chromosome 2 of the mouse genome. IL-1, especially IL-1 β has diverse actions in the brain and there is considerable evidence implicating it in neurodegeneration. Injection of IL-1 β has been shown to exacerbate ischemic brain damage and injection of IL-1 receptor antagonist leads to a decrease in infarct volumes. However, the mechanisms regulating the action of IL-1 are poorly understood.

There is increasing evidence available to indicate that nitric oxide (NO) may be an important mediator of ischemic brain injury. NO is produced by nitric oxide synthase (NOS), and reacts with the superoxide anion (O₂⁻) to form peroxynitrite (ONOO⁻), a powerful oxidant, in postischemic reperfusion. ONOO⁻ directly oxidizes sulfhydryl groups in L-tyrosine and there by alters or prevents the normal functioning of those proteins. Although it is difficult to detect ONOO⁻, 3-nitro-L-tyrosine (3-NT), the reaction product of ONOO⁻ can be detected using immunohistochemical techniques and is considered to be a reliable marker for ONOO⁻. On the other hand, the addition of IL-1 β into cultured human microglia and rat microvascular endothelial cells express inducible NOS (iNOS) mRNA and generate NO. These results suggest that NO and ONOO⁻ formation may be associated with the neurodegenerative mechanism of IL-1 after ischemia/reperfusion. However, the relationship between IL-1 and ONOO⁻ formation after ischemia is still unclear.

Thus, the purpose of the present work is to determine the neurodegenerative mechanism of IL-1 to focus on the generation of ONOO⁻ and NOS after transient ischemia.

Methods

Mice with homozygous disruption of both IL-1 α and β genes (IL-1 KO) that had been backcrossed for six to nine generations into BALB/c strain were used in these experiments. Wild-type mice were generated from the same chimeric founder. Adult male mice were subjected to focal cerebral ischemia. Anesthesia was induced by inhalation of 2.0 % sevoflurane in N₂O/O₂ (70/30 %) through a facemask. Ischemia was induced by occlusion of the left middle cerebral artery (MCA) using the intraluminal filament technique. At 1 h after ischemia, the mice were reanesthetized and the suture was withdrawn.

plasma layer by centrifuge for measurement of nitric oxide and arginine content. Moreover, the mice were determined regional cerebral blood flow (rCBF) during and after CCAO by using laser-doppler flowmeter with/without nucleoprotamine diet mice.

Results and Discussion

After severe ischemia, the survival rate of the NF-group was lower than those of the group fed standard diet or NP. The survival rate of standard diet mice showed 80 % 1 day after severe tCCAO. While the mice which fed NF diet decreased clearly the survival rate to 20 % as compare with standard diet group, the mice fed NP diet recovered it up to 60 %. The result shows that the deficient of nucleoprotamine in the diets affects the survival rate.

Morphological changes in the hippocampal CA1 region were estimated 48 h after mild ischemia by toluidine blue- and TUNEL-staining. The CA1 pyramidal layer in the hippocampus in the NP group remained morphologically relatively intact at day 2 after tCCAO, and the staining intensity and number of TUNEL-positive cells was low. Cells in these areas in the PT group also remained relatively intact. However, the neuronal cell death in DNA group was similar to that compared to the NF group, with many TUNEL-positive cells clearly observed. The number of cell death in the DNA-group, however, was affected similar to that of the NF-group.

The arginine contents in both the NP and PT diets were about two times higher than that of the NF diet. The plasma arginine levels at day 0 in the NP and PT group were higher than that of the NF group. The arginine levels in urine were similar to that in plasma. Urinary NO_2^- and NO_3^- levels in the NP and PT groups were higher than those in the NF group. In order to determine the possibility that the experimental diet could alter CBF during ischemia, we measured rCBF in the NF and NP groups. The rCBF in NP group 10 min after ischemia was significantly higher than NF groups.

Our data suggests that the nucleoprotamine content in salmon soft roe could be a useful nutritional resource for the prevention of cell damage caused by ischemia such as those occurring with cerebral and/or heart infarction.

Acknowledge

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α -synuclein as a peripheral biomarker for Parkinson's disease

Michell, AW; Luheshi LM; Spillantini MG; Barker RA

A biomarker able to reflect the pathological changes that occur within the brain of patients with Parkinson's disease (PD) would find several uses. Potentially it might help diagnose and subdivide this heterogeneous disease, thus helping to select appropriate patients for clinical trials of neuroprotective and neuroregenerative therapies.

