Abstracts

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DEMONSTRATION OF ANTIGENIC AND SPECIFIC OUTER MEMBRANE PROTEIN(S) OF *ACINETOBACTER BAUMANNII*

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Introduction: *Acinetobacter baumannii* has been recognized as an emerging nosocomial pathogen and is often multi-resistant to antibiotics. Current identification of *A. baumannii* is by conventional culture method and biochemical tests, which take about 2 to 7 days to produce results. Hence, there is a need for a new rapid, sensitive, and specific test that would allow better management of nosocomial infections. The aim of this study was to detect the presence of a specific and antigenic biomarker for *A. baumannii* from the outer membrane protein (OMPs) that can be used for the development of a rapid and specific diagnostic test.

Material and Methods: Protein profiles of OMP lysates from the ATCC strain and clinical isolates of *A. baumannii* were obtained by SDS-PAGE and compared. The protein profiles of the clinical isolates were 95% identical to that of the ATCC strain. Following this, the protein electrophoretograms were subjected to Western blot using sera from patients infected with *A. baumannii*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and sera from normal healthy subject using labeled anti-IgM, IgA, and IgG to check for cross reactivity.

Results: There was 1 band of molecular weight 34.4 kDa that was found only in clinical isolates of *A. baumannii* and it does not cross react with other tested sera. The observations suggested that the protein was specific for *A. baumannii* and can be used as a biomarker for development of a diagnostic test.

Conclusion: The results are encouraging in that the 34.4 kDa protein identified is specific for *A. baumannii* and can be used as a biomarker for development of a diagnostic test that would be faster and more specific than the current techniques of diagnosis. However, further studies need to be done to measure the antibody level against this specific protein, the sensitivity and specificity of the protein, and the retention time of the antibody detectable in the serum of the infected patients.

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CLONING, EXPRESSION, AND PURIFICATION OF TOXOCARA CANIS RECOMBINANT ANTIGENS (rTES-32, rTES-120) AND DEVELOPMENT OF SERODIAGNOSTIC TEST FOR TOXOCARIASIS

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Introduction: Routine serodiagnosis of human toxocariasis is based on indirect IgG-ELISA kits which employ native *Toxocara canis* excretory-secretory (TES) antigen. However, these assays lacked specificity especially when used in tropical countries where multi-parasitism are prevalent. In an effort to improve the diagnostic test for infection, we have developed an IgG4-ELISA assay that uses 3 recombinant antigens.

Material and Methods: Recombinant *T. canis* DNA that encodes for rTES-26, rTES-32, and rTES-120 were produced by cloning of open-reading frames (ORF) of the respective genes via reverse-transcriptase-PCR (RT-PCR) using mRNA extracted from a culture of *T. canis* second stage larvae into PCR2.1 TOPO vector. Sequence analysis revealed that TOPO/TES-32 and TOPO/TES-120 were 100% similar to the reported sequences in the GenBank; however, TOPO/TES-26 gene fragment had 4 mutations. After all mutations in TOPO/TES-26 gene fragment had been corrected, TES-26 and TES-32 were subsequently subcloned into a GST-tagged prokaryotic expression vector, while TES-120 was subcloned into a HIS-tagged vector. All constructs were expressed in *E. coli* BL21(DE3) expression host.

The recombinant proteins were subsequently purified under native condition by affinity chromatography using GST and His-Trap resins, since these recombinant proteins are abundantly expressed in soluble form. The site-specific protease, Factor Xa, was used to remove GST tag in the TES-26 and TES-32 fusion proteins. Western blot analysis revealed that these recombinant antigens were immunologically reactive and specific.

Results: Sera from patients infected with toxocariasis had IgG4 antibodies that recognized these recombinant antigens, while sera from individuals with other infections and healthy controls did not. When the 3 recombinant antigens

were tested in ELISAs specific for immunoglobulin IgM and IgE classes, as well as IgG subclasses (IgG1-IgG4), the results clearly showed that only IgG4 assay displayed good specificity. The diagnostic utility of each purified recombinant antigen and rTES-30USM (previously produced in our laboratory) was further evaluated by IgG4-ELISA assay using 242 serum samples, which included 30 sera from patients with clinical, haematological, and serological evidence of toxocariasis. Both rTES-26 and rTES-32 IgG4-ELISAs demonstrated sensitivity of 80.0%, while rTES-120 IgG4-ELISA showed sensitivity of 93.3%, which is similar to that previously reported for rTES-30USM IgG4-ELISA. The sensitivity of rTES-120/rTES-30USM IgG4-ELISA was found to be significantly higher than rTES-26/rTES-32 IgG4-ELISA ($P < 0.001$). However, the mean ODs of the 30 toxocariasis samples among the IgG4 assays using the 4 recombinant antigens were shown not to be significantly different. There was marginally no significant difference between the specificities of rTES-26 and rTES-120, rTES-26 and rTES-30USM, or rTES-30USM and rTES-120.

