DRUGS FOR INFECTIOUS DISEASE

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ANTIMALARIAL ACTIVITY AND PHARMACOKINETIC PROPERTIES OF NEW CHEMICAL ENTITIES

Dambuza N., Smith P., Chibale K., Wiesner L.

Background: Malaria is a disease caused by *Plasmodium falciparum* and is transmitted to a human host when an infected female *Anopheles gambiae* mosquito bites the host. It is estimated that 81% cases and 91% of deaths cause by malaria occurred in the WHO African Region, with children under 5 years of age and pregnant women being the mostly affected. *P. falciparum* has also developed resistance to most antimalarials and this has encouraged the development of new candidates of effective antimalarial drugs.
Method: Twelve new chemical entities were tested against *P. falciparum* chloroquine sensitive (D10 and 3D7) and resistant strains (Dd2 and K1). These compounds were tested for cytotoxicity against Chinese Hamster Ovarian (CHO) cell lines. Their pharmacokinetic properties were determined using a mouse model and blood samples were collected at different time intervals and analysed using LC-MS/MS. For *in vivo* efficacy, the mice were infected with a *P. berghei* strain in a 4-day Peter's test. The parasitaemia was determined from day 3 and the course of the infection was followed for 24 days by microscopic examination of stained blood films every 2–3 days.
Results: IC_{50} values for sensitive and resistant strains were 0.006 to 4 μg/ml (0.014 to 7 μM) and 0.007 to 51 μg/ml (0.017 to 105 μM), respectively. Of the twelve compounds only seven were active against malaria parasites. No significant cytotoxicity was observed. Five of the active compounds possessed low oral bioavailability and because of low blood concentration levels they were unlikely to exert any therapeutic effect on a mouse infected with plasmodium parasites. Two other potent compounds had high bioavailability at 60% and 69%, respectively and this resulted in improved *in vivo* efficacy.
Conclusion: The compounds evaluated in this study were from different structural classes and were very active antimalarials. The data collected showed that there was a relationship between the class of the compounds and their bioavailability. This was confirmed by high bioavailability of the two potent compounds which were both 2-aminopyridines while other classes evaluated in this study showed low oral bioavailability *in vivo*.

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ANTIRETROVIRAL INDUCED ADVERSE DRUG REACTIONS IN HIV INFECTED PATIENTS IN MALI: A RESOURCE-LIMITED SETTING

Oumar A., Dogoni O., Abdi-Bogoreh R., Cisse M., Landouré G., Dembélé J., Maiga I., Maiga A., Koita O., Dao S.

Purpose: To our knowledge, there is a rare report regarding antiretroviral induced adverse drug reactions (ADRs) in Malian patients who were infected with the human immunodeficiency virus (HIV). We have evaluated the frequency of antiretroviral therapy (ART) induced ADRs in this population and have assessed some risk factors of these reactions.
Methods: This is a prospective cohort study that was performed in the Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome Research Center (The CESAC) of Mali during years 2010–2011. Adults patients, who were infected with HIV and newly started on ART, were included in this study and by laboratory and clinical follow-up was performed for at least 6 months to detect the occurrence of any ADR. Naranjo's scale of classification has been used to characterize the side effects.
Results: During this study 94.6% of patients showed at least one ADR and 5.3% at least two ADRs. Prevalence of ADRs based on affected organ was 3.1% gastrointestinal (GI), 15.4% hematological, 45.9% neurological, 10.6% cutaneous, 1.4% hepatic, and 20.4% metabolic adverse effects. Adverse events were highly probable according to the Naranjo score (83.7%). The use of Zidovudine and Stavudine was observed as risk factors for anaemia, and peripheral neuropathy, and lipodystrophy, respectively, while nevirapine and female gender were identified as risk factors for skin reactions, lipohypertrophy by bivariate logistic regression.
Conclusions: Side effects were frequently encountered in our study. The nature of these adverse events was mostly peripheral neuropathy, lipoatrophy, lipodystrophy, and anemia. The link between the use of antiretroviral drugs and adverse events was highly probable according to the Naranjo probability scale. We recommend an active clinical and laboratory monitoring of antiretroviral therapy to strengthen pharmacovigilance in Mali

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ASSESSING THE EFFECTIVENESS OF PLASMODIUM LACTOSE DEHYDROGENASE (PLDH) BASED RAPID TEST DEVICE VS. STANDARD MICROSCOPY FOR DIAGNOSIS OF MALARIA IN PLASMODIUM BERGHEI IN ANIMALS

Uyaiabasi G., Adeyemi O., Oreagba I., Umaru L., Aina O.

Prompt and accurate diagnosis are key to effective management of malaria and in preventing the emergence of resistance. HPR2 based Rapid diagnostic tests (RDTs) kits are heavily relied upon in the diagnosis of malaria in areas where there are limited or no access to microscopy, but their effectiveness is greatly compromised by false positives results. pLDH based RDTs are viable alternative but discrepancies in reported sensitivities has limited it's use. This study assessed the effectiveness of pLDH based RDTs kits and time-taken for total antigen clearance following effective drug treatment. Microscopy was used as the standard.
Swiss albino mice were inoculated with 0.2 ml of blood from an animal already infected with *Plasmodium berghei* NK65 strains. After confirmation of the infection using microscopy; animals were randomly placed into four groups of five animals each and treated with 10 ml/kg po distilled water, 30 mg/kg chloroquine, 4/24 mg/kg po artemether/lumefantrine and 2.1/16.8 mg/kg po dihydroartemisinin/piperaquine respectively. Assessments were done on the first 4 days and on Days 7, 14, 21 and 28, by collecting small amount of blood from the tail of the animals for preparing thick and thin slides for microscopy and also testing with Acon® Malaria P.f/Pan RDTs. The sensitivity, specificity, positive predictive value and the negative predictive value were calculated.
A total of 146 observations were assessed in all the treatment groups, out of which 70 were true positives (TP), 69 were true negatives (TN), 2 were false positives (FP) and 5 were false negatives (FN). The Acon® Malaria *P.f*/Pan RDT was very effective in detecting pLDH, with sensitivity and specificity of 93.3% and 97.2% respectively. The

positive predictive value and the negative predictive value were 97.3% and 93.4% respectively.
The pLDH based RDT kits showed high efficacy and effectiveness for malaria parasitological diagnosis and is a viable alternative to HPR2 based RDTs. It is however recommended that all RDT kits for malaria diagnosis should consist of both HPR2 and pLDH based antigen detecting antibodies for more efficient results.