There is increasing evidence that α -synuclein plays a central role in the pathogenesis of PD. Point mutations in this protein have been shown to cause autosomal dominant PD¹, and recently a kindred has been described in which triplication of the α -synuclein gene locus led to familial PD². In sporadic PD α -synuclein has been shown to be present in Lewy bodies³. Furthermore, there is evidence of phosphorylation and other post-translational modifications of α -synuclein in human synucleinopathies⁴.

We looked at peripheral α -synuclein expression in blood samples of patients with PD versus controls to determine whether it might be a useful biomarker of disease. α -synuclein was found in the platelets of all subjects, but the absolute amounts were extremely variable, and there did not seem to be a correlation between the level of expression and the disease status.

Many patients with PD show abnormal autonomic function, including excess sweating. We looked for α -synuclein in skin biopsies from patients and controls, and found that there was a high level of expression in some patients and controls, but that it was absent in most others.

Platelet and skin levels of α -synuclein are therefore unlikely to be useful diagnostic biomarkers for PD. The present study did not contain sufficient numbers to determine whether the levels of this protein might be able to subdivide PD on the basis of disease severity, duration or other parameters, although an ongoing collaboration looking at plasma may help to answer these questions.

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Pattern of „spinal cord regeneration” in surgical treatment of syringomyelia

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Syringomyelia is the development of a syrinx within the spinal cord and hydromyelia is a dilatation of the central canal by cerebrospinal fluid. The exact pathogenesis is still unknown.

The spinal cord is composed of an outer layer of white matter and a central area of gray matter and in the white matter are major fiber tracts. The syrinx is surrounded by marked gliosis and compresses adjacent spinal cord structures causing a focal demyelination and atrophied fibers, especially the pain and temperature fibers that cross the cord near the central canal.

The treatment depends on the cause of the syringomyelia; for the syringomyelia with fourth ventricle communication, syringomyelia due to spinal cord injury and idiopathic syringomyelia the best treatment seems to be drainage of the fluid-filled cavity (1,2). A shunt is placed into the syrinx cavity to drain CSF, this shunt is left in place and fluid drains from the cavity, shrinking the syrinx (3,4).

Prognosis of surgically-treated syringomyelia in this way is variable; there is usually an improvement of clinical symptoms, but in some patients only a stabilization of symptoms is achieved. In others, a slow but persistent progression occurs (2).

Twelve patients with syringomyelia are analyzed in this study and the surgical results are presented.

The resultant hypothesis is that clinical improvement can be caused by the “spinal cord regeneration” as a remyelination of fiber tracts.

In cases of syringomyelia simultaneous therapy is therefore proposed: surgical therapy and regenerative and remyelination therapies such as axonal growth inhibitor blockade, antibody remyelination therapies etc.(5)

Systematic randomized clinical studies with large numbers of patients are strongly recommended to fully validate these results.

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Nucleoprotamine supplement protects mouse hippocampal neurons from delayed cell death after global ischemia

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Introduction

According to the Japanese government the catch of salmon in 2001 was 211,000 metric tons, for which the amount of soft roe associated with this catch is thought to account for 5,000 metric tons. Fish soft roe is rarely used as a food because of difficulties associated with its processing, its tendency to spoil, and other reasons. The nutritional significance and usefulness of fish soft roe, which mainly contains DNA and protamine, as an oral supplement, are not well known. Oral supplements of nucleotides may affect cellular metabolism, since most nucleotides and their derivatives act mediators in signal transduction resulting in the proliferation, differentiation and apoptosis as well as serve as genomic materials. In addition, protamine contains high levels of arginine, but that the exact content of arginine depends on the stage of maturation of the soft roe. In living organisms, arginine is donor of nitric oxide (NO), which plays key roles of vasodilation, the endocrine system and inflammation. Therefore, it is possible that nucleoprotamine could be more appropriate than nucleotides as a functional food.

The neuronal cell death causing by brain and cardiac infarction often give a severe influence for the patients subjected to these diseases. Although many therapeutic efforts in clinical scene is performing and developing, it doesn't go far enough. To solve this issue, the preventive medicine for supplement of beneficial food component might be one of strategy.

In the present study, the preventive effect of oral supplements of nucleoprotamine and its derivatives, DNA and protamine, extracted from salmon soft roe, on survival rate and hippocampal cell death induced by transient brain ischemia, was evaluated in mice.

