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108. CYCLOSPORIN ANALYSIS IN BLOOD BY AUTOMATED RESERVED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD COUPLED WITH SOLID PHASE EXTRACTION AND SPEED VACUUM EVAPORATION

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Cyclosporin A (CyA) is a cyclic undecapeptide drug used in combating tissue rejection after organ transplant. High cyclosporin doses may lead to nephrotoxicity while a dose below the therapeutic level increases the probability of transplant rejection. A rapid HPLC analysis was developed for the estimation of cyclosporin in blood using a PC 1000 software and autosampler for routine analysis. The mobile phase consisted of acetonitrile: methanol: water (50:30:20) while the analytical column was a C_{18} column maintained at 75°C with UV detection set at 214 nm. Whole blood samples, spiked with the internal standard cyclosporin D (CyD), was added with protein precipitating agent, centrifuged, and applied to a disposable solid phase C_{18} column to rapidly extract the CyA and CyD. The extracting solvent was removed by using a speed vacuum apparatus. Average retention times were 8.1 min for CyA and 10.0 min for CyD. Linear calibration curves were obtained from 0-500 mg/mL with average correlation coefficient of 0.995. Calibration standards with increasing concentrations of CyA and fixed concentration of CyD were spiked in blood from healthy volunteers and subjected to the same preparation as CyA-containing blood samples. CyA concentrations in blood samples were determined using internal standard addition mehod (by area ratio of CyA to CyD) and the obtained calibration curve. This analytical technique is useful in monitoring cyclosporin level in transplant patients.

Key words: cyclosporin, high performance liquid chromatography, reversed-phased, solid phase extraction, speed vacuum, immunosuppressive drug, whole blood, chromatography, internal standard addition method

109. GROWTH PATTERNS AND INFECTIVITY OF A DENGUE-2 VIRUS STRAIN PROPAGATED IN THE HUMAN MYELOMONOCYTIC CELL LINE K562

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Dengue virus is the causative agent of the disease dengue, which is manifested in different degrees of severity. There are 4 serotypes of the virus namely, Dengue 1, 2, 3 and 4. The genomic nucleotide sequences of representative strains of all 4 serotypes have been determined. Recently, Mangada and Igarashi (1998) reported the sequencing of the entire genome of three Dengue 2 virus strains from Thailand. These are ThNH-p11/93, ThNH-28/93 and ThNH-7/9, which were isolated form Thai patients exhibiting dengue fever, dengue haemorrhagic fever, and dengue shock syndrome, respectively. Differences in the secondary structure in the 3 non-coding region, as well as significant amino acid replacements, which could potentially alter the nature of the viral proteins, have been noted. In the present study, the growth patterns and infectivities of these three virus strains were compared. The viruses were initially propagated in the mosquito cell line, C6/36 *Aedes albopictus*, maintained in Eagle's minimal essential medium containing 2% fetal calf serum (FCS) and incubated at 28oC. After 1 week, the infected culture fluids (ICF) were collected and pre-incubated with or without enhancing antibodies. These 2 types of ICFs were then inoculated at the same multiplicity of infection into K562 human myelomonocytic cells. After two hours of viral adsorption, cells were cultured in 24-well plates at a concentration of 2×10^5 cells/ml per well in 2% FCS-supplemented RPMI at 37°C in a CO_2 incubator. Cells were harvested everyday for 7 days. Virus growth was quantified by focus formation unit assay in BHK Percent of infected K562 cells was detected through immunoflourescence test and correlated with severity of disease.