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CLINICIAN COMPLIANCE WITH LABORATORY MONITORING AND PRESCRIBING GUIDELINES IN PATIENTS RECEIVING TENOFOVIR

De Waal R., Cohen K., Fox M., Stinson K., Maartens G., Boulle A., Davies M.

Background: Tenofovir has been part of the preferred first-line regimen for HIV-infected patients in South Africa since 2010, but is associated with kidney toxicity. South African antiretroviral guidelines recommend creatinine monitoring at baseline, 3, 6 and 12 months, and substituting tenofovir should creatinine clearance decrease to <50 ml/min. Some authors argue that tenofovir can be used safely without monitoring. We assessed clinician compliance with tenofovir monitoring and prescribing guidelines at two South African HIV clinics, where routine clinical data are prospectively collected.
Methods: We included patients ≥16 years old who started first-line antiretroviral treatment between 2010 and 2012. We summarised the proportion of patients who had creatinine concentrations recorded at the recommended time points; the proportion started on tenofovir despite creatinine clearance <50 ml/min; and the proportion switched off tenofovir if their creatinine clearance decreased to <50 ml/min.
Results: We included 13,228 patients. Creatinine concentration was recorded at baseline in 6616 (50%). In 13,012 patients on tenofovir at the start of 3 months, 5916 (45%) had ≥1 creatinine concentration recorded between 0.5–4 months. In 12,895 patients on tenofovir at the start of 6 months, 3793 (29%) had ≥1 creatinine concentration recorded between 4–8 months. In 12,721 patients on tenofovir at the start of 12 months, 3277 (26%) had ≥1 creatinine concentration recorded between 8–18 months.
Of those who had creatinine concentration recorded, 125 (2%) received tenofovir despite creatinine clearance <50 ml/min at baseline. While on tenofovir, 339 patients had ≥1 creatinine clearance <50 ml/min. Of 206 patients with ≥3 months' follow-up after an abnormal result, 79 (38%) were switched off tenofovir within 3 months. In 97 patients who were not switched and had further creatinine clearances available, 83 (86%) had a creatinine clearance >50 ml/min at their next visit.
Conclusions: Based on routinely collected data, a large proportion of patients on tenofovir did not have creatinine concentration recorded in the database. Clinician compliance with prescribing guidelines was good at baseline, and over a third of patients were switched off tenofovir if creatinine clearance decreased to <50 ml/min. Many patients with creatinine clearance <50 ml/min recovered despite continuing tenofovir.

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CMIA: A POTENT AND SELECTIVE BACTERICIDAL COMPOUND AGAINST *ACINETOBACTER BAUMANNII*

Sadeghian A., Sadeghian H.

Introduction: *Acinetobacter baumannii* is an increasingly nosocomial pathogen throughout the world, and the occurrence of multidrug-resistant (MDR) species is increasing.The aim of this study is to present the antimicrobial effects of a newly synthesized imidazoacridine, 11-chloro-3-methyl-3*H*-imidazo(4,5-a) acridine (CMIA), against MDR clinical isolates of *A. baumannii*.
Material and methods: Standard dilution tubetest assay was performed to determine the MBC of CMIA for 91 clinical isolates of highly antibiotic-resistant bacteria with 28 of *A. baumannii* in them. The MBCs were determined by dilution tube-test method, introduced by National Committee for Clinical Laboratory Standards. A serial dilution of tested compounds (final concentration of 200–0.4 mg/l), were added to the test bacteria in Mueller-Hinton broth and were incubated at 37°C for 24 h (10^5–10^6 CFU/ml).
Results and discussion: The MBCs of CMIA ranged from 2.0 to 10.9 mg/l for Acinetobacter isolates while it was more than 47.9 mg/l for other clinical strains. The findings demonstrate that CMIA is a potent and selective antimicrobial agent against clinical strains of antibiotic-resistant *A. baumannii*.
Conclusion: It is well documented that the CMIA is the most potent and selective bactericidal analog against clinical isolates of highly antibiotic-resistant *A. baumannii*. The observed bactericidal activity of CMIA represents a potentially attractive alternative for topical treatment of *A. baumannii* infections.

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CO-ADMINISTRATION OF ARTEMETHER AND NEVIRAPINE HAS NO UNDESIRABLE EFFECT ON BLOOD GLUCOSE LEVEL IN WISTAR RATS

Anafi S., Kwanashie H., Anuka J., Muktar H., Agbaji A.

Background: Interactions between antimalarials and antiretroviral drugs may significantly affect the efficacy or side effects associated with combination therapy in malaria and HIV infections. This study investigated the effect of co-administration of nevirapine (NVP) and artemether (ART) on blood glucose level, with and without immunosuppression in Wistar rats.
Methods: Experiments were carried out according to international ethical standards regarding the handling and use of laboratory animals. Rats were divided into six groups of six per group. Groups 4, 5 and 6 received 30 mg/kg NVP daily for 21 days. From days 15–21, groups 2 and 5 received 5 mg/kg ART (ART_5) while groups 3 and 6 received 10 mg/kg ART (ART_{10}). All other animals received the vehicle (3% v/v Tween 80), up to day 21. All drugs administrations were through intraperitoneal route. In the latter 12 h of day 21, all rats were fasted and on day 22, the fasting blood glucose level was determined using a drop of blood from the animals' tail and a programmed digital glucometer. In a separate experiment, the above protocol was repeated in rats immuno-compromised with dexamethasone 20 mg/kg on day 1 followed by booster doses of 10 and 5 mg/kg on days 8 and 15 respectively. Data were analysed using ANOVA followed by Dunnett's post-hoc test.
Results: There was no statistically significant alteration in the blood glucose levels of normal rats administered with NVP + ART_5 (69.0 ± 5.0 mg/dl) and NVP + ART_{10} (72.0 ± 0.6 mg/dl) compared with Tween 80 vehicular control (87.7 ± 1.9 mg/dl); and immunocompromised rats administered with NVP + ART_5 (77.0 ± 1.0 mg/dl) and NVP + ART_{10} (81.0 ± 3.0 mg/dl) compared with Tween 80 vehicular control (88.0 ± 1.0 mg/dl).
Conclusion: Co-administration of ART and NVP had no significant effect on blood glucose level in Wistar rats, and may possibly not affect glucose levels in HIV/AIDS patients with malaria and diabetic co-morbidities.

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COMBINED VITAMINS E AND C IMPROVE NEPHROTOXICITY IN COLISTIN TREATED RATS

Zeghal K., Ghlissi Z., Hakim A., Mnif H., Rebai T., Sahnoun Z.