Methods

Male C57BL/6 mice aged 8 weeks were supplemented the experimental diets which were based on a normal chow diet, an artificially formulated nucleoprotamine-free diet (NF) with/without 1.2% nucleoprotamine (NP), 1.2% DNA sodium salt (DNA), or 1.2% protamine (PT), that were extracted from salmon soft roe. Seven days after supplement these diets (day 0), the mice were subjected to severe (25 min) or mild (15 min) fore brain ischemia by the model of transient common carotid artery occlusion (tCCAO). Severe ischemic mice were determined the survival rate for 7 days and mild ischemic mice were evaluated neuronal cell death of hippocampal CA1 region 2 days after ischemia. In some mice which dieted NF, NP and PT for 7 days, urine by agitated by handling and heparinized blood samples were taken and was separated the

Table 2. The comparative analysis of data obtained by different studies about skewed

| Number of patients | Number of patients with skewed X-inactivation (%) | Authors |
|--------------------|---|------------------------|
| 11 | 36 | Zoghbi et al., 1990 |
| 30 | 53 | Camus et al., 1996 |
| 23 | 65 | Krepischi et al., 1998 |
| 34 | 9 | Amir et al., 2000 |
| 39 | 28 | Auranen et al., 2001 |
| 72 | 43 | Weaving et al., 2003 |
| 70 | 38.5 | Present study |

X-inactivation in RTT girls.

The incidence of skewed X-inactivation is quite different depending on technique applied and heterogeneity of RTT patients group studied. Summarizing the data obtained by different studies it can be noticed that incidence of chromosome X inactivation skewing is higher than in normal females (the number of individuals with non-random X-inactivation in normal females vary from 3 to 17%). Regarding our present data we suggest the skewed X-inactivation to be a feature of RTT.

The higher incidence of skewed X-inactivation may be explained by development disadvantage of cells with mutated MECP2 gene being active leading to death of these cells. Thus, MECP2 mutations seem to be involved in transcriptional activity of X-linked genes. Together replication delay of active chromosome X and higher incidence of skewed X-inactivation show the effect of mutations in transcriptional regulator gene (MECP2) and reveal the epigenetic consequence of this anomaly regarding to complex neurological malformations occurring in RTT.

The study of genetic and epigenetic aspects of this severe neurodegenerative disorder could be applied for developing the approaches of RTT treatment based on changing X-inactivation patterns by some chemicals. Thus, the study is basic for the development of neuroprotection and treatment of X-linked neurological diseases affecting the function of CNS cells. The work was supported in part by COPERNICUS 2 grant.

The Neuroprotective Effects of Phytoestrogens on Amyloid β Protein-induced Toxicity Are Mediated by Abrogating the Activation of Caspase Cascade in Rat Cortical Neurons*

Azad Mohammad Jasim

Amyloid β protein ($A\beta$) elicits a toxic effect on neurons *in vitro* and *in vivo*. In present study we attempt to elucidate the mechanism by which $A\beta$ confers its neurotoxicity. The neuroprotective effects of phytoestrogens on $A\beta$ -mediated toxicity were also investigated. Cortical neurons treated with 5 μ M $A\beta$ -(25-35) for 40 h decreased the cell viability by 45.5 \pm 4.6% concomitant with the appearance of apoptotic morphology. 50 μ M kaempferol and apigenin decreased the $A\beta$ -induced cell death by 81.5 \pm 9.4% and 49.2 \pm 9.9%, respectively. $A\beta$ increased the activity of caspase 3 by 10.6-fold and to a lesser extent for caspase 2, 8, and 9. The $A\beta$ -induced activation of caspase 3 and release of cytochrome c showed a biphasic pattern. Apigenin abrogated $A\beta$ -induced cytochrome c release, and the activation of caspase cascade. Kaempferol showed a similar effect but to a less extent. Kaempferol was also capable of eliminating $A\beta$ -induced accumulation of reactive oxygen species. These two events accounted for the remarkable effect of kaempferol on neuroprotection. Quercetin and probucol did not affect the $A\beta$ -mediated neurotoxicity. However, they potentiated the protective effect of apigenin. Therefore, these results demonstrate that $A\beta$ elicited activation of caspase cascades and reactive oxygen species accumulation, thereby causing neuronal death. The blockade of caspase activation conferred the major neuroprotective effect of phytoestrogens. The antioxidative activity of phytoestrogens also modulated their neuroprotective effects on $A\beta$ -mediated toxicity.

Genomic variations as a possible cause of cell death in the human brain: the study of chromosomes in neuronal cells of the developing and adult brain using interphase Multicolor Fluorescence In Situ Hybridization (MFISH).

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The human brain is the control center that stores, computes, integrate and transmit information contains about one trillion (10^{12}) neurons. The brain contains an enormous amount of glial cells, which occupy the spaces between neurons and modulate their functions. Up to date, there were no direct studies of large scale genomic variations and chromosomal complement in the central nervous system in man. It was postulated (without experimental proofing) that the neuronal cells of the normal brain contain normal (diploid) chromosomal complement.

