The following selection—alphabetical by title—reflects Cuban publishing in international medical and population health journals over the last quarter on an array of topics. Links to these journal articles may be found at www.medicc.org/mediccreview.


The occurrence of injection site reactions following immunization is the most frequently reported toxicity manifestation of vaccines; however, the different types of local reactions and the different mechanisms involved are still unclear. Here, the current advances in adjuvants and the role that adjuvants play in local reactions are reviewed. The role of adjuvants in the formation of the loco-regional complex (LRC), which consists of the injection site, draining lymphatic vessels and regional lymph nodes, is also discussed. Finally, strategies and recommendations for the rational design of adjuvanted vaccines are discussed, with a particular interest in the reduction of local inflammation.


Recent neuroimaging studies show that brain abnormalities in neuromyelitis optica (NMO) are more frequent than earlier described. Yet, more research considering multiple aspects of NMO is necessary to better understand these abnormalities. A clinical feature of relapsing NMO (RNM) is that the incremental disability is attack-related. Therefore, association between the attack-related process and neuroimaging might be expected. On the other hand, the immunopathological analysis of NMO lesions has suggested that CNS microvasculature could be an early disease target, which could alter brain perfusion. Brain tissue volume changes accompanying perfusion alteration could also be expected throughout the attack-related process. The aim of this study was to investigate in NMN patients, by voxel-based correlation analysis, the assumed associations between regional brain white (WMV) and grey matter volumes (GMV) and/or perfusion on one side, and the number of optic neuritis (ON) attacks, myelitis attacks and/or total attacks on the other side. For this purpose, high resolution T1-weighted MRI and perfusion SPECT imaging were obtained in 15 RNMO patients. The results showed negative regional correlations of WMV, GMV and perfusion with the number of ON attacks, involving important components of the visual system, which could be relevant for the comprehension of incremental visual disability in RNMO. We also found positive regional correlation of perfusion with the number of ON attacks, mostly overlapping the brain area where the WMV showed negative correlation. This provides evidence that brain microvasculature is an early disease target and suggests that perfusion alteration could be important in the development of brain structural abnormalities in RNMO.


Background Lipid extracts of Roystonea regia (D-004) and saw palmetto (SP) fruits have been shown to prevent experimental prostate hyperplasia in rodents, and to produce antioxidant effects in experimental and clinical studies.

Objective To compare the effects of D-004 and SP extracts on the International Prostate Symptomatic Score (IPSS) and plasma oxidative variables in men with benign prostate hyperplasia (BPH).

Methods This randomized, double-blind study was conducted in patients with moderate BPH. Forty-eight eligible subjects (average age: 65 years) were randomised to D-004 (320 mg/day) or SP (320 mg/day) capsules for 8 weeks. Decrease on IPSS was the primary efficacy variable. Oxidative markers were secondary outcomes. Data were analysed as per intention to treat.

Results D-004 and SP significantly decreased mean IPSS values by 33.9% (p<0.0001) and 24.4% (p<0.001), respectively, as compared to baseline. D-004 (p=0.0001) reduced plasma malondialdehyde (MDA) (32.6%), protein-linked carbonyl groups (CG) (25.2%) and increased (p<0.0001) catalase (CAT) activity. SP treatment lowered (p=0.0001) MDA (28.2%), CG (23.4%) and raised (p<0.0001) CAT activity. Effects on oxidative variables were similar in both groups. D-004, not SP, significantly lowered (p<0.05) prostate specific antigen (PSA) values. Both treatments were well tolerated. Only 2 SP-treated patients withdrew from the study. No adverse experiences were reported.

Conclusions Treatment with D-004 or SP (320 mg/day) for 8 weeks decreased significantly IPSS values in patients with moderate BPH, the effect of D-004 being the better, but further studies should confirm this result. Both treatments favourably and similarly modified plasma MDA (lipid peroxidation marker), GC (protein oxidation marker) and CAT activity.


