acyclovir-resistant and wild-type strains of herpes simplex virus type 1

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The antiherpes agents acyclovir (ACV), ganciclovir (GCV) and penciclovir (PCV) are potent inhibitors of herpes simplex viruses in vivo and in vitro and virus-selective nucleoside analogs. Their agents are converted to monophosphate by viral thymidine kinase (TK). Cellular enzymes convert the nucleoside analog monophosphate to its triphosphate, which is a potent inhibitor of the HSV-encoded DNA polymerase. To investigate metabolism of ACV, GCV and PCV in cell culture system, we infected Vero, cellular TK-deficient 143B and viral TK expressing FTK/143B cells with herpes simplex virus type 1 (HSV-1) strain F and AR1 - AR2, the laboratory derived TK-deficient ACV-resistant mutants of HSV-1 strain F. The level of phosphorylation of nucleoside analogs was dependent upon virus type and cell line. In HPLC assay, the level of phosphorylation of nucleoside analog in AR1 infected cells was lower than HSV-1 strain F infected cells and similar to uninfected cells. However, AR2 infected cells was lower than HSV-1 strain F and higher than AR1 infected cells. The order of phosphorylation level of ACV and PCV was triphosphate ≥ diphosphate > monophosphate., and GCV was monophosphate ≥ diphosphate > triphosphate. It was also observed that phosphorylation of nucleoside analogs in virus infected cells varied within the different cell type. The order of phosphorylation levels of GCV in these cell types was 143B ≥ FTK/143B > Vero, and PCV was FTK/143B ≥ 143B > Vero.

[PC1-2] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Effects of antioxidants and an anticancer drug against H2O2-induced cell injury

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The induction of apoptosis by the oxidative stress was investigated in SK-MEL-2 cells with hydrogen peroxide(H_2O_2). And the effects of antioxidants and an anticancer drug were observed against H2O2-induced apoptosis.

Various concentrations of H_2O_2 were treated to SK-MEL-2 cells for different incubation times and the cytotoxicity was evaluated by MTT assay. In addition, to determine the defense effect of antioxidants or an anticancer drug on apoptosis, the cells were each or pretreated with them, and then exposed to H_2O_2.

Thus, antioxidants such as vitamin E inhibited the apoptotic cell death by oxidative stress, but co-treatment of H_2O_2 and cisplatin seemed to be additive effect by production of free radicals.

[PC1-3] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Development of Stable Assay System for Monitoring NF-Kappa B Expression in Human HaCaT Cells

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A stable assay system for monitoring NF-kappa B activation was developed to determine the influence of activated NF-kappa B in human HaCaT cells. The pNF-kappa B-SEAP-NPT vector that permits for expression of the secreted alkaline phosphatase (SEAP) gene and contains the neomycin phosphotransferase (NPT) gene for the antibiotic G-418 resistance was constructed. Human HaCaT

cells transfected with pNF-kappa B-SEAP-NPT vector secreted the SEAPs into the culture medium as a time-dependent manner until 48h. The SEAPs were measured using fluorescent assay method. The treatment of transfected cells with antioxidants *N*-acetyl-*L*-cysteine (10 mM) and Vitamine C (10 mM) inhibit NF-kappa B activation up to 50% and 25% compared to a control, respectively. This system can be used for determining the effect of various chemicals and natural products to NF-kappa B activation in human HaCaT cells.

[PC1-4] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Development of in vitro assay system for the screening of type specific 5α-reductase inhibitors

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In many androgen-responsive organs, such as prostate and skin, testosterone is converted into 5α-dihydrotestosterone (5α-DHT) by 5α-reductase. 5α-DHT then binds to androgen receptors and functions in the nucleus to regulate specific gene expression. Human 5α-reductase has at least two isoforms, designated types I and II. The type I 5α-reductase expression predominates in skin, prostatic epithelia, and type II isoform predominates in human accessory sex tissues. Since 5α-DHT promotes the development of acne, male pattern alopecia and benign prostatic hyperplasia, inhibitors of 5α-reductase may be useful for treatment of these conditions. For the screening of 5α-reductase inhibitors, human prostate cancer cell lines (LNCaP, DU145 etc) were used. But type-specific inhibitors were effective for the exclusion of side effects. We constructed cell lines that express the type specific 5α-reductase. For type specific cell lines construction, DU145 and LNCaP were used respectively, type I and II. From each cell line, RNA were extracted and synthesized cDNA containing 5α-reductase open reading frame (ORF) by using RT-PCR method, and then cloned into mammalian expression vector, pTarge T vector. 293 cell line, which don't express 5α-reductase, were transfected by the electrophoration method. These cell lines were tested by using of a 5α-reductase inhibitor, finasteride, is being evaluated as the chemoprevention agent of prostate cancer in a clinical trial and have been used successfully for treatment of benign prostatic hyperplasia. In these system, each cell line expressed type specific 5α-reductase and was inhibited by finasteride effectively. These results suggest that these system are effective for the screening of type specific 5α-reductase inhibitor for treatment of benign prostatic hyperplasia and alopecia.

[PC1-5] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

The anticoagulant activity of chondroitin sulfate proteoglycan derived from human placenta

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Chondroitin sulfates proteoglycans were isolated from human placenta. For the identification of enzymatic digestion products of isolated proteoglycan, strong anion exchange-high performance liquid chromatography (SAX-HPLC) was performed. By the action of chondroitin ABC and chondroitin B lyase, three unsaturated disaccharides 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-D-galactose (ΔDi-OS), 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose (ΔDi-6S) and 2-acetamide-2-deoxy-3-O-(β-D-gluco-4-enepyranosyluronic acid)-4-O-sulfo-D-galactose (ΔDi-4S) were produced from the human placenta proteoglycan. The anticoagulant activity of chondroitin sulfate proteoglycan was evaluated by activated partial thromboplastin time (aPTT) assay and thrombin time (TT) assay. The clotting times of aPTT and TT were increased from