Chromosomal abnormalities are the most common cause of genomic mutations in somatic and germ-line cells with the frequency of 0.1-2.0% per single chromosome depending of many genetic, epigenetic and environmental factors. The high frequencies of spontaneous chromosomal mutations allow proposing that the normal human brain could contain several billions of aneuploid neuronal cells. However, direct proof of chromosomal mutations, leading to large-scale genomic alterations in neuronal cells, has been missing in the human brain. Fully differentiated neurons in adult human (mammalian) brains do not divide; consequently, theirs chromosomal complements have not been examined by standard cytogenetic techniques. Only interphase karyotyping based on application of chromosome-specific DNA probes and Multicolor Fluorescence In Situ Hybridization (MFISH) could be considered as an efficient approach for analysis of chromosomal complement in neuronal human cells. The availability of DNA probes to all human chromosomes and multi-color FISH protocols allow to plan the studies of chromosomes in non-dividing interphase cells of the fetal and adult human brain.

Indirect evidences for some form of somatic genomic alterations have been obtained (reviewed by Rehen et al., 2001) in mice. Precedent exists for aneuploidy during early mammalian development, where it is thought to result in cell death (Voullaire et al., 2000; Harrison et al., 2000). Visualization of metaphase chromosomes by a nuclear transfer technique in mouse cortical neurons has indicated that the majority of them have abnormal karyotype (Osada et al., 2002). Direct FISH and spectral karyotype analysis of neurons in the developing and adult nervous system of mouse embryonic cerebral cortical neuroblast have been performed (Rehen et al., 2001). These approaches provide the identification of more than 30% of neuroblasts as aneuploid (Rehen et al., 2001). Therefore, it is possible that genomes in developing and adult neurons can be different at the level of whole chromosomes.

There are only limited number of molecular-cytogenetic studies, utilizing interphase FISH for the studies of the human brain. Yang et al., (2001) reported

Table 1. Molecular-cytogenetic studies of chromosome X inactivation patterns of

We have tested this approach using the most common assay for determining of X-inactivation patterns (AR assay) and have found that both techniques provide practically the same results.

The data obtained shows that maternal chromosome X could also be preferentially inactivated in RTT. We suggest the reason of preferential inactivation of maternal X is that MECP2 mutation is located in maternal chromosome X because the phenotype of girl was less severe comparing to others. It conflicts with data previously obtained, as it was shown that MECP2 mutations are exclusively of paternal origin.

We propose two possible epigenetic mechanisms, affecting clinical manifestation of the disease: 1) skewed X inactivation of mutated allele and 2) activation of normal MECP2 allele in inactive chromosome X when active chromosome X has the mutated copy of MECP2 (functional disomy). X inactivation pattern and functional disomy

| #pats | Ratio of active to inactive X chromosomes with small C-heterochromatin | Origin of chromosome X with small C-heterochromatin | Status of X inactivation |
|-------|--|---|--------------------------|
| 1 | 74 : 26 | - | Skewed |
| 2 | 55 : 45 | Pat | Random |
| 3 | 38 : 62 37 : 63 | Mat | Skewed(maternal X) |
| 4 | 18 : 82 | Pat | Skewed (paternal X) |
| 5 | 7 : 93 9 : 91 | Pat | Skewed (paternal X) |
| 6 | 64 : 36 | - | Skewed |
| 7 | 91 : 9 | Mat | Skewed (paternal X) |
| 8 | 69 : 31 | - | Skewed (paternal X) |

of MECP2 in the brain cells could affect expression of MECP2 at RTT. The study of chromosome X inactivation patterns using a common AR assay revealed a higher incidence of skewed X-inactivation in RTT girls (38.5%) comparing to control group of normal females (6.5%). We have compared the results obtained with previous data about X chromosome inactivation patterns in RTT girls. The comparative analysis of data about skewed X-inactivation in RTT girls is presented in Table 2:

Genetic and epigenetic mechanisms of neurodegenerative diseases: the study of children with Rett syndrome.

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Rett syndrome (RTT) is a severe neurodegenerative disorder with the incidence of 2.5% in mentally retarded girls in Russia (Vorsanova et al., 1999). We have performed molecular genetic and cytogenetic studies of 75 patients (72 girls and 3 boys) with clinical picture of RTT, selected according to the criteria for diagnosis of RTT. The spectrum of mutations in the responsible gene, encoding methyl-CpG binding protein (MeCP2) and skewed chromosome X inactivation have been analysed. MECP2 mutations were found in 38 from 50 (76%) RTT girls and one boy (Vorsanova et al., 2002). Skewed X-inactivation was detected in 27 from 70 (38.6%) RTT girls.