Pathogenic CAG repeat expansion in the ataxin-2 gene (ATXN2) is the genetic cause of spinocerebellar ataxia type 2 (SCA2). Recently, it has been associated with Parkinsonism and increased genetic risk for amyotrophic lateral sclerosis (ALS). Here we report the association of de novo mutations in ATXN2 with autosomal dominant ALS. These findings support our previous conjectures based on population studies on the role of large normal ATXN2 alleles as the source for new mutations being involved in neurodegenerative pathologies associated with CAG expansions. The de novo mutations expanded from ALS/SCA2 non-risk alleles as proven by meta-analysis method. The ALS risk was associated with SCA2 alleles as well as with intermediate CAG lengths in the ATXN2.
risk for ALS was associated with pathogenic CAG repeat as revealed by meta-analysis.


Background/Aims Nonalcoholic fatty liver disease (NAFLD) is intimately related to insulin resistance and ranges from a benign course to liver fibrosis and cirrhosis. NAFLD management mainly involves dietary modification and weight loss. Although no fully successful pharmacological intervention is available, alternative therapies to treat NAFLD have shown promising results. Experimental studies have shown that D-002, a mixture of beeswax alcohols with antioxidant effects, is hepatoprotective. The aim of this study was to investigate the efficacy and safety of D-002 in patients with NAFLD.

Methods Fifty patients with NAFLD were randomized to receive a placebo or D-002 (100 mg/day) for 24 weeks. The primary endpoint was a significant ultrasonography-detected reduction of liver fat infiltration versus a placebo. Secondary endpoints were decreases in the homeostatic model assessment (HOMA) index, insulin levels, serum liver enzymes, increases in plasma total antioxidant status (TAS) and improved clinical symptoms versus the placebo recipients. Results At randomization, all indicators were comparable in both groups. At study completion, seven (28.0%) D-002-patients, but none of the placebo recipients, exhibited a normal liver echo pattern on ultrasonography (p < 0.01). Also, D-002 significantly reduced (p < 0.01 vs. baseline and placebo) the HOMA index and insulin levels and increased the TAS, but did not affect other parameters. The proportion of D-002-patients (12/25, 48.0%) showing symptom improvement was higher (p < 0.001) than that of the placebo group (1/25, 4.0%). The treatment was safe and well tolerated. Three patients in each group withdrew from the study.

Conclusions D-002 (100 mg/day) improved ultrasonographic findings, indicators of insulin resistance, plasma TAS and clinical evolution on NAFLD patients. Further studies, however, are needed to confirm these results.


Murine hybridoma monoclonal antibodies (MAbs) were produced against the capsular polysaccharide (CPs) of serogroups A, C, W135 and Y meningococci (MenA, MenC, MenW, MenY) in order to develop immunological reagents for the identification of meningococcal polysaccharides. Each serogroup-specific MAb reacted with the CPs from its homologous serogroup only and did not react with CPs from the other three serogroups. The affinity constant (Ka) of the four MAbs measured by non-competitive ELISA was 6.62 x 10(9), 2.76 x 10(9), 1.48 x 10(9) and 3.8 x 10(9) M(-1) for MenA, MenC, MenW and MenY MAbs respectively. The application of these MAbs for identity tests was demonstrated by their abilities to correctly identify the CPs from serogroups A, C, W135 and Y in meningococcal CPs-based vaccines through ELISA. The MAbs obtained in this work are a very useful set of tools for study meningococcal polysaccharides vaccines.


Huntington disease is the most frequent polyglutamine disorder with variable worldwide prevalence. Although some Latin American populations have been studied, HD prevalence in Cuban population remains unknown. In order to characterize the disease in Cuba, the relative frequency of HD was determined by studying 130 patients with chorea and 63 unrelated healthy controls, emphasizing in the molecular epidemiology of the disease. Sixty-two patients with chorea belonging to 16 unrelated families carried a pathological CAG expansion in the HTT gene, ranging from 39 to 67 repeats. Eighty-three percent of them come from the eastern region of the country. A significant inverse correlation between age at onset and expanded CAG repeats was seen. Intermediate alleles in affected individuals and controls represented 4.8% and 3.97% respectively, this being this a putative source of de novo mutation. This study represents the largest molecular characterization of Huntington Disease in the Cuban population. These results may have significant implications for an understanding of the disease, its diagnosis and prognosis in Cuban patients, giving health professionals the tools to implement confirmatory genetic testing, pre symptomatic testing and clinical trials in this population.


