ABSTRACTS

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ORAL PRESENTATIONS

The Role of Protein Kinase C in the Synergistic Interaction of Safingol and Irinotecan in Colon Cancer Cells

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Colon cancer represents one of the most common solid tumors in adults. Although 5-fluorouracil (5-FU) and irinotecan have been frequently administered in colon cancer patients, low response rates to these single drug therapies were reported. It is therefore imperative to search for new targeted combination therapies that are effective. In this study, we have investigated the anti-cancer effect of safingol as a single agent or in combination with irinotecan using HT-29 and LS-174T colon cancer cells as our in vitro models. The anti-cancer effects of safingol as single agent or in combination with irinotecan in HT-29 and LS-174T colon cancer cells were determined using MTT assay. The combination index (CI), based on the median effect principle by Chou and Talalay, and was computed to determine drug synergism. The expression levels of phosphorylated PKC and its downstream substrate, MARCKS, were determined using Western blot. Cell adhesion was examined using a Matrigel-based *in vitro* assay. As a single agent, safingol was more potent than irinotecan and 5-FU, with IC50 values of 2.5±1.1 μM and 3.4±1.0 μM achieved in HT-29 and LS-174T cells, respectively. The combination of safingol/irinotecan at 1:1 molar ratio was found to be additive in HT-29 cells (CI= 0.94) and synergistic in LS-174T cells (CI = 0.68), and resulted in concentration- and time-dependent down-regulation of p-PKC and p-MARCKS. The drug effect of the safingol/irinotecan combination could be further modulated in the presence of a PKC stimulator (phorbol-12-myristate-13-acetate) or a PKC inhibitor (staurosporine). Furthermore, the 1:1 safingol/irinotecan combination inhibited the adhesion of colon cancer cells to the extracellular matrix 4 h post-treatment. Modulation of the PKC pathway could be a possible molecular basis for the observed synergism of the safingol/irinotecan combination. However, PKC was not inhibited with concentrations of safingol which could induce substantial cell kill. Our results show that inhibition of PKC by safingol/irinotecan combination could be a potentially effective strategy for colon cancer treatment.

Fractionated Aqueous Extract of Leaves of DBT/DM/06: Characterization, *In Vivo* Evaluation and Formulation Development

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The objective of present study was to characterize the fractionated aqueous extract of DBT/DM/06 by chromatographic technique and evaluate its *in vivo* antihyperglycemic activity in diabetic rats. Another objective was to study the feasibility of development of a suitable dosage form for the extract and evaluate its stability. TLC method was developed for the identification of marker compounds in the extract. HPTLC fingerprint of the extract was taken and the marker compounds were identified. HPTLC was used as a suitable analytical method for quantification of the marker compounds. Streptozotocin-induced diabetic rat model was used to evaluate the antihyperglycemic activity of the extract. The fractionated aqueous extract was formulated as tablets using appropriate excipients. The tablets were evaluated for various quality control parameters and the keeping properties were evaluated by storing them at accelerated condition for a period of three months. Rutin, isoquercitrin and inositol were identified as marker compounds in the extract. Rutin was selected as an analytical marker for further studies with the extract. HPTLC analytical method was developed for quantification of rutin and was found to be linear in the concentration range of 200-1600 ng. In streptozotocin-induced diabetic rat model, the extract showed a statistically significant decrease in the fasting blood sugar at all three dose levels (10, 50 and 100 mg/kg) when compared to disease control after fifteen days of treatment. The maximum fall in blood glucose levels was found with 50 mg/kg dose of the extract. Tablets of the fractionated aqueous extract possessing good stability were prepared successfully.