Replication timing of methyl-CpG-binding protein 2 (MECP2) gene (Xq28) has been analysed by FISH in 20 girls with RTT and 14 healthy girls. We applied FISH technique with cloned MECP2 gene (PAC 671D9) to analyse the time of replication of this locus (Soloviev et al., 1995) and chromosome X-specific alphoid DNA probe to mark early and late replicating chromosome X. We have detected that MECP2 locus is subjecting to X inactivation; i.e. early replicating MECP2 allele is located on active chromosome X, and late replicating allele – on inactive chromosome X. Alleles with mutation of MECP2 gene showed delayed replication on both active and inactive chromosome X. Therefore, mutations of MECP2 gene could lead to epigenetic “silencing” of this locus – delayed or late replication of mutated MECP2 allele “switch off” transcription of the locus. Thus one can suggest that study of replication timing is quite informative in analysis of transcriptional activity of X-linked genes. This phenomenon seems to reduce pathogenic effect of MECP2 mutations in actively replicating and developing neuronal cells. We can propose that the neuronal cells in the stage of active divisions during early development are not produced abnormal MeCP2 protein, while both normal and mutated MECP2 loci are expressed in mature neurons. Therefore, epigenetic “silencing” of mutated MECP2 gene could explain the phenomenon specific for RTT, i.e. why RTT girls are characterized by a period of normal early development followed by stage of dramatic regression.

In order to evaluate the origin of inactivated chromosome X we have developed an approach allowing to differentiate homologous chromosomes X in metaphase chromosomes and interphase nuclei by Fluorescence In Situ Hybridisation technique (FISH) with chromosome X-specific alpha-satellite DNA probe. FISH analysis of metaphase chromosomes in cohort of 33 girls with Rett syndrome (RTT) allowed us to detect 8 girls with structurally different chromosomes X - one chromosome X with a large and other one with a small centromeric heterochromatin. Step-wise application of differential replication staining and FISH technique to identify inactivation status of paternal and maternal chromosome X in RTT girls was applied. Non-random or skewed X inactivation in five RTT patients with preferential inactivation of one X chromosome over the other X chromosome in 60%-90% of cells was detected. Therefore, non-random or skewed X inactivation with variable penetrance in blood cells could take place at RTT. This Molecular-cytogenetic approach could be applied to study skewed X inactivation into neurones. The data obtained is presented in table 1:

the use of FISH to examine the chromosomal complement of interphase nuclei in the adult human brain. They were able to demonstrate that a significant fraction of the hippocampal pyramidal and basal forebrain neurons in Alzheimer disease have fully of partially tetraploid chromosome complement. They propose that this imbalance in chromosome complement is the direct cause of neuronal loss in Alzheimer's disease. Molecular-cytogenetic study utilizing MFISH of post-mortem brain of schizophrenic patients has been performed (Yurov et al., 2001). A statistically significant level of aneuploidy (up to 4% of neurons) was detected in the brain of patient with schizophrenia. The result indicated that low-level of aneuploidy (or chromosomal mosaicism) could be involved in the pathogenesis of schizophrenia. Therefore, neuropsychiatric diseases might have a special interest for extended molecular-cytogenetic analysis as they could be associated with chromosomal and gene mutations involving in regulation of neurodevelopmental processes in the brain.

The aim of present study is analysis of chromosomal complement in neuronal cells of the post-mortem brain tissue using modern molecular-cytogenetic techniques (interphase MFISH). Fluorescence in situ hybridization (FISH) was used for study of interphase chromosomes in human embryonic central nervous system (CNS) cells cultured in vitro and in autopsy samples of the adult human brain. Six primary cultures of embryonic human CNS cells (Vostrikov et al., 2002) were analyzed. Six control cell cultures obtained from human peripheral blood lymphocytes, six samples of post-mortem brains were used as control in FISH experiments. 1000-3000 hybridized cells were analyzed for each sample. The level of aneuploidy (per individual chromosome) was in range of 0.1 - 0.8% in post-mortem brain cells and 4-7% in embryonic CNS cells. However, significant level of aneuploidy (0.5-4%) has been detected in post-mortem brains of patients with schizophrenia (Yurov et al., 2001).