Key words: dengue virus, dengue fever, haemorrhagic fever, dengue shock syndrome . Ades albopictus, enhancing antibodies, myelomonocytic cell, K562 cells, immunofluorescence assay test, dengue-2 viruses

110. THE ANALGESIC ACTIVITY OF THE ALKALOIDS OF *(Ipomea Muricata)* Jacq. FAM. CONVOLVLACEAE: A CORRELATION OF IN VIVO AND IN VITRO STUDIES

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The analgesic activity of the indolizidine alkaloids from the seeds of Ipo*moea muricata*, namely ipomine, ipalbidine, ipalbine, and ipalbinium were studied on mice using the hot-plate method. At a dose of 1 mg/kg BW, all alkaloids except ipalbine, elicited higher threshold to pain in mice than the 5 mg/kg-BW dose of indomethacin. The dose-activity relationship indicate that for all the alkaloids, 5 mg/kg/BW was observed as the dose that produced a time-course curve ideal of all analgesic agents. Based on the degree and nature of analgesic action, the alkaloids are arranged as follows: ipalbidine > ipalbinium > ipomine > ipalbine.

The alkaloids were assayed for their ability to inhibit prostaglandin synthesis in isolated rat leukocytes. Only ipalbadine showed significance inhibition of prostaglandin release at a concentration of 100μM. The results implicate the phenylindolizidine ring for the observed analgesic activity in vivo.

Key words: *Ipomoea muricata*, alkaloids, analgesic, ipomine, ipalbidine, ipalbinium, ipalbine

111. CERVICAL ADENOCARCINOMA IN FILIPINOS

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Paraffin blocks of cervical adenocarcinoma tissues taken from 23 patients were processed by light microscopy and morphometry. Three different grades or cervical adenocarcinoma, namely well-glandular lining, cell and nuclear pleomorphisim, stromal invasions, and lymphatic involvement were observed. Mor-

phometry of transformed cells revealed that small cells predominate in the poorly differentiated stage. No notable differences in nuclear sizes were observed among the three histological anaplasia, that is, the nucear-cytoplasmic ratio increases as the cell becomes more anaplastic.

Key words: cervix, adenocarcinoma, histology, morphometry

112. CYTOGENETIC ABNORMALITIES IN FILIPINO COLON AND COLORECTAL CARCINOMA PATIENTS

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Cancer of the colon and rectum is a common and often fatal disease. It is one of the three leading causes of cancer mortality worldwide. There is a dearth of cytogenetic data on solid tumors such as colon and rectal cancer, primarily because of the inherent technical problems associated with these studies. Identification of tumor-specific chromosomal abnormalities, important determining clinical remission or relapse, was conducted. Tumor tissues, all described as adenocarcinomas by histopathological examination, were surgically removed from one colorectal and 8 colon patients (7 males and 2 females). Primary cultures were prepared and chromosomes were stained using the Trypsin G-banding method. Structural aberrations included 3p and 5p deletions. Numerical aberrations such as hypodiploids, polyploids, absence of the Y chromosome, and presence of marker chromosomes were also observed. Cytogenetic findings were correlated with histopathological and flow cytometry data as well as cancer stage using the TNM and Duke's systems. Genetic abnormalities confirmed by fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH).

Key words: adenocarcinoma, cancer stage, colon cancer, colerectal cancer, cytogenetics, flow cytometry, histopathology, hypodiploid, malignancy, polyploid, trypsin G-banding

113. THE INDOLE ALKALOIDS FROM THE LEAVES OF *Alstonia scholaris* (L.) Don (APOCYNACEAE) – COMPARATIVE ANTIMYCOBACTERIAL ACTIVITY AND ANTICARCINOGENICITY AGAINST HUMAN ORAL EPIDERMOID CARCINOMA CELL-LINES

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The indole alkaloids comprise the second largest single group of plant bases reputed for their pharmacological and therapeutic properties. In this study, the indole alkaloids from the air-dried leaves of *Alstonia scholaris* (L.) Brown and *Catharanthus roseus* (L.) G. Don (both from family Apocynaceae), were studied for their potential antitubercular activity and comparative anticarcinogenicity against human-oral epidermoid carcinoma cell lines (KB cells). Radiorespirometric assay of the crude alcohol leaf extracts and the crude alkaloids revealed a pronounced inhibition of *Mycobacterium tuberculosis* H37Rv and *Mycobacterium avium*. However, only the *A. scholaris* crude alcohol extract and the crude alkaloids were active against both mycobacterial species. Sulforhodamine-B colorimetric assay of the crude, *C. roseus* alcohol extracts and alkaloids showed broad spectrum cytotoxicity against the KB cancer cells at LC_{50} (0.3 and 0.2 mcg/mL). *Crude A. scholaris* alcohol extracts exhibited a very slight cytotoxicity (18.8 mcg/mL) whereas its crude alkaloids did not demonstrate a significant cytotoxicity at LC_{50} (>20).