POSTER PRESENTATIONS

Templating of Polymeric and Lipid Nanocarriers Using Biocompatible Solvents

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The objective of the present investigation was to evaluate utility of various pharmaceutically acceptable biocompatible solvents in the fabrication various polymeric and lipid nanoparticles. The biocompatible solvents evaluated in the investigation were N-methyl pyrrolidone (Pharmasolv®; NMP), 2-pyrrolidone (Soluphor® P; SP), tetrahydrofurfuryl alcohol PEG ether (Glycofurol; GF) and diethyleneglycol monoethylether (Transcutol® P; TCP). The ability of these solvents to solubilize various biodegradable and nonbiodegradable polymers was evaluated at 25º using a modified method. The ability of these solvents to solubilize various phospholipids (Phospholipon 90G and 90H) and solid lipids was also evaluated at 75º. Based on the solubility studies data, the feasibility of fabrication of polymeric nanoparticles, solid lipid nanoparticles and phospholipid nanoparticles was evaluated by using nanoprecipitation method. Various surfactants were evaluated for identifying the best surfactant that results in the smallest nanoparticle size with optimum polydispersity index. The effect of type of the biocompatible solvent on the particle size of the nanocarriers was also evaluated. The colloidal stability of the fabricated nanocarriers was evaluated. The biocompatible solvents employed in the investigation showed varying ability to solubilize various polymers, phospholipids and solid lipids. All the biocompatible solvents showed ability to solubilize various polymethacrylates or Eudragits at the concentration as high as 100 mg/ml whereas biodegradable polymers such as PLGA were soluble in only SP and NMP at the concentration of 50 mg/ml. All the solvents showed ability to solubilize soy lecithin at the concentration as high as 600 mg/ml and solid lipids (Compritol, Precirol and glycerylmonostearate) at the concentration of 500-1000 mg/ml. Feasibility studies indicated that polymeric nanoparticles could only be formed with Eudragit RSPO and RLPO whereas other polymers yielded either microparticles or polymeric strands. It was possible to fabricate phospholipid nanoparticles using all these solvents. Among the solid lipids evaluated, only glycerylmonostearate (GMS) could form solid lipid nanoparticles. The polymeric and lipid nanocarriers had particle size ranging from 90-220 nm and exhibited good colloidal stability. The biocompatible solvents explored in the investigation had ability to solubilize polymers, phospholipids and solid lipids due to their amphiphilic nature. The formation of nanoparticles was dependent on the chemical structure of the polymer and lipid and also their concentration and the type of the surfactant used. The difference in the nanoparticle size observed with the different biocompatible solvents showed better correlation with solubility parameter of the biocompatible solvents. The feasibility of single step fabrication of polymeric and lipid nanocarriers using pharmaceutically acceptable biocompatible solvents was established for the first time.

Radiotracer Uptake Studies of Technetium Labeled Thymoquinone Using Gamma Scintigraphy Imaging Technique

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To investigate the tissue uptake of technetium (^{99m}Tc) labeled thymoquinone (TQ), in mice by scintigraphic imaging. TQ was radiolabeled by direct labeling method using stannous chloride as reducing agent. The conditions required for maximum labeling efficiency such as pH, incubation time, concentration of stannous chloride were optimized for maximum radiolabeling efficiency, ascertained by ascending thin layer chromatography. The stability of the radiolabeled complex in saline and serum at 37º was assessed for a period of 24 h. For *in vivo* studies ^{99m}Tc-TQ complex was administered intravenously at 10 mg/kg body weight as a single dose in normal swiss albino mice. The mice were placed in a ventral (sternal) recumbent position with head extended forward under the Millenium MPS single head Gamma camera (GE) fitted with LEGP pin hole collimator and images were acquired by using GENIE acquisition station. Dynamic images were acquired for 30 min while the static images were acquired for 1 min at hourly intervals, for 24 h post injection. The images acquired were transferred

to eNTEGRA workstation for further processing. The scintigrams obtained were analyzed by drawing region of interests (ROIs) on the dynamic and static images. Time activity curves (TAC) of 30 min were obtained from the dynamic images for the organs of interest. In addition, the animals were sacrificed by cervical dislocation at 0.5, 2, 6, 8 and 15 h and radioactivity counts of organs were counted using a dose calibrator (Capintec 127R) and expressed as percent injected dose per gram/ tissue after decay correction. The radiolabelling efficiency of 95% could be achieved by direct radiolabeling with 250 µg/ml of stannous chloride at pH 7 and gently mixed and incubated for 30 min. The *in vitro* stability in serum and physiologic saline showed that radiolabeled complex was stable for 24 h. Based on the percent injected dose (% ID) the highest uptake of the ^{99m}Tc-TQ was found in the RES even after 24 hour post injection. The % ID obtained from the static images from the RES (lung, liver and spleen) were 84.866, 72.670, 64.981, 62.783, 57.278% after 0.5, 2, 6, 8 and 15 h respectively which correlated well with the organ distribution obtained after the sacrifice of animals at the same time points. Scintikinetic study of Thymoquinone demonstrate the maximum % uptake of the radiolabeled TQ is in the RES organs like liver, lungs and spleen.