Our results indicate that embryonic CNS cells and adult brain cells differ in aneuploidy frequency. Therefore, selective loss of chromosomally abnormal developing neuronal cells have place during ontogenesis. However, relatively small, but significant, amount of aneuploid neurons could survive and exist in the adult brain. High level of chromosomal aneuploidy in embryonic human neuronal cells could, therefore, leads to genetic mosaicism in fetal and, probably, adult CNS. “Cryptic” chromosomal mosaicism of the brain have no phenotypic appearance, but should have substantial negative effect on normal brain development and functions, and, therefore, may have relevance to many neuropsychiatric diseases. Supported in parts by grant Copernicus 2 and INTAS.

Antiepileptic Drugs of IV Generation and Neuroprotection – our experience

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Neuroprotection is a widely used term for preventing neuronal damage after different injuries of the central nervous tissue. Most often it is used for prevention of apoptotic neuronal death after traumatic or ischaemic events or in cases of possible or suspected neuronal hyperexcitation. We present 20 patients treated with antiepileptic drugs of IV generation (lamotrigine, topiramate and gabapentine), who were referred to our Centre for epilepsy after craniocerebral trauma or neurosurgical operations, without clinical signs of symptomatic epileptic seizures. To all patients we performed MR of the brain at the beginning of the treatment and we followed them for at least 6 months after introducing mentioned AED's. During this period we performed electroencephalography three times (before treatment, after six months of treatment). We compared this results with the results of the group of 20 patients who had similar diagnosis and initial findings, but were treated with standard antiepileptic drugs.

Our results suggest that the group of patients treated with antiepileptic drugs of IV generation have better prognosis (according to the results of psychological tests electroencephalographic findings as well as clinical symptoms) then the group of patients treated with standard AED.

Those results can speak in favour of antiepileptic drugs of IV generation in the sense of neuroprotection and can justify their use in this indications, although a longer follow-up period should be recommended.

All patients were evaluated on the Unified Parkinson's Disease Rating Scale (UPDRS) and underwent timed motor tests at baseline and after 3, 6, 12, 18 and 24 months. The impact of GDNF infusion on quality of life was assessed using validated quality of life questionnaires: the 39-item Parkinson's Disease Questionnaire PDQ-39 and the 36-item Medical Outcomes Study short form health survey SF-36 and were used before surgery and after 3, 6, 12, 18 and 24 months. Neuropsychological outcomes were assessed once before surgery and then at 12 and 24 months after surgery. Additionally, the patients were assessed pre-operatively and at 6 and 12 months postoperatively with ¹⁸F-dopa PET.

Results:

After 2 years, there were no serious clinical side effects, a 57% improvement in the off-medication motor subscore of the Unified Parkinson's Disease Rating Scale (UPDRS) and a 63% improvement in the activities of daily living subscore. Health-related quality of life measures (PDQ-39 and SF-36) showed general improvement over time, with the overall scores tending towards levels expected in a control population. Neuropsychological assessment results indicated, both at one and two years, no significant detrimental effects of GDNF infusion on cognition. In addition there was evidence at 2 years of an improvement in verbal anterograde memory. ¹⁸F-dopa PET scans showed an 18 – 24 % increase in putaminal and nigral dopamine signal.

Conclusions: Chronic infusion of GDNF into the posterior putamen for 2 years is safe and results in improvement of parkinsonian symptoms and quality of life. GDNF may represent a potential neuroprotective and neurorestorative therapy for PD.

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A Mechanism for the Neuroprotective Effect of Apolipoprotein E Isoform-Specific Modification by the Lipid Peroxidation Product 4-Hydroxynonenal

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Inheritance of the apolipoprotein E (apoE) 4 allele increases the risk of Alzheimer's disease and may also influence the pathogenesis of other neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). The influence of ApoE genotype on disease susceptibility must ultimately be explained by the fact that ApoE proteins differ in only two amino acids: apoE2 has two cysteine residues, apoE3 has none. We previously reported increased protein modification by the lipid peroxidation product 4-hydroxynonenal (HNE), which covalently binds to proteins on cysteine residues, in human ALS Lumbar spinal cord. We now report increased levels of HNE-modified apoE in lumbar spinal cord samples from mice expressing an ALS-linked mutation in Cu/Zn-superoxide dismutase relative to controls. Studies of interactions of pure apoE proteins with apoE proteins with HNE showed that the isoforms differ in the amount of HNE they can bind, with the order E2 > E3 > E4. This correlated with the differential ability of apoE isoforms to protect against apoptosis induce by HNE in cultures of mouse spinal cord motor neurons and by the amyloid β -peptide in cultures of rat hippocampal neurons. These data suggest that ApoE plays a major role in detoxifying HNE, and the differential neuroprotective effect of its isoforms may help explain the relationship between apoE genotype and the susceptibility to neurodegenerative diseases.

ductive stress and prevent neuronal cell death. The Food Standards Agency reported that 5% of the UK population over the age of 60 years are vitamin B3 deficient. Therefore nicotinamide deficiency may be an additional, but easily correctable, complication in the treatment of PD.