Objective: We evaluated the reno-protective effect of combined vitamins E (vit E) and C (vit C) on colistin methanesulfonate (CMS) induced-nephrotoxicity in rats.
Methods: Animals were treated with sterile saline, CMS at 450,000 IU/kg/day, CMS + combined vitamins (E and C) or only with combined vitamins (E and C) for 7 days. Sterile saline and CMS were administered intramuscularly (i.m.) in twice daily doses. Vitamins were given by oral gavage in once daily dose. Afterwards, the urine N-ace-

tyl-b-D-glucosaminidase (NAG) and γ-glutamyl-transferase (GGT) levels, the plasma creatinine (Cr), urea, vit E and vit C levels, and the renal tissue malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) levels, as well as renal histology were performed.
Results: CMS induced an acute tubular necrosis, increased the level of NAG, GGT and MDA, and reduced the vit E, vit C, SOD, CAT and GPx activities. The co-treatment with combined vitamins (E and C) plus CMS restored all biochemical parameters cited above and improved the histopathological damage.
Conclusion: It appears that CMS-induced nephrotoxicity is at least partly due to oxidative stress. The co-treatment with combined vitamins (E and C) seems to protect against oxidant damage which may be due to their antioxidant properties.

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CORRECT INTERPRETATION OF ACTS DOSAGE INSTRUCTION: IMPLICATION IN MALARIA DRUG RESISTANCE

Ebong O., Ogbuehi I., Siminialayi I., Asuquo E.

Background: Drug resistant *P. falciparum* usually develops following continued exposure of parasites to sub-therapeutic levels of antimalarial drugs[1]. Ensuring that drugs are taken in correct doses for the prescribed time reduces this risk of development of drug resistance. This study examined the level of understanding and interpretation of antimalarial drug dosage instructions among caregivers in Rivers State, Nigeria.
Methods: Respondents at a medical outreach program in Isiokpo community in Rivers State were studied using self administered questionnaires and in-depth interviews. They were asked to interpret dosage instructions for different available Artemisinin-based combination therapies (ACTs). Respondents also gave reasons for their interpretations. Data was statistically analyzed using SPSS software version 17.0 and Chi-square tests.
Results: Of 225 respondents consulted, 202 (61% females and 39% males) consented to participate in the study. Majority of the respondents, 73.5%, had secondary or less and 26.5% had tertiary education. Respondents interpreted drug instructions for three brands of ACTs. Seventy-nine percent of respondents misunderstood instructions, while 21% answered correctly.
The prevalence of incorrectly interpreting one or more dosage instructions among respondents with tertiary, secondary, primary and no education was 11%, 15%, 27% and 47%, respectively ($P < 0.001$).
In multivariate analyses, prescription instructions that gave specified hourly intervals (0 and 8 h) followed by time periods (morning, evening) were significantly more likely to be misinterpreted compared to once daily dosed drugs, that says, 'take 1 tablet in one intake per day' or 'take 2 tabs, day 1, 2 and 3.' With regards to the understanding of using body weight (kg) and age (yrs) to determine the right number of pills, some respondents may have mistaken the two different parameters as only 56% of the responses were correct, 44% were wrong.
Discussion and conclusion: These results show 75% respondents misinterpreting one or more dosage instructions. For some respondents, interpreting drug instructions may pose a cognitive burden which may result in drug misuse and subsequent drug resistance. This is a public health problem in the sustenance of gains made in malaria elimination.

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CYTOCHROME P450 2B6 GENETIC VARIANTS, NEVIRAPINE PLASMA LEVELS AND CLINICAL RESPONSE IN HIV-1-INFECTED KENYAN WOMEN

Oluka M., Okalebo F., Guantai A., McClelland R., Graham S.

Background: The pharmacokinetics of nevirapine (NVP) is subject to pharmacogenetic variability leading to inter-individual differences in efficacy and toxicity. Two variants in *CYP2B6*, 516G>T and 983T>C, are common in African populations, but data on their impact on NVP plasma levels and clinical response in African women are limited. We tested the association between 516G>T and 983T>C and NVP plasma concentrations and clinical outcomes. The relationship between NVP levels and clinical outcomes was also explored
Methods: Study subjects were 66 HIV-1-seropositive women taking NVP-based ART. Plasma collected at week 12 was analyzed for NVP concentrations by high performance liquid chromatography (HPLC). *CYP2B6* 516G>T and 983T>C were genotyped by real-time polymerase chain reaction (PCR). CD4 cell count, viral load, and genotypic drug resistance in plasma and genital secretions were assessed at baseline and during follow up. The data was analyzed to evaluate the effects of each genotype on plasma nevirapine levels at week 12 and on change in CD4 cell count at month 3, 6 and 12. Associations between plasma NVP levels and outcomes were analyzed by logistic or linear regression.
Results: Women bearing the *CYP2B6* 516 TT genotype (n = 9) had higher mean nevirapine plasma levels (14.33 μg/ml) compared to those with heterozygous 516 GT (9.18 μg/ml; n = 25) and wild- type 516 GG (7.95 μg/ml; n = 32) ($P = 0.01$) genotypes. Women who were heterozygous for the *CYP2B6* 983 TC genotype (n = 13) had higher mean nevirapine plasma levels (12.94 μg/ml), compared to women with the homozygous 983 TT (8.35 μg/ml; n = 53) ($P = 0.007$) genotype. Each $\log_{10}$ increase in nevirapine plasma levels was associated with a significantly higher CD4 cell count at month 3 (adjusted beta 122 cells/μl, 95% confidence interval [CI] 20–223 cells/μl, $P = 0.02$) and month 6 (adjusted beta 102 cells/μl, 95% CI 11–194 cells/μl, $P = 0.03$) but not at month 12 (adjusted beta 113 cells/μl, 95% CI −15 to 241 cells/μl, $P = 0.07$).
Conclusions: *CYP2B6 516* G>T and *CYP2B6* 983T>C genotypes were strongly associated with plasma NVP levels, which predicted immunologic response in women on NVP-based ART. These data support continued work on the potential utility of human genetic testing to inform NVP dosage optimization for individual patients.

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D-ENANTIOMERIC ANTIMICROBIAL PEPTIDES AS ANTIBACTERIAL AGENT AGAINST DRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS*

Lam J., Lan Y., Mason J.

Background: Tuberculosis (TB) control efforts have taken on increased urgency due to the emergence of multidrug-resistance (MDR) and extensive drug-resistant (XDR) TB. Effective antibacterial therapeutics are desperately needed to combat the drug resistant TB. Antimicrobial peptides (AMPs) have the ability to target microbial pathogens within eukaryotic cells. In the present study, we investigated the activity of a family of structurally related D-enantiomeric AMPs (D-LAK peptides) against clinical isolates of MDR-TB and XDR-TB.
Methods: The antibacterial activity of six D-LAK peptides (with different hydrophobicity and structural conformation) were tested against clinical isolates of drug susceptible, MDR and XDR *Mycobacterium tuberculosis* (Mtb) using broth micro-dilution assay in 96 well plates. The cytotoxicity of the D-LAK peptides on human macrophage-like cells (THP-1) was examined by lactate dehydrogenase (LDH) and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) assay. In addition, the effect of combining D-LAK peptides with isoniazid was also assessed against MDR-TB.

