P-11 In Vitro Neural Cell Differentiation of Genetically Modified Human Embryonic Stem Cells Expressing Tyrosine Hydroxylase

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Background & Objectives: This study was to examine in vitro neural cell differentiation pattern of the genetically modified human embryonic stem cells expressing tyrosine hydroxylase (TH).

Method: Human embryonic stem (hES, MB03) cell was transfected with cDNAs cording for TH. Successful transfection was confirmed by western immunoblotting. Newly transfected cell line (TH#2/MB03) was induced to differentiate by two neurogenic factors retinoic acid (RA) and b-FGF. Exp. I) Upon differentiation using RA, embryoid bodies (EB, for 4 days) derived from TH#2/MB03 cells were exposed to RA (10^{-6} M)/AA (5×10^{-2} mM) for 4 days, and were allowed to differentiate in N2 medium for 7, 14 or 21 days. Exp. II) When b-FGF was used, neuronal precursor cells were expanded at the presence of b-FGF (10 ng/ml) for 6 days followed by a final differentiation in N2 medium for 7, 14 or 21 days. Neuron differentiation was examined by indirect immunocytochemistry using neuron markers (NF160 & NF200).

Results: After 7 days in N2 medium, approximately 80% and 20% of the RA or b-FGF induced Th#2/MB03 cells were immunoreactive to anti-NF160 and anti-NF200 antibodies, respectively. As differentiation continued, NF200 in RA treated cells significantly increased to 73.0% on 14 days compared to that in b-FGF treated cells (53.0%, $p<0.05$), while the proportion of cells expressing NF160 was similarly decreased between two groups. However, throughout the differentiation, expression of TH was maintained (90%). HPLC analyses indicated the increased levels of L-DOPA in RA treated genetically modified hES cells with longer differentiation time.

Conclusions: These results suggested that a genetically modified hES cells (TH#2/MB03) could be efficiently differentiated in vitro into mature neurons by RA induction method.

P-12 전뇌 허혈성 뇌졸중 (Global Ischemia) 동물 모델 Mongolian Gerbil에 대한 인간 배아줄기세포 이식효과

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Background & Objectives: 전뇌 허혈성 뇌졸중 동물모델에서 중풍 치료제로서 인간배아줄기 세포의 인지 및 기억력 장애에 대한 기능 회복의 효능 및 신경세포 보호효과를 검토하고자 실시하였다.
Method: 전뇌 허혈성 동물 모델은 웅성 모래쥐 (Mongolian gerbil, 70~80 g) 경부를 절개하여 흉골허근과 흉골저작근 사이의 양쪽 총경동맥을 5분 동안 폐색시켜 만들었다. 본 연구에 사용된 인간배아줄

기 세포(미국 국립보건원에 등록된 MB03세포)는 냉동 보관된 배아로 제작하였으며, 신경세포로 분화를 유도하기 위하여 retinoic acid를 이용한 4-/4+ 방법을 사용하였다. 전뇌 허혈을 유도한 후 2주 째에 3×10^4개와 1×10^5개의 세포를 각각 중풍 동물모델의 내측중격 위치에 이식하였다. 인간배아줄기 세포의 신경세포 보호효과를 규명하기 위하여 면역조직화학 염색법을 이용하여 해마내의 세포사멸을 측정하였으며, 인지 및 기억증진의 작용을 규명하기 위하여 수중미로 학습 및 아세틸콜린성 신경세포의 활성도를 측정하였다.

Results: 수중미로 학습의 획득시행에서 4일째에 3×10^4개군 (22.96초)과 1×10^5개군 (16.42초)군이 control군 (47.54초)에 비하여 현저한 학습능력의 증진효과를 보였다. 또한 파지시행에서 1×10^5개군이 기억력증진 효과를 보였다. 면역조직화학 염색법을 이용한 조직검사결과 기억을 담당하는 신경세포의 생성이 증가되었다.

Conclusions: 본 연구결과는 인간배아줄기 세포가 기억력과 관련된 아세틸콜린성 세포로 분화됨을 증명하며, 인간배아줄기 세포가 중풍에 의한 뇌 손상을 회복시키는 치료에 이용될 수 있음을 의미한다.

P-13 Preventive Effect of Korean Red Ginseng Total Saponin on Rat Infertility Induced by Polycystic Ovaries

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Background & Objectives: Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age. There is some evidence that nerve growth factor (NGF) is involved in the pathogenesis of PCOS. The activation of NGF may be a factor involved in enhancing norepinepherine outflow to the gland in the EV-induced polycystic ovary. In this study, we investigated the effect of Korean red ginseng total saponin (GTS) on the ovarian morphology and NGF expressions in the ovaries, adrenal glands, pituitary and hippocampus.

Method: PCO was induced by a single injection of EV (4 mg i.m.). During the experimental period of 60 days, GTS-treated group was administered with GTS every other day, and this group was compared with a vehicle treated control group and an estradiol-injected group not subjected to GTS. At day 60, the expressions of NGF in the ovaries, adrenal glands, pituitary and hippocampus were examined by immunohistochemistry.

Results: The main findings of the present study were (1) PCO were fully developed in rats with a single i.m. injection of EV, (2) PCO showed the increased expression of NGF in the ovaries, adrenal glands, pituitary and hippocampus, and (3) GTS administration decreased NGF expressions in the ovaries and adrenal glands without affecting pituitary and hippocampus significantly.