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Intrapatamenal infusion of glial cell line-derived neurotrophic factor in Parkinson's disease: A two-year clinical, cognitive and quality of life outcome study.

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Objective:

To evaluate the safety and potential neuroprotective and neurorestorative effects of glial derived neurotrophic factor (GDNF) infusion into the putamen in patients with advanced Parkinson's disease (PD).

Background:

Glial derived neurotrophic factor (GDNF) promotes recovery of the injured nigrostriatal dopamine system and improves motor function in both rodent and non-human primate models of Parkinson's disease (PD). Only one study has attempted to examine the effect of GDNF in humans. This study utilised a monthly intraventricular bolus approach to delivering GDNF; no clinical benefit was obtained. Intracerebral administration is probably necessary because of the limited penetration into the brain tissue from either the blood or the cerebrospinal fluid. The neuroprotective and neurorestorative properties of GDNF seen in preclinical studies suggest that trophic factors may play an important role in treating PD, and intraparenchymal infusion of GDNF may represent a new treatment option.

Methods:

In this phase I safety study, 5 PD patients with a previous good response to levodopa underwent unilateral or bilateral insertion of cannulae into the posterior putamen through which human recombinant GDNF has been chronically infused via indwelling pumps. Clinical evaluations were based on the Core Assessment Program for Intracerebral Transplantations (CAPIT), a validated protocol for evaluating surgical treatments of idiopathic PD.

Neuroprotective Effects of Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) in Rat Models of Different Types of Brain Injuries

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Pituitary adenylate cyclase activating polypeptide (PACAP) was discovered as a hypothalamic peptide that stimulates cAMP production in the pituitary gland. Since its discovery, widespread distribution of PACAP in the central and peripheral nervous systems has been described. PACAP has numerous actions in the nervous system, including neurotrophic and neuroprotective effects. It has been reported that PACAP protects hippocampal CA1 neurons in global cerebral ischemia, it has protective effects in fornix transection, facial nerve axotomy, spinal cord injury and optic nerve injury.

In the present study we report our recent results on the neuroprotective effects of PACAP. We investigated its effects in focal cerebral ischemia, in a model of Parkinson's disease and in traumatic brain injury. In focal cerebral ischemia, induced by the intraluminal filament model, we found that PACAP was able to reduce the infarct size by 40-50% in both permanent and transient occlusion of the middle cerebral artery. PACAP treatment also ameliorated the functional deficits, and reduced the number of apoptotic neurons. In a rat model of Parkinson's disease, induced by unilateral 6-hydroxydopamine lesion of the substantia nigra, PACAP proved to be a very efficient neuroprotective agent. PACAP treated animals showed no hypokinetic signs, and the asymmetrical neurological signs recovered by 10 days postlesion in contrast to control animals. The number of dopaminergic cells was 50% on the ipsilateral side when compared to the uninjured side, while it was less than 5% in control rats. In a rat model of traumatic brain injury, induced by compact acceleration, PACAP treatment reduced the number of injured axons.

Our results demonstrate that PACAP is an effective neuroprotective agent in various models of brain injuries. The exact mechanism awaits further investigation, but according to presently available data, various factors can play a role, including the antiapoptotic anti-inflammatory and antioxidant effects of PACAP.

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Nicotinamide : Neuroprotective effects against Dopamine and 1-Methyl-4-phenylpyridinium Ion (MPP⁺)

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder, characterised by the selective destruction of dopaminergic neurones of the substantia nigra. Inhibition of mitochondrial Complex I and oxidative stress are key features of the nigral dopaminergic neuronal destruction in Parkinson's disease. Additionally, within the compromised neurone, the inherent presence of dopamine may itself be a risk factor. Enzymatic oxidation and auto-oxidation of dopamine result in the production of hydrogen peroxide, superoxide and toxic quinone species. In-vitro experiments have highlighted the cytotoxicity of L-dopa and dopamine in dopaminergic cell lines, although there is no direct evidence to show that the same is true in vivo. Nevertheless, this has led to the speculation that L-dopa therapy may potentially have neurotoxic effects [1]. We therefore sought to characterise the neuroprotective effects of nicotinamide using heme oxygenase-1 induction and lactate dehydrogenase (LDH) release as indicators of oxidative stress and cell death respectively.