In Cuba the endemic species of scorpion Rhaphalus junceus has been used in traditional medicine for cancer treatment. However, there is little scientific evidence about its potential in cancer therapy. The effect of a range of scorpion venom concentrations (0.1, 0.25, 0.5, 0.75 and 1mg/ml) against a panel of human tumor cell lines from epithelial (Hela, SiHa, HeLa-2, NCI-H292, A549, MDA-MB-231, MDA-MB-468, HT-29), hematopoietic origins (U937, K562, Raji) and normal cells (MRC-5, MDCK, Vero) was determined by the MTT assay. Additionally, the effect of venom on tumor cell death was assayed by Fluorescence microscopy, RT-PCR and western blot. Only the epithelial cancer cells showed significant cell viability reduction, with medium cytotoxic concentration (IC50) ranging from 0.6–1mg/ml, in a concentration-dependent manner. There was no effect on either normal
or hematopoietic tumor cells. Scorpion venom demonstrated to induce apoptosis in less sensitive tumor cells (Hela) as evidenced by chromatin condensation, over expression of p53 and bax mRNA, down expression of bcl-2 mRNA and increase of activated caspases 3, 8, 9. In most sensitive tumor cells (A549), scorpion venom induced necrosis evidenced by acridine orange/ethidium bromide fluorescent dyes and down-expression of apoptosis-related genes. We concluded the scorpion venom from R. junceus possessed a selective and differential toxicity against epithelial cancer cells. This is the first report related to biological effect of R. junceus venom against a panel of tumor cell lines. All these results make R. junceus venom as a promise natural product for cancer treatment.


In 2001 a program for predictive testing of Spinocerebellar Ataxia type 2 was developed in Cuba, based on the detection of an abnormal CAG trinucleotide repeat expansion in the ATXN2 gene. A descriptive study was designed to assess the implications of ATXN2 large normal and intermediate alleles in the context of the SCA2 Prenatal Diagnosis Program. Four clinical scenarios were selected based upon the behaviour of large normal and intermediate alleles when passing from one generation to the next, showing expansions, contractions, or stability in the CAG repeat size. In some populations, traditional Mendelian risk figures of 0 or 50% may not be applicable due to the high frequency of unstable large normal alleles. Couples with no family history of SCA2 may have a 50% risk of having an affected offspring. Similarly, couples in which there is both an expanded and a large normal allele may have a recurrence risk >50%. It is imperative that these issues be addressed with these couples during genetic counselling. These recurrence risks have to be carefully estimated in the presence of such alleles (particularly alleles ≥27 CAG repeats), carriers need to be aware of the potential risk for their descendants, and programs for prenatal diagnosis must be available for them.


To define the molecular basis of secondary resistance to epidermal growth factor receptor (EGFR)-specific antibodies is crucial to increase clinical benefit in patients. The limited access to posttreatment tumor samples constitutes the major barrier to conduct these studies, repre-senting preclinical experimentation as a useful alternative. Anti-EGFR antibody-based therapy has been reported to mediate tumor regression by interrupting oncogenic signals and, more recently, by inducing antitumor immunological responses. However, resistance models have been focused only on tumor escape associated with EGFR blockade, whereas studies describing immune-associated escape mechanisms have not been reported thus far. To address this issue, we modeled distance and resistance in D122 metastasis-bearing C57BL/6 mice treated with 7A7 (an anti-murine EGFR antibody). Similarly to patients receiving EGFR-specific antibodies, 7A7 resistance promotion represents an important drawback to successful therapy. Characterization of primary cultures derived from metastasis in 7A7-treated mice revealed a high frequency of tumor variants resistant to in vivo and in vitro antibody treatment. We showed, for the first time, the convergence of alterations in oncogenic and immunological pathways in 7A7-resistant variants. To identify key molecules behind resistance, seven 7A7-resistant variants were screened. HER3 overexpression and PTEN deficiency leading to hyperactivation of protumoral downstream signaling were found in these variants as a consequence of 7A7-mediated EGFR inhibition. Concomitantly, we found a high percentage of resistant variants carrying abnormalities in the constitutive and/or interferon gamma (IFN-γ)-inducible major histo-compatibility complex I (MHC-I) expression. A significant decrease in mRNA levels for MHC-I heavy chains, β-,microglobulin and antigen processing machinery genes as well as transcriptional alterations in IFN-γ pathway components were identified as the main mechanisms underlying MHC-I expression defects in 7A7-resistant variants. Notably, these defects have not been previously associated with EGFR-specific antibody resistance, providing novel immunological escape mechanisms. This study has strong implications for the development of new combination strategies to overcome anti-EGFR antibody resistance.