Vacuum liquid chromatography of the crude *A. scholaris* and *C. roseus* alkaloid fractions resulted in several alkaloid-positive fractions. Radiorespirometric assay of the alkaloid fractions (first two for *A. scholaris*) indicated a higher inhibition for fraction As and Cr B. Sequential gravity liquid column chromatography of the two bioactive fractions afforded a white amorphous solid (As-ISO) and a yellow solid (Cr-ISO). One-dimensional nuclear magnetic resonance analysis (^1H and ^{13}C) of the isolates revealed the structure of As-ISO as akuammidine, a sampagine type (1) and Cr-ISO as vindoline, a plumeran type (2).

Key words: *Alstonia scholaris*, *Catharanthus roseus*, indole alkaloid, antimycobacterial, anticarcinogenicity, Mycobacterium tuberculosis H37Rv, *Mycobacterium avium*, human oral epidermoid carcinoma lines, sampagine plumen

114. MOLECULAR DETECTION OF ENTEROVIRUSES ASSOCIATED WITH DILATED CARDIOMYOPATHY

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Viral infections are the most common cause of inflammatory heart muscle disease aside from other known factors. Enteroviruses, particularly cocksakieviruses B (CVBs), have been implicated in the pathogenesis of dilated cardiomyopathy (DCM). However, viral cultures of myocardial tissue are almost always negative, even when the clinical history or serological studies indicate viral infections. In this study, a right ventricular biopsy (5 mg wet weight) was performed in patients with clinically suspected DCM. The myocardial biopsies were used in the detection of enteroviral RNA by the Polymerase Chain Reaction (PCR). PCR is ideally suited for this study since it can detect low copy numbers of viral genome in small tissue samples. Two sets of primers (A/B and C/D) are from two different consensus sites in the enteroviral genome which allowed the detection of either CVB or poliovirus (PV). Primers A/B are most homologous to CVB3, whereas primers C/D are 100% homologous with six virus types (CVB1, CVB3, CVB4, PV1, PV2, and PV3). Of the 28 patients enrolled in the study, 3 were positive for the first set of primers, while 11 were positive for the second set of primers. Two patients were found positive for both set of primers. Samples are also processed for electron microscopy but so far viral inclusion bodies or particles have not been detected. The results of this study indicate a link between viral infection and dilated cardiomyopathy in some patients.

Key words: enterovirus, cocksakie virus, poliovirus, endomyocardial, biopsies, dilated cardiomyopathy, RNA, cDNA synthesis, PCR, electron microscopy, inclusion bodies

115. DETECTION OF *Helicobacter pylori* FROM FORMALIN-FIXED, PARAFIN-EMBEDDED GASTRIC BIOPSY SPECIMENS; A STRATEGY FOR *vacA* GENOTYPING

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Helicobacter pylori is an important human pathogen, having been identified as the major causative agent of chronic gastritis. Although it is well-studied in the US, Europe, Latin America, Africa, and some Asian countries, i.e., Japan, Korea, and Thailand, there are no available data on its epidemiology in the Philippines. Due to the difficulty in culturing this bacterium, we established a method to detect *H. pylori* from formalin-fixed, paraffin-embedded gastric biopsy specimens. Forty-two samples from the Institute of Pathology of St. Luke's Medical Center were evaluated using polymerase chain reaction (PCR). The amplification targets are the genes for urease A (*ure A*), urease B (*ure B*) and urease C (*glmM*). Once a sample has been confirmed to be positive for *H. pylori*, the presence of the vacA gene was also evaluated. This gene encodes the vacuolating cytotoxin which induces the formation of intracellular vacuoles in epithelial cells. The signal region of the vacA gene occurs as either s1 or s2 allele, while the middle region is present as m1 or m2 allele. Different combinations of these alleles give rise to a specific vacA genotype and correlate with the severity of the disease. In this study, the PCR-based system developed by Atherton (*1999 J Clin. Microb. Vol. 37:9*) was used to determine the vacA genotype of 42 gastric samples obtained from paraffin blocks.

