

B-NP-4

Emodin Abrogates Glucose Induction of Fibronectin Synthesis in Human Peritoneal Mesothelial Cells (HPMC)

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Introduction: The peritoneal membrane is continuously exposed to unphysiological glucose concentrations during peritoneal dialysis. Glucose induction of peritoneal matrix protein synthesis by HPMC leads to the thickening and fibrosis of the peritoneal membrane and failure of peritoneal dialysis. These abnormalities are mediated through the activation of PKC induction of TGF-beta1. Recently, emodin (1,6,8 trihydroxyanthraquinone) has been reported to abrogate increased fibronectin (FN) synthesis in an animal model of glomerulosclerosis. This study investigates the effect of emodin on FN synthesis in HPMC under elevated glucose.

Methods: Confluent growth arrested HPMC were pre-conditioned in either 5mM or 30mM D-glucose for 2-3 weeks prior to the addition of emodin (20micrograms/ml) for 24 h. Transcriptional and translational modulations of FN and TGF-beta1 synthesis in HPMC were investigated by RT-PCR, TGF-beta1 ELISA, Western blot analysis, and immunohistochemistry. 30mM mannitol was used as the hexose control.

Results: Increased TGF-beta1 synthesis was observed when HPMC were cultured under elevated glucose concentrations (278.5 ± 30.4pg/ml) vs 433.1 ± 50.2pg/ml, 5mM vs 30mM D-glucose, $P < 0.0001$). 30mM D-glucose increased FN synthesis through increased activation of PKC-alpha. Localization studies demonstrated enhanced cytoplasmic FN synthesis particularly around the peri-nucleus, and the presence of intense fibrils within the extracellular milieu. 30mM mannitol did not increase TGF-beta1 nor FN synthesis above basal levels. Concomitant addition of emodin and 30mM D-glucose to HPMC reduced TGF-beta1 synthesis (433.1 ± 50.2pg/ml vs 309.1 ± 37.9pg/ml, 30mM D-glucose alone vs 30mM D-glucose and emodin respectively, $P < 0.0001$). Emodin also decreased cytoplasmic and extracellular accumulation of FN, but did not reduce peri-nuclear FN staining. Further studies demonstrated that these modulatory effects of emodin were mediated through the inhibition of PKC activation.

Conclusion: We conclude that emodin can reduce glucose induced TGF-beta1 and FN synthesis in HPMC and represents a novel strategy in the prevention and treatment of peritoneal fibrosis.

B-NU-1

Neuropeptide Y and Its Analogs Modulate Cell Viability in *in vitro* Models of Ischemia

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Introduction: Neuropeptide Y (NPY) has been implicated in the pathogenesis of many human diseases. Middle cerebral artery occlusion (MCAO) experiments have previously shown that exogenous NPY or an Y1 agonist, [Leu,³¹ Pro³⁴]-NPY, worsened the infarct volume and impaired the regional cerebral blood flow (rCBF), that an Y1 antagonist, BIBP3226, reduced the infarct volume without affecting the rCBF, and that an Y2 agonist, NPY3-36, did not influence the infarct volume or rCBF. To directly investigate the cellular effects during cerebral ischemia, we applied oxygen and glucose deprivation (OGD) to two types of neuroblastoma cell lines and primary astrocyte culture. SK-N-MC cells express the Y1 receptors, and SH-SY5Y cells express the Y2 receptors.

Methods: SK-N-MC cells and SH-SY5Y cells in monolayer were subjected to OGD for 1h. NPY (at 10⁻⁸ M, 10⁻⁹ M, or 10⁻¹⁰ M), [Leu,³¹ Pro³⁴]-NPY (at 10⁻⁶ M, 10⁻⁷ M, or 10⁻⁸ M), BIBP3226 (at 10⁻⁵ M, 10⁻⁶ M, or 10⁻⁷ M) or the vehicle only was added to the SK-N-MC cells and NPY (at 10⁻⁸ M, 10⁻⁹ M, or 10⁻¹⁰ M), NPY3-36 (at 10⁻⁷ M, 10⁻⁸ M, or 10⁻⁹ M) or the vehicle only was added to the SH-SY5Y cells immediately before OGD. Cultured primary astrocytes in monolayer were subjected to OGD for 4h. NPY (at 10⁻⁸ M, 10⁻⁹ M, or 10⁻¹⁰ M), [Leu,³¹ Pro³⁴]-NPY (at 10⁻⁶ M, 10⁻⁷ M, or 10⁻⁸ M), BIBP3226 (at 10⁻⁵ M, 10⁻⁶ M, or 10⁻⁷ M), NPY3-36 (at 10⁻⁷ M, 10⁻⁸ M, or 10⁻⁹ M) or the vehicle only was added to the astrocytes immediately before OGD. At 24 h after the OGD, cell viability was assessed using tetrazolium salt to evaluate the mitochondrial functions, and results of all OGD groups were expressed as percentages of that from the sham OGD group. Student's *t* test was used to compare the cell viability between the vehicle-treated group and the other groups.

Results: In SK-N-MC cells, NPY at 10⁻⁸ M, 10⁻⁹ M and 10⁻¹⁰ M or [Leu,³¹ Pro³⁴]-NPY at 10⁻⁶ M worsened the cell viability after OGD for 1h; BIBP3226 at 10⁻⁶ M and 10⁻⁷ M improved the cell viability. In SH-SY5Y cells, NPY or NPY3-36 did not affect the cell viability after OGD for 1h. In primary astrocyte culture, different treatments did not significantly affect the cell viability after OGD for 4h.

Conclusion: Inhibition of NPY Y1 receptors using BIBP3226 can directly protect neuronal cells against ischemic injury.