Results: All the D-LAK peptides tested could successfully inhibit the growth of Mtb *in vitro* to a certain extent and this was similarly effective against MDR and XDR strains. D-LAK peptides effectively broke down the heavy clumping of mycobacteria, consistent with a 'detergent-like effect' that could reduce the hydrophobic interactions between the highly lipidic cell walls of the mycobacteria, preventing bacteria cell aggregation. D-LAK peptides could not eradicate Mtb at non-toxic concentration, however they were effective as adjunct agent at low concentration to potentiate the efficacy of isoniazid against drug-resistant Mtb without inducing cytotoxicity to mammalian cells, possibly by facilitating the access of isoniazid into the mycobacteria by increasing the surface permeability of the pathogen.
Conclusions: We have identified that out of the six tested D-LAK peptides, D-LAK120-A is the optimal peptide within the family based on the balance between anti-TB activity and the low cytotoxicity towards mammalian cells. Although D-LAK peptides alone may not be sufficiently potent at their nontoxic concentrations as anti-TB agent, they could be used as adjunct agent to improve the efficacy of the existing antibiotics against drug-resistant TB.

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DISCOVERY OF THE 2-AMINOPYRIDINE MMV390048 AS AN ANTI-MALARIAL WITH THE POTENTIAL TO BE A COMPONENT IN A SINGLE DOSE CURE

Paquet T., Chibale K., Street L., Younis Y., Gonzalez Cabrera D., Le Manach C., Feng T., Nchinda A., Waterson D., Witty M., Wittlin S., Charman S., White K., Avery V., Bashyam S.

Background: With the aim to identifying a new treatment for malaria, a collection of 36,000 BioFocus SoftFocus compounds were screened against the malaria parasite, *Plasmodium falciparum*, sensitive (3D7) and resistant (Dd2) strains. 222 "hits" were identified that displayed >80% inhibition at 1.82 μM against both resistant and sensitive strains, and in addition had >100 fold selectivity in terms of cytotoxicity in various human cell lines.
Methods and results: Re-synthesis and testing of selected hits confirmed potency, and compounds with sufficient potency, good metabolic stability, and good physio-chemical properties were furthered to hit-to-lead optimisation.
Among the compounds were a series of 2-aminopyridines, which were optimised to a lead compound, MMV017007. The lead displayed good pharmacokinetics, with bioavailability and half-life in rats of 83% and 8.7 h respectively. Efficacy in *Pf* SCID mice (severe combined immunodeficient mice infected with *Plasmodium falciparum*) was demonstrated with an ED_{90} of 3.6 mg/kg. Unfortunately, the lead displayed potential cardiotoxicity risks (hERG IC_{50} = 5.5 μM) and had a high predicted human dose.
Further lead optimisation to overcome the abovementioned issues delivered MMV390048. This compound showed an improved *in vitro* potency, had excellent metabolic stability, but moreover, overcame hERG issues (IC_{50} >11 μM). In addition, single dose efficacy was achieved in a *P. berghei* model, curing infected mice with a 1 × 30 mg/kg dose. Efficacy in the *Pf* SCID mouse model was also demonstrated (ED_{90} = 0.57 mg/kg). MMV390048 further proved to have good pre-clinical pharmacokinetics and safety data and was selected as clinical candidate to enter phase I trials in 2014.
Since compound development was based on phenotypic observations, it became necessary to investigate the mode of action of MMV390048. Pull-down experiments with immobilised analogues of MMV390048, along with competitive binding studies (GSK Cellzome), indicated *Pf*PI4K as the potential target. Resistant mutant generation and subsequent sequencing (David Fidock, Columbia) revealed single nucleotide polymorphisms in the *Pf*PI4K locus only, providing additional confidence regarding MMV390048's mode of action.
Conclusion: The program therefore not only delivered a clinical candidate with the potential to be used as part of a single dose cure, but also revealed a novel drug target to combat malaria.

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DISINTEGRIN, RHODOSTOMIN, INHIBITS THE ACTIVATION OF TOLL-LIKE RECEPTOR 2 THROUGH ALPHAVBETA3-DEPENDENT SIGNALING PATHWAY

Huang T., Hsu C.

Background: In comparison with the well-characterized Toll-like receptor (TLR) 4 for Gram-negative bacteria, the Gram-positive bacteria receptor TLR2 is still poorly defined. Previously, we proved that rhodostomin (Rn), a snake venom disintegrin, interacts with αVβ3 integrin on phagocytes to execute its protection on LPS-induced endotoxemia both *in vitro* and *in vivo*. Moreover, we further evaluated the effects of Rn on Pam3CSK4 (a TLR2-specific agonist)-activated moncyte cell line, THP-1.
Methods: We evaluated adhesion, migration and cytokine release and determined binding site with flowcytometry. We also investigated the signalling pathway with RNA interference and Western-blotting.
Results: Rn significantly inhibited adhesion and migration, and decreased cytokine release of Pam3CSK4-activated THP-1. Transfection of integrin αV siRNA similarly inhibited Pam3CSK4-induced activation of monocyte, including cytokine release and cell adhesion. Rn also concentration-dependently attenuated the Pam3CSK4-induced phosporylation of JNK, ERK and p38 and degradation of IκB. Moreover, Rn reduced the phosporylation of FAK, Akt, c-Src and Syk, which are important molecules in transducing the signal pathway of integrin. We further revealed that Pam3CSK4/TLR2-induced translocation of MyD88, a central adaptor of TLR2, to the cell membrane was also inhibited by Rn treatment.
Conclusions: Taken together, Rn interferes with the activation of monocytes triggered by TLR2 ligation through interrupting the crosstalk between αVβ3 and TLR2/MyD88- dependent signaling pathways, implying that the protective function of Rn against both Gram-negative and -positive bacteria activated phagocytes may be attributed to its anti-TLRs activation.

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DRUG SUSCEPTIBILITY TESTING OF *MYCOBACTERIUM TUBERCULOSIS* TO ISONIAZID AND FLUOROQUINOLONE ENTRAPPED 1,2 DIPALMITOYL-L-α-PHOSPHATIDYLCHOLINE LIPOSOMES

Van Rensburg L., Van Zyl J., Smith J., Wiid I.