Stability Indicating Assay Method for Simultaneous Estimation of Dutasteride and Tamsulosin Hydrochloride

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Dutasteride (DTS) is a competitive and specific inhibitor of both Type 1 and Type 2, 5-alpha reductase (5-AR) isoenzymes, an intracellular enzyme that converts testosterone to dihydrotestosterone (DHT). Tamsulosin hydrochloride (TMS) is a sub type selective α1A and α1D adrenoreceptor antagonist that causes a decrease in smooth muscle tone in the prostate and prostatic urethra and thus the increase of a urinary flow. The combination of both these drugs is used for the treatment of patients with symptomatic benign prostatic hyperplasia. Literature survey revealed no reported method for the simultaneous estimation of these drugs. Thus, the objective of this project was to develop a validated stability indicating assay method (SIAM) for DTS and TMS in accordance to ICH guidelines by HPLC analysis. The study protocol included stress degradation of DTS and TMS, isolation of degradants and its characterization by spectroscopic methods, and finally the development and validation of SIAM for simultaneous estimation of these drugs. Stress degradation studies were performed on bulk drugs using 1N HCl, 1N NaOH, oxidation using 3% and 20% H_2O_2, photo degradation as per ICH with sunlight exposure for 45 days (to solution form) and accelerated stability at 40º and 75% RH (for 3 mo). All the degradants were separated by preparative HPTLC and the chemical structure determination of these were done by IR, Mass and NMR spectroscopy. A Jasco HPLC system was used. A Hi Q Sil C18W (250×4.6 mm, 5 µm id) column was used with a mobile phase consisting of methanol:ammonium acetate buffer pumped in linear gradient mode at a flow rate of 1.2 ml/min. The eluents were monitored at 225 nm. The output signal was monitored and processed using BORWIN software. IR was carried out on Jasco FTIR, Mass spectroscopy and NMR spectroscopy was done on Varian 500 MS and quanta-mercury h1 frequency instrument. Degradation of DTS was observed only under acidic and alkaline hydrolytic condition. Degradation of TMS was observed under hydrolytic conditions and photolytic degradation. The retention times for standard TMS and standard DTS in combination was 4.585 min and 27.438 min, respectively when analyzed by HPLC using UV detector. The peak purity determination was performed by HPLC analysis using a PDA detector. The retention time and percent purity for DTS and TMS was 27.871 min and 99.6% and 5.395 min and 99.98%, respectively in presence of their degradants. The accuracy of the method was estimated by analyzing marketed tablets. The ICH validation parameters and results for DTS were as follows: Assay 98.3%, Percent recovery 99.39%, LOD and LOQ 0.02 µg/ml and 0.2 µg/ml, respectively. Repeatability with % RSD 1.248., linearity range 0.25-200 µg/ml and linearity equation was y= 18250x+6466.9 with coefficient of correlation of 0.9995. The results of validation parameters for TMS were as follows: Assay 99.48%, Percent recovery 99.44%, LOD and LOQ 0.01 µg/ml and 0.1 µg/ml respectively, Repeatability with % RSD 0.326, Linearity range 0.1-75 µg/ml and linearity equation was y = 32489x-1319.1 with coefficient of correlation of 0.9992. A systems suitability test was applied to representative chromatograms to check tailing, column efficiency, peak area and resolution. The proposed HPLC method was found to be accurate, precise, and specific. The stress degradation studies have also helped to identify the degradation pathways of DTS and TMS.