Heme oxygenase exists as three isozymes (HO-1, HO-2 and HO-3) which catalyse the degradation of heme to biliverdin, carbon monoxide and iron. HO-1 is an inducible form and HO-2 and HO-3 are non-inducible. HO-1 is induced by a large number of chemical agents and cellular stresses, many of which facilitate the production of reactive oxygen species (ROS) and/or modify glutathione levels. HO-1 is induced in nigral dopaminergic neurones and produces intense immunohistochemical staining around the peripheries of Lewy bodies in PD. The significance of HO-1 induction under conditions of oxidative stress is not clearly understood. It has been suggested that HO-1 may have a role in antioxidant defence, as biliverdin is converted to the antioxidant bilirubin. However, catabolism of heme results in the production of free iron. Given that dopaminergic neurones naturally produce large amounts of hydrogen peroxide, it is possible that this could result in free radical damage to the cell, (reviewed in [2]).

Nicotinamide is the amide form of vitamin B3 and is a precursor in the NADH synthetic pathway. Nicotinamide is also the substrate for the enzyme nicotinamide N-methyltransferase (NNMT), which forms N-methylnicotinamide in an irreversible reaction that ultimately removes available nicotinamide from the cell. Individuals with PD have been shown to have higher levels of NNMT expression compared to age matched controls [3]. Hence, it is hypothesised that such individuals could be vitamin B3 deficient and have reduced levels of NADH, thereby impairing the mitochondrial electron transport chain. It is therefore postulated that nicotinamide may have beneficial effects for the neurone.

Aims

The aims of this investigation were to characterise the ability of nicotinamide to reduce oxidative stress and cytotoxicity caused by L-dopa, dopamine and MPP⁺ treatment individually and in combination. Cells were exposed to nicotinamide prior to, during, or subsequent to MPP⁺ exposure to determine whether nicotinamide could prevent the actions of MPP⁺ and rescue cells from the actions of MPP⁺.

Methods

TE 671 cells were used as an in-vitro neuronal cell line model.

Preventative actions of nicotinamide

Cells were treated with the test compound for 24 hours or with nicotinamide pre-treatment for 24 hours followed by combined test compound and nicotinamide for 24 hours. Concentrations of test compounds used: dopamine 0, 1, 10, 50, 100, 200 mM, L-dopa 0, 10, 50, 100, 200 mM, MPP⁺ 0, 0.1, 1, 10, 100, 400 mM, nicotinamide 0.1, 1mM.

Cell Rescue

Cells were treated with MPP⁺ 0, 0.1, 1, 10, 100, 400 mM for 1h, then nicotinamide 0.1 mM or an equivalent volume of vehicle were added and cells were cultured for a further 23 hours.

HO-1 Induction

Levels of HO-1 mRNA were measured relative to HO-2 (housekeeping gene) using semi-quantitative RT-PCR. Results are expressed as the ratio HO-1:HO-2 and given arbitrary units.

Cytotoxicity

Percentage cytotoxicity was measured using LDH release determined by CytoTox 96 Non-Radioactive Assay (Promega, UK).

Results

Preventative Actions of Nicotinamide (Vitamin B3)

Both dopamine (DA) and L-DOPA caused statistically significant, dose-related increases ($p < 0.001$) in HO-1 expression and LDH release over the range of concentrations tested. Nicotinamide treatment caused dose related reductions in HO-1 induction and LDH release. Typical results are illustrated below (n=6).

| DA (mM) | 0 | 1 | 10 | 100 |
|---------------------|-------------|-------------|-------------|--------------|
| HO-1 : HO-2 [SEM] | 1.28 [0.27] | 1.14 [0.09] | 1.89 [0.40] | 4.36 [0.76] |
| LDH (%) [SEM] | 0.00 [1.82] | 3.80 [4.56] | 4.01 [8.24] | 37.97 [5.46] |
| DA + 1mM Vitamin B3 | | | | |
| HO-1 : HO-2 [SEM] | 0.97 [0.40] | 1.04 [0.53] | 1.12 [0.38] | 1.14 [0.48] |
| LDH [SEM] | 0.00 [1.24] | 0.00 [0.90] | 3.75 [1.62] | 16.87 [1.47] |

MPP⁺ caused increased LDH release but no increase in HO-1 induction. Nicotinamide treatment abolished the increase in LDH release.

Cell Rescue

Addition of nicotinamide after 1 hour treatment with graded doses of MPP⁺ resulted in a significant decrease in cytotoxicity (Chi squared test for linear trend, $p = 0.037$, n=4).

Discussion and Conclusions

These data demonstrate the neuroprotective properties of nicotinamide to reduce oxi-