Background: The pulmonary route is very attractive for drug delivery as it offers an alternative mode of delivery to intravenous and oral systems. Therefore, mixing of pharmaceutically active agents with pulmonary surfactant may provide an attractive method of improving drug delivery. In this study, 1,2 Dipalmitoyl-L-α-phosphatidylcholine (DPPC) was evaluated as a possible drug carrier for antimycobacterial drugs isoniazid (INH), ofloxacin (OFX) and moxifloxacin (MXF).
Methods: Drug entrapped liposomes of dipalmitoylphosphatidylcholine, DPPC, were developed and evaluated for antimycobacterial activity, size, drug entrapment. Clinical isolates of M.tuberculosis X51 XDR strain and M.tuberculosis H37Rv reference strain was cultured on L-J slant cultures and used for BACTEC analysis. The Radiometric BACTEC 460TB method for the drug susceptibility testing was used where the mycobacteria are grown in 7H12 culture medium containing ^{14}C-labeled palmitic acid. All drug-entrapped DPPC liposomes were tested at concentration comparable to their minimum inhibitory concentrations keeping the Drug: DPPC ratio 1:1 w/w.
Results: *M. tuberculosis* displayed susceptibility to the anti-tuberculosis drugs in liposomal formulations. The drug-entrapped DPPC liposomes did not inhibit the antimycobacterial activity of the respective drugs. In addition, results demonstrate a definite increase in antimycobacterial activity of the drugs in combination with DPPC than that of free unformulated drug. INH displayed a 22% increase in bacterial susceptibility when in combination with the DPPC-liposomes at half of the MIC. The same increased bacterial susceptibility was found for the fluoroquinolones. Ofloxacin-DPPC liposomes displayed a 25%

increase in susceptibility at 1 μg/ml and, although the population was deemed to be resistant, 0.5 μg/ml displayed an increased susceptibility of 23%. Moxifloxacin-DPPC liposomes, with increased susceptibility of 4%, did not alter the MIC to such an extent as ofloxacin and INH. Un-loaded DPPC liposomes displayed a 3% decrease in mycobacterium growth for the H37Rv strain and a 20% decrease in growth for the XDR strain X51.
Conclusion: The data demonstrate that the liposomal-drug preparations do not negatively affect the antimycobacterial of the antitubercular drugs. This finding warrants further investigation on the clinical relevance of DPPC containing surfactant as a future drug delivery agent.

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EARLY AND LATE OUTCOMES FOLLOWING TREATMENT FOR SICK-CHILD VISITS AT A RURAL LEVEL IV HEALTH CENTER IN UGANDA

Wiens M., Gan H., Kabakyenga J., Barigye C., Zhou G., Karlen W., Ansermino J., Larson C., MacLeod S.

Background: Infectious diseases continue to plague children in developing countries. While significant focus is placed on treatment in hospitals and health centers (HC), there has been little research on outcomes following sick-child visits (SCV) at HC. The objective of this study was to determine outcomes 4 months following a SCV at a level IV HC in Uganda. We also sought to determine predictors of early and late outcomes following the initial SCV.
Methods: Children aged 0–12 presenting to a level IV HC October 2012 to January 2013 were eligible. Baseline diagnostic and clinical data were collected and children were followed-up approximately 16 weeks following their visit. Health care utilization and vital status were collected. Events were classified as early and late. Early event included admission, referral and death during the visit as well as a repeat SCV, admission or death within 1 month of the visit. Late events included repeat SCV, admissions and deaths between 1 and 4 months of initial visit. Predictors were analyzed using multivariable logistic regression.
Results: 717 subjects were enrolled and 604 (84%) completed follow-up. The median time to follow-up was 127 days (IQR 115–141). During the SCV 36% were diagnosed with a non-pneumonia respiratory infection, 31% with malaria, 23% with pneumonia, 5% with gastroenteritis, 5% with skin and soft tissue infections, 10% with another infection, and 60% with a non-infectious disease. There was significant overlap between diagnoses. Early and late events occurred in 16% and 34% of subjects, respectively. Predictors of early events included temperature, OR 1.54 (95%CI 1.25–1.84), respiratory rate, OR 0.98 (95% CI 0.95–1.00), heart rate, OR 1.02 (95%CI 1.00–1.03), age (months), OR 0.99 (95%CI 0.98–1.00), and weight-for-age Z-score, OR 0.78 (95%CI 0.61–0.95). There were no predictors of late events.
Conclusions: Health care utilization following SCV is high both for both early and late events. While there were few predictors of these outcomes, health care providers should be aware that either illness recurrence or treatment failures are common among children seen in rural outpatient settings. Parents of children should be made aware of the vulnerability for subsequent illness following SCV.

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EARLY TREATMENT RESULTS OF TELAPREVIR BASED TRIPLE REGIMENS IN CHRONIC HEPATITIS C PATIENTS IN TURKEY

Aksu F., Kurtaran B., İnal A., Ulu A., Kömür S., Tasova Y., Aksu H.

Background: In chronic hepatitis C patients (CHC), triple drug regimens (TDR) containing a protease inhibitor, pegylated interferon and ribavirin were found to significantly increase sustained virologic response rates compared to dual drug regimen containing pegylated interferon and ribavirin especially in genotype 1. In Turkey, telaprevir has been used since March 2013. We aimed to evaluate early results of CHC patients treated with telaprevir, pegylated interferon and ribavirin.
Methods: We evaluated 15 patients treated with TDR containing telaprevir, in a University hospital in Turkey, retrospectively. Demographic data of patients, treatment indications and genotypes were recorded. HCV-RNA levels at 4, 12 and 24th weeks, virological response and treatment duration were analysed. Rapid virologic response (RVR) is defined as detection of HCV-RNA negative in fourth and early virologic response (EVR) is defined as detection of HCV-RNA negative in 12th week.
Results: The mean age of patients was 56 (41–74) years. Twelve relapse and three non-responder CHC patients were retreated with telaprevir based TDR. All patients were genotype 1. Nine patients had liver biopsy. Of the nine patients with biopsy results, one patient was compensated cirrhosis. The mean histologic activity index was 7.1 (3–10) and fibrosis score was 1.6 (1–3) according to Ishak fibrosis score. The mean value of HCV-RNA was 1,866,360 (90,400–7,890,000) IU/ml. TDR were stopped due to severe skin rash in one patient in seventh week of treatment. HCV-RNA detected negative in all patients in the fourth week of therapy. In 12th week HCV-RNA was detected negative and early virological response was obtained in 13 of 14 patients that completed 12 week treatment. Eleven patients completed 24 weeks therapy, negative HCV-RNA detected in eight of these patients, HCV-RNA was positive in three patients at the end of treatment. The treatment of three patients is ongoing.
Conclusions: Though small number of patients and early to see sustained virological response, our early results, high RVR and EVR, with telaprevir based treatment regimen seems to be promising for CHC patients.