Background The prognosis of patients bearing high grade glioma remains dismal. Epidermal Growth Factor Receptor (EGFR) is well validated as a primary contributor of glioma initiation and progression. Nimotuzumab is a humanized monoclonal antibody that recognizes the EGFR extracellular domain and reaches Central Nervous System tumors, in nonclinical and clinical setting. While it has similar activity when compared to other anti-EGFR antibodies, it does not induce skin toxicity or hypomagnesemia. Methods A randomized, double blind, multicentric clinical trial was conducted in high grade glioma patients (41 anaplastic astrocytoma and 29 glioblastoma multiforme) that received radiotherapy plus nimotuzumab or placebo. Treatment and placebo groups were well-balanced for the most important prognostic variables. Patients received 6 weekly doses of 200 mg nimotuzumab or placebo together with irradiation as induction therapy. Maintenance treatment was given for 1 year with subsequent doses administered every 3 weeks. The objectives of this study were to assess the comparative overall survival, progression free survival, response rate, immunogenicity and safety. Results The patients who received dose = 3200 mg of nimotuzumab given over a median number of 16 doses. The combination of nimotuzumab and RT was well-tolerated. The most prevalent related adverse reactions included nausea, fever, tremors, anorexia and hepatic test alteration. No anti-idiotypic response was detected, confirming the antibody low immunogenicity. The mean and median survival time for subjects treated with nimotuzumab was 31.06 and 17.76 vs. 21.07 and 12.63 months for the control group. Conclusions In this randomized trial, nimotuzumab showed an excellent safety profile and significant survival benefit in combination with irradiation.


Analysis of premature chromosome condensation (PCC) mediated by fusion of G0-lymphocytes with mitotic CHO cells in combination with rapid visualization and quantification of rings (PCC-Rf) is proposed as an alternative technique for dose assessment of radiation-exposed individuals. Isolated lymphocytes or whole blood from six individuals were γ-irradiated with 5, 10, 15 and 200Gy at a dose rate of 0.5Gy/min. Following either 8- or 24-h post-exposure incubation of irradiated samples at 37°C, chromosomal spreads were prepared by standard PCC cytogenetic procedures. The protocol for PCC fusion proved to be effective at doses as high as 20Gy, enabling the analysis of ring chromosomes and excess PCC fragments. The ring frequencies remained constant during the 8-24-h repair time; the pooled dose relationship between ring frequency (Y) and dose (D) was linear: $Y=(0.088±0.005)×D$. During the repair time, excess fragments decreased from 0.91 to 0.59 chromatid pieces per Gy, revealing the importance of information about the exact time of exposure for dose assessment on the basis of fragments. Compared with other cytogenetic assays to estimate radiation dose, the PCC-Rf method has the following benefits: a 48-h culture time is not required, allowing a much faster assessment of dose in comparison with conventional scoring of cell-fusion-mediated PCC. Additionally, the PCC-CRf method is well suited for chemically-induced premature chromosome condensation (PCC-Rch), and it allows the...
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Analysis of heavily irradiated lymphocytes that are delayed or never reach mitosis, thus avoiding the problem of saturation at high doses. In conclusion, the use of the PCC fusion assay in conjunction with scoring of rings in G0-lymphocytes offers a suitable alternative for fast dose estimation following accidental exposure to high radiation doses.