Key words: *Helicobater pylori*, PCR, *ureA*, *UreB*, *glmM*, vacuolating cytotoxin, *vacA*, signal region, middle region, gastric biopsy.

116. PRIMERS FOR CYSTEINE PROTEINASE GENE COULD DISTINGUISH PATHOGENIC *Entamoeba histolytica* FROM NON-PATHOGENIC *E. dispar*

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Cysteine proteinases and pore-forming peptides (amoebapores) have been found to be responsible for tissue lysis and cytopathic effects described in invasive amebiasis. A total of six distinct *Entamoeba histolytica* cysteine proteins genes (ehcp 1-ehcp6) have been identified and sequenced. Three of these genes, ehcp 1, 2, and 5 are expressed at high levels in the pathogenic *E. histolytica*. In this study, an Oligo 4.0 software was used to design primer pairs WRG 1&2 and WRG 3&4 from consensus sequences of this gene. Amoeba were isolated from stool samples, cultured, and maintained in Robinson's medium. Genomic DNA was extracted using phenol-chloroform method. Polymerase Chain Reaction (PCR) using both primer sets could distinguish pathogenic *E. histolytica* from the non-pathogenic *E. dispar*. A 570-bp PCR amplicon was observed in 6 out of the 8 samples using WRG 1&2, while a 755-bp product was obtained in 4 samples using WRG 3&4. These results were further compared with the PCR products generated by primers designed by Tachibana (1991) which also distinguish pathogenic from non-pathogenic *Entamoeba* species. Amplified products from the cysteine proteinase gene primers are currently being cloned for sequence analysis.

Key words: *Entamoeba histolytica, E. dispar,* PCR, cysteine proteinases, *E. histolytica* gene (ehcp1-ehcp 6), amoebapores, Robinson's medium, genomic DNA extraction, cloning, gene sequencing.

117. POLYMERASE CHAIN REACTION-SEQUENCE SPECIFIC PRIMER (PCR-SSP) SYSTEM FOR BONE MARROW TYPING

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The Human Leukocyte Antigen (HLA) genes are highly polymorphic, co-dominantly expressed recognition genes that play an important role in determining tissue compatibility for transplantation. Because of this, HLA compatibility between donor and recipient must be determined prior to transplantation. Although HLA types have been defined using serology for many years, the resolution capacity of this method is limited. The advent of DNA-based techniques has allowed the identification of DNA sequence variations which cannot be distinguished by serologic typing. Sequence-specific primer (SSP) typing is DNA-based method that allows discrimination between the different alleles amplified by polymerase chain reaction (PCR). In this technique, PCR primers are designed to anneal only to a single or specific set of alleles. These primers contain sequences unique to the allele(s) and both primers must anneal to the DNA to get a positive amplification of the test sequence. The 96-well format for PCR allows simultaneous determination of 24 allelic groups 4 samples belonging to potential donors and the recipient. Amplified DNA fragments are separated by agarose gel electrophoresis and visualized by staining with ethidum bromide and exposure to UV light. Interpretation of the results is based on the presence or absence of amplified DNA fragment corresponding to the specific primer set. Determination of HLA type is by analysis of the pattern of positive wells either using an Analysis Program software or a reaction pattern worksheet.

Key words: HLA, transplantation, tissue compatibility, bone marrow, polymerase chain reaction, sequence-specific primer, serologic typing, donor, alleles, tissue typing