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EFFECT OF CO-ADMINISTRATION OF ARTEMETHER AND NEVIRAPINE ON LIVER ENZYMES AND KIDNEY INDICES IN WISTAR RATS

Anafi S., Kwanashie H., Anuka J., Muktar H., Agbaji A.

Background: Malaria and HIV occur together in many parts of the world creating the need for co-administration of antimalarial and antiretroviral drugs with potential for drug interactions. The objective of this study was to investigate the effect of administration of artemether (ART) and nevirapine (NVP) separately, and in combination on liver enzymes and kidney functions in both non-immuno-compromised and immuno-compromised Wistar rats.
Methods: Experiments were carried out according to international ethical standards regarding the handling and use of laboratory animals. Rats were divided into six groups of six per group. Groups 4, 5 and 6 received 30 mg/kg NVP daily for 21 days. From days 15 to 21, groups 2 and 5 received 5 mg/kg ART (ART_5) while groups 3 and 6 received 10 mg/kg ART (ART_{10}). All other animals received the vehicle (3% v/v Tween 80), up to day 21. All drugs administration were through intraperitoneal route. On day 22, animals were sacrificed and sera obtained. Alanine transaminase (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) were determined using standard kinetic methods. Total protein, albumin, creatinine and urea levels were determined using enzyme selectra XL machine. In a separate experiment, the above protocol was repeated in rats immuno-compromised with dexamethasone 20 mg/kg on day 1 followed by booster doses of 10 and 5 mg/kg on days 8 and 15 respectively.
Results: In non-immuno-compromised rats, statistically significant increases ($P < 0.05$) in ALP were observed in NVP and NVP-ART_{10} groups. In immuno-compromised rats, significant increases ($P < 0.05$) in ALP were also observed in NVP-ART_{10} group while ALT was significantly increased in both NVP and NVP-ART_{10} groups. No statistically significant changes were observed in total protein, albumin and urea in both non-immuno-compromised and immuno-compromised rats. However, a significant increase ($P < 0.05$) in creatinine was

observed in NVP-ART_{10} administered group in both non-immuno-compromised and immuno-compromised rats.
Conclusion: Alterations in ALP, ALT and creatinine observed might impair normal liver and kidney functions, hence the need for precautionary measures when ART and NVP are co-administered.

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EFFECT OF ETHANOLIC EXTRACT OF *OLEA EUROPAEA* ON *PLASMODIUM FALCIPARUM* INDUCED MICE

Sangodele J.O., Okere S.

Background: Malaria persists as a major health burden in African spite of all efforts at prevention and control. It is a major health problem with in Nigeria with stable transmission throughout the country. Malaria accounts for about 50% of out-patient consultations, 15% of hospital admissions and is the prime amongst the top three causes of death in the country. Parasite resistance to anti-malarial drugs remains a major threat to the control of malaria. This study aimed at evaluating the therapeutic effect of *Olea europaea* leaf extracts on malaria parasite induced mice.
Methods: Thirty Swiss albino mice were divided into six groups of five rats each. Group A normal control (no induction, no treatment); group B experimental control (O^+ human parasitized blood induced but no treatment). Group C, D, and E were induced with *Plasmodium falciparum* intraperitoneally treated with 40, 80 and 120 mg/kg body weight (bwt) ethanolic extract of the leaves of *Olea europaea* respectively. Malaria parasite was confirmed 72 h after induction using a compound microscope. Treatment continued for 3 days. Group F standard control induced with O^+ human parasitized blood and treated with a reference drug (Artesunate).
Results: Phytochemical screening of *Olea europaea* leaf extracts revealed high content of alkaloids, saponin, flavonoids. The proximate analysis of *Olea europaea* leaf revealed that it contains 68.80% carbohydrate content, 10.60% moisture content, 37.00% crude fiber, 39.68% crude fat, 14.60% ash content. Parasitaemia steadily decreased as the treatment progressed. Parasitaemia decreased in the 120 mg/kg body weight treated groups by a maximum of 79.1% compared with the standard drug (artesunate 50 mg/kg). The 40 mg/kg and 80 mg/kg bwt of the extract were able to inhibit 33.4% and 59.0% of parasitaemia respectively throughout the treatment period. All infected animals had reduced packed cell volume following infection and an increase was noted with treatment.
Conclusion: The dose 120 mg/kg bwt of *Olea europaea* showed a higher level of potency when compared to a known antimalarial drug; artesunate.

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EFFECTS OF QUERCETIN INHALATION ON ANTI-ACUTE RADIATION PNEUMONITIS IN THE RATS' MODEL

Chunling Z., Zhang W.

Background: The large volume of irradiation can cause radioactive pneumonia. It is a hotspot for radioactive pneumonia to look for safe and effective drugs from traditional Chinese herbal medicine. The aim of this study was to investigate the effects of quercetin on the rats with radiation pneumonia by inhalation.
Methods: 48 adult male wistar rats were randomly divided into four groups: the blank group (group A), the model group (group B), the positive control group (group C) and the quercetin group (group D). The model of radiation pneumonitis was established by single thoracic irradiation in the dose of 15 Gy using 6 mV linear X-ray. Group A was without any treatment, the other three groups were equal atomization inhaled from 1 week before irradiation until 4 months after irradiation. The rats were inhaled 20 ml saline in group B every day until the day before executed, group C were inhaled 20 ml drug solution with combination of dexamethasone (5×10^{-4} mg/100 g) and cephalexin (20 mg/100 g), and group D were given quercetin solution (10 mg). Six rats were randomly sacrified in the second and fourth month. The quantity of WBC and its classfication in blood and BALF were detected, the lung tissues were pathologically observed, and the quantity of hydroxyproline in the lungs was detected by Chloramine T method.
Results: The WBC in group D was lower than that of group B. Pathological photograph study showed that there was significant marked lung injury in the model group, while in the quercetin group was only slight injury and reduceed the level of TNF-β1 and IL-6. Compared with group B, there was a certain extent decline of hydroxyproline content in group D.
Conclusions: Quercetin can alleviate the pathological changes of radiation pneumonitis and has protective efections, which may be a new therapeutic method for the prevention/treatment of acute radiation pneumonitis.

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EFFICACY OF TRADITIONAL ANTI-HELMINTHIC PLANT MEDICINE FOR THE TREATMENT OF SOIL TRANSMITTED INTESTINAL WORMS IN HUMANS: A SYSTEMATIC REVIEW

Nikodem C., Silaigwana B.