Context Microencapsulation of antigens has been extensively studied over the last decades aiming at improving the immunogenicity of vaccine candidates. Objective Addressing microparticles (MPs) toxicity in rats. Material and methods Spray-dried Eudragit® L 30 D-55 MPs and Eudragit® L 30 D-55 alginate MPs were elaborated and characterized. MPs obtained were administered to rats, three groups were defined: G1, control group; G2, administered with Vibrio cholerae (VC)-loaded MPs; G3, receiving VC-loaded alginate MPs. Animals received three vaccine doses. Body weight, food and water intake were controlled during the study. Haematological parameters, virobiocidal titres, organ weight and histology in necropsy were also analyzed. Results All animals grew healthy. Body weight gain, food and water intake and haematological parameters remained within physiological values, showing no treatment-related differences. Moreover, organ weight changes were not detected and animals developed protective virobiocidal titres. Conclusion VC-loaded MPs and VC-loaded alginate MPs have proved to be safe and effective in the assessed conditions.


Background Cuba is a unique country, and despite limited economic development has an excellent health system. However, the prevalence of asthma symptoms in children in Havana, Cuba, is unusually high. Aim Since early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental in early life exposures are critical to the aetiology of asthma, we have studied environmental...

Recent results with respect to the secretory production of bio-active *Mycobacterium tuberculosis* proteins in *Streptomyces* have stimulated the further exploitation of this host as a bacterial cell factory. However, the rapid isolation of a recombinant protein by conventional procedures can be a restrictive step. A previous attempt to isolate recombinant antigens fused to the widely used 6His-tag was found to be relatively incompatible with secretory production in the *Streptomyces* host. As an alternative, the eight-residue Strep-tag® II (WSHPQFEK), displaying intrinsic binding affinity towards streptavidin, was evaluated for the secretory production of two *M. tuberculosis* immunodominant antigens in *Streptomyces lividans* and their subsequent downstream processing. Therefore, the genes ag85A (Rv3804c, encoding the mycolyl-transferase Ag85A) and Rv2626c (encoding hypoxic response protein 1), were equipped with a 3’-Strep-tag® II-encoding sequence and placed under control of the *Streptomyces venezuelae* CBS762.70 subtilisin inhibitor (vsi) transcriptional, translational and signal sequences. Strep-tagged Ag85A and Rv2626c proteins were detected in the spent medium of recombinant *S. lividans* cultures at 48h of growth, and purified using a Strep-Tactin Superflow® matrix. Recombinant Ag85A appeared as a 30-kDa protein of which the N-terminal amino acid sequence was identical to the expected one. Rv2626c was produced in two forms of 17 and 37kDa respectively, both with the same predicted N-terminal sequence, suggesting that the 37-kDa product is an Rv2626c dimer. The obtained results indicate that the Strep-tagII is proteolytically stable in *Streptomyces* and does not interfere with the membrane translocation of Ag85A and Rv2626c. A comparison of reactivity of serum from tuberculosis patients versus healthy persons by ELISA showed that both *S. lividans*-derived antigens were recognized by sera of individuals infected with *M. tuberculosis*, indicating that they remained antigenetically active. To our knowledge, this is the first report showing the usefulness of an affinity peptide for detection and efficient downstream processing of recombinant proteins produced in *Streptomyces*. The present results add up strength to the significance of *S. lividans* as a valuable host to produce *M. tuberculosis* proteins with vaccine and diagnostic potential.


**Introduction** Zolpidem is a non-benzodiazepine drug used for the therapy of insomnia, which has selectivity for stimulating the effect of GABA-A receptors. Recently, a paradoxical arousing effect of zolpidem in patients with severe brain damage has been repeatedly reported. **Methods** A placebo-controlled magnetic resonance study was conducted to evaluate its effect on BOLD and metabolites spectral signals in a patient with severe brain injuries and an age-matched healthy volunteer. A multi-modal analysis was used to assess aspects in the pharmacologically-induced changes in the resting-state brain metabolism. **Results** A significantly increased BOLD signal was transiently localized in the left frontal cortices, bilateral anterior cingulated areas, left thalamus and right head of the caudate nucleus. The healthy subject showed a deactivation of the frontal, parietal and temporal cortices. BOLD signal changes were found to significantly correlate with concentrations of extravascular metabolites in the left frontal cortex. It is discussed that, when zolpidem attaches to modified GABA receptors of neurodormant brain cells, brain activation is induced. This might explain the significant correlations of BOLD signal changes and proton-MRS metabolites in this patient after zolpidem. **Conclusion** It was concluded that proton-MRS and BOLD signal assessment could be used to study zolpidem-induced metabolic modulation in a resting state.