In the final assay, rTES-32 was excluded since it did not show better sensitivity or specificity than rTES-26. Instead, rTES-30USM was included due to its high sensitivity and the fact that a 100% detection of toxocariasis cases was achieved with the combined use of rTES-30USM and rTES-120 in IgG4-ELISA.

Conclusion: A final assay which is sensitive (80.0%–93.3%) and specific (92.0%–96.2%) for detection of toxocariasis was successfully developed using 3 adjacent wells, each separately coated with rTES-26, rTES-30USM, and rTES-120. This study is novel in several ways: it is the first report on the use of multiple recombinant antigens for serodiagnosis of toxocariasis, the use of rTES-26 (and rTES-32) in *Toxocara* serodiagnosis, the use of IgG4 assay for rTES-120 and rTES-26, and the use of GST tag in the expression and purification of *Toxocara* recombinant proteins. The test may provide a significant improvement over commercially available tests for diagnosis of toxocariasis and may be use especially in countries co-endemic with other soil-transmitted helminthes.

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CYTOCHROME P450 2C8: AN INVESTIGATION OF TYPES AND FREQUENCIES OF CYP2C8 POLYMORPHISM IN MALAYSIA AND IN VITRO ANALYSIS OF CATALYTIC ACTIVITY

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Introduction: CYP2C8 is a polymorphic gene. Polymorphisms and drug interactions may imply susceptibility to adverse effects with drugs of CYP2C8 substrate. The objective of this study is to investigate the types and frequencies of CYP2C8 polymorphism in Malaysia and to analyze the in vitro catalytic activity of CYP2C8.

Materials and Methods: Subjects were healthy adult Malays (n = 313), Chinese (n = 335), and Indians (n = 200), as well as male Malay patients with hypertension (n = 154). DNA extracted from the blood was subjected to PCR method developed for CYP2C8 genotyping. HPLC method was developed using Amodiaquine for the in vitro catalytic activity and inhibitory study of CYP2C8 utilizing recombinant CYP2C8 protein expressed in E. coli. The in vitro inhibitory study with natural products consisted of herbs extracts of *Eurycoma longifolia Jack* (ELJ) or locally known as Tongkat Ali, *Labisia pumila* (LP) or Kacip Fatimah, *Andrographis paniculata* (AP) or Hempedu Bumi, *Echinacea purpurea* (EP) or Purple Coneflower, and *Ginkgo biloba* (GB), as well as Tualang Honey (TH) and Policosanol, a cholesterol lowering tablet produced from sugarcane wax.

Results: The allele frequencies of CYP2C8*2 and *3 were 0.2% and 0.3% in Malays and 1.5% and 2.3% Indians. In Malay patients with hypertension, CYP2C8*2 and *4 were detected with allele frequencies of 0.3% each. The kinetic parameters, Vmax and Km for CYP2C8 were 2.41 (SD 0.014) pmol/min/pmol CYP2C8 and 1.28 (SD 0.047) µM respectively. Extracts of LP, AP, TH, and Policosanol inhibited Amodiaquine metabolism via the CYP2C8 pathway in an uncompetitive (LP and AP), competitive (TH), and noncompetitive (Policosanol) inhibition mechanism.

Conclusion: We have successfully developed a specific, sensitive, and less tedious allele-specific multiplex PCR method for genotyping CYP2C8 polymorphism in Malaysia. We also have successfully expressed recombinant CYP2C8 protein and developed a specific and sensitive HPLC method for the in vitro analysis of CYP2C8 catalytic activity and the herb and food inhibition effect on CYP2C8 activity. Future studies are required to investigate the inhibitory effects of natural products on CYP2C8 activity to improve the understanding between genotypes and drug-herb or drug-food interactions in relation to susceptible diseases.

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