Soil transmitted helminthiasis (STH) is a common infectious disease caused by parasitic worms or intestinal nematodes (Keiser &Utzinger, 2008). The most common causative agents of STH infections in humans are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Necator americanus* or *Ancylostoma duodenale*) (Taylor-Robinson *et al*., 2012). The disease remains a serious public health problem worldwide (Mascarini-Serra, 2011). Approximately more than 4.5 billion people globally are at risk of STHs infections (Keiser &Utzinger, 2008; Uneke, 2010). It is estimated that *A. lumbricoides* infects approximately 1.2 billion people worldwide, and 600–800 million infections are caused by *T. trichiura* and hookworms respectively (de Silva *et al*., 2003; Saboya *et al*., 2011; Taylor-Robinson *et al*., 2012; Uneke 2010). China accounts for approximately 50% of the total cases of infections caused by *Ascaris*. On the other hand, *Trichuris* infections and hookworms are more prevalent in central Africa and Sub-Saharan Africa respectively (de Silva *et al*., 2003). The global prevalence of STH varies disproportionately, infections are widespread in tropical and subtropical areas, and are generally associated with poverty and poor sanitation (Uneke, 2010; Taylor-Robinson *et al*., 2012). Concerns about the sustainability of periodic deworming with anthelmintic dugs and the emergence of resistance have prompted considerable efforts to use medicinal plants as potential alternative treatment of STHs (Tandon *et al*., 2011; Khayde *et al*., 2012).—Medicinal plants show high parasite reduction rates however results are based on low quality, small- scale trials. —Proper double-blind randomised clinical trials are needed to determine their safety and efficacy before they can be recommended for healthcare policy. Policy recommendations to conserve indigenous plant biodiversity without threatening the discovery of future disease cures are also imperative.

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ENZYMATIC AND CHEMICAL MODIFICATIONS OF ERYTHROCYTE SURFACE ANTIGENS TO IDENTIFY PLASMODIUM FALCIPARUM MEROZOITE BINDING SITES

Baron K., Cromarty D.

Background: Erythrocyte membrane proteins are essential for cells to perform vital physiological functions. Utilisation and/or alteration of these membrane proteins occur in multiple diseases, including malaria. At least 40% of the global population is at risk of malaria infection annually, with 0.7–2.7 million people dying as a result. Erythrocyte

invasion by the merozoite form of the malaria parasite is a complex multiphase process essential to the rapid development of malaria. This erythrocyte invasion utilises a sequence of multiple erythrocyte surface receptor-parasite ligand interactions. The erythrocyte surface receptor proteins involved can serve as potential antimalarial drug or vaccine targets. Thus determining the relative importance of erythrocyte receptors involved in invasion using modern proteomic techniques, may provide further information and focus for antimalarial drug and vaccine strategies.

Methods: Uninfected erythrocytes were treated with trypsin, tris (2-chloroethyl) amine (TCEA) and sodium periodate to alter surface and cytoskeletal proteins. Protein changes were assessed by Sodium Dodecyl Sulphate (SDS) Polyacrylamide Gel Electrophoresis (PAGE), and analysed by liquid chromatography-mass spectrometry (LC-MS/MS).

Parasite invasion assays were performed in microtiter plates to assess the effects of enzymatically/chemically-treated erythrocytes on malaria parasite invasion efficiency. Wells contained healthy, untreated erythrocytes or treated erythrocytes, and invasion efficiency was defined as the rate of invasion achieved with enzymatically/chemically-treated erythrocytes expressed as a percentage of the rate of invasion achieved with untreated (control) erythrocytes.

Results: Erythrocyte surface protein changes brought about by enzyme and chemical treatments were clearly visualised using SDS PAGE, and were identified and tracked using LC-MS/MS. Changes in surface proteins included those to spectrin, glycophorin and band 3. The effects of chemically/enzymatically-treated erythrocytes on parasite invasion efficiency were successfully assessed, where the outcome of the treatments varied from haemolysis through to reduction (up to approximately 50%) in invasion efficiency. Based on these results, the relative importance of erythrocyte receptors in the invasion process was determined.

Conclusion: Utilising modern proteomic techniques offers a fresh approach into the relative importance of erythrocyte surface proteins involved in the invasion process of the malaria parasite.

This information lays a foundation for the discovery of new key targets for drug- and vaccine-based anti-malarial strategies.

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EVALUATION OF IN VIVO ANTITRYPANOSOMAL ACTIVITY OF CRUDE EXTRACTS OF *ARTEMISIA ABYSSINICA* AGAINST *TRYPANOSOMA CONGOLENSE* FIELD ISOLATE

Feyera T., Terefe G., Shibeshi W.

Background: African trypanosomiasis is a major disease of economic and public health importance affecting agricultural and human development. The search for alternative compounds against African trypanosomiasis is justified by various limitations of existing chemotherapeutic agents. This study was aimed at screening the hydromethanolic and dichloromethane (DCM) crude extracts of aerial parts of *Artemisia abyssinica* for in *vivo* antitrypanosomal activity against *Trypanosoma congolense* field isolate in mice.

Methods: The aerial parts of the plant was extracted by maceration technique using dichloromethane and 80% methanol to obtain the corresponding crude extracts. The plant extracts at doses of 100, 200 and 400 mg/kg body weight were administered intraperitoneally daily for 7 days to mice with an established infection with Trypanosoma congolense. Diminazene aceturate and distilled water were used as positive and as negative controls respectively. The level of parasitaemia, body weight, packed cell volume, differential leukocyte counts and mean survival period were monitored.

Results: The study showed that the DCM extract at 200 and 400 mg/kg, and the hydromethanolic extract at 400 mg/kg reduced parasitaemia ($P < 0.05$), ameliorated anaemia ($P < 0.05$), prevented body weight loss ($P < 0.05$) and modified ($P < 0.05$) differential lymphocyte and neutrophil counts compared to the negative control.

Conclusions: This study established that aerial parts of *A. abyssinica* have antitrypanosomal potential and can be considered a potential source of new drugs for the treatment of tropical diseases caused by trypanosomes.

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EVALUATION OF SUPPRESSIVE AND CURATIVE ANTIPLASMODIAL ACTIVITIES OF *OLDENLANDIA AFFINIS* (RUBIACEAE), A TRADITIONAL HERB INCLUDED IN ANTIMALARIAL THERAPY

Onyeto C.

Aim of study: This ongoing study is aimed at evaluating and developing the herb, *Oldenlandia affinis*, as alternative therapy for the treatment of malaria.

Introduction: Despite the availability of many orthodox anti malaria medicines the menace continued to be a burden in sub Saharan Africa. Many herbs have been used successfully to treat malaria hence the search for alternative and complementary anti malaria therapy from medicinal plants become imperative. *Oldenlandia affinis* (Rubiaceae) is a scrambling perennial herb widespread in tropical Africa and used for the treatment of cancer, malaria, HIV, anti-infertility etc.

Outcome of our preliminary investigation: We undertook a preliminary investigation of suppressive and curative antiplasmodial activities of the aqueous methanol extract of the aerial parts of *Oldenlandia affinis* in rodent models. The methanol extract (ME) obtained by 48 h cold maceration was subjected to antimalaria investigation using suppressive and curative models. Intraperitoneal treatment of mice with the aqueous methanol extract (100, 200 and 400 mg/kg) for 4 days exhibited a dose dependent suppression of mean plasmodium parasitaemia in treated group by as much as 11.76 %, 61.4 %, and 75 % respectively when compared to the mean parasitaemia in the negative control. Similarly, ME, at the same doses, caused significant ($P < 0.05$) and dose-related decreases in parastaemia in the curative model. These reductions in parastaemia were similar to the reductions in parasitaemia recorded with the positive control (artemether 50 mg/kg) given parenterally. Preliminary phytochemical analysis of the extract revealed the presence of saponins, tannins, reducing sugar, flavonoid, glycosides, steroids, terpenoids, carbohydrates, protein, alkaloids and acidic compounds. These findings suggest that aqueous methanol extract of *O. affinis* possesses anti-plasmodial activity in rodents and deserve further detailed studies with a view to harnessing its potential.

Future perspective: In view of the promising outcome of our preliminary investigation, we intend to carryout bioactivity-guided fractionation and separation to determine the active principles responsible for the observed activities. We will carry out more *in vivo* and *in vitro* assays to fully characterize the bioactivity of the herb, *Oldenlandia affinis*.

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IMMUNOMODULATORY ACTIVITY OF LACTOBACILLUS PARACASEI ON ENTEROPATHOGEN *SALMONELLA* TYPHI; IN VITRO & IN VIVO STUDIES

Hamzawy M., Mazaya B., Wael T., Khalil M.

Background: Multidrug resistances of Salmonella strains represents a significant threat to human health. Consequently, great efforts are being made to attain alternative therapeutic tools against typhoid fever to overcome of drug resistance. Indeed, salmonellosis-induced diarrhea, are one of the major causes of childhood morbidity and mortality in developing countries. Lactobacillus is a type of probiotic bacteria, the mode of action of these bacteria is not fully understood yet.

Aim: The current study aimed to evaluate the enteropathogenic action of *S. Typhi* and the potential protective effect of *L. paracasei* against salmonella infections.

Method: Four groups of male CD1-mice were treated for 8 days included negative control; the group challenged with single inoculation

of S. *Typhi* (200 µl aliquot of 1X 10^8/P.O) at the first day, the group treated with lactobacilli (200 µl aliquot of 1X 10^8/P.O) for 7 days, and the group challenged with single inoculation of S. *Typhi* one day prior daily treatment with lactobacilli for further 7 days.
Results: Animals challenged with *Salmonella Typhi* showed significant disturbance in inflammatory cytokines; TNF-α and IL-1β and positive value of widal test, severe changes of histological and histochemichal examinations of alimentary tracts and livers and increase the innate immunity of hepatocytes and villi destruction . Lactobacilli succeeded to improve the inflammatory cytokines and histological and histochemichal pictures in the infected mice and complete remission of widal test comparable to control group.
Conclusion: It can be concluded that lactobacilli has a potential protective effects of lactobacilli against enteropathogenic S. Typhi due to its immunomodulatory mechanism that implicated to be an alternative mode for eradication of *S. Typhi* infection.

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IN VITRO AND IN VIVO EFFECT OF LUMEFANTRINE ON *TRYPANOSOMA CRUZI*, THE ETIOLOGIC AGENT OF CHAGAS DISEASE

Garcia Bournissen F., Gulin E., Solana M., Novoa M., Alba-Soto C., Altcheh J., Flordellis C., Senard J., Gales C.

Background: Lumefantrine (LMF) is an anti-malarial drug with a long half life and still unclear mechanism of action. Lumefantrine activity against kinetoplastids has not been fully studied.The objective of this study was to evaluate LMF activity against *T cruzi*.
Methods: *In vitro* studies: Vero cells were infected with *T cruzi* trypomastigotes, VD strain, for 24 h. Cultures were then exposed to LMF (0.5 to 50 µM) for 72 h. After treatment, cells were stained with Giemsa, and infected cells and number of amastigotes per cell were counted using ImageJ software.
In vivo studies: 16 female BALB/c mice (4 per group) were infected with 500 trypomastigotes (VD strain), and LMF administered at peak parasitaemia, 12 days post infection (dpi), for 20 days. Group 1 received 80 mg/kg/day LMF, Group 2 160 mg/kg/day LMF, Group 3 was the untreated control, and Group 4 was a positive control treated with benznidazole 100 mg/kg/day. Mice weight, parasitaemia and mortality were registered daily.
Results: No effect of LMF on *T cruzi* infection was observed in the *in vitro* studies, except for the 50 µM dose which showed significant parasite reduction ($P < 0.01$). The *in vivo* studies showed decreased mice mortality, decreased parasitaemia and lower weight loss in the animals treated with LMF compared to the untreated group, suggesting a partial effect of the drug on the infection. A further experiment combining LMF (150 or 300 mg/kg/day) with low-dose benznidazole (5 mg/kg/day) in mice failed to find a synergic effect of the drug.
Discussion: In spite of a modest effect of LMF *in vivo* against *T cruzi*, the activity of the drug in this model was considered limited and non-synergic with benznidazole, even at high doses. We do not believe, based on these results, that LMF is a good candidate for further development in Chagas disease.

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NOVEL ANTIBODY-BASED BIOTECHNOLOGICAL APPROACH OF INFLUENZA MANAGEMENT: SUMMARY OF PRECLINICAL AND CLINICAL STUDIES

Tarasov S., Gorbunov E., Myslivets M., Rodionova N., Epstein O.

Background: Influenza is one of the most serious infectious diseases confronting the world today. Effective treatment is limited by the necessity of early treatment initiation and by a high genetic variability of influenza virus. Anaferon® is antiviral drug containing release-active dilution of polyclonal antibodies to interferon gamma manufactured by technology of ultrahigh dilution and licensed in several countries. Drug mechanism of action is modification of interferon gamma conformation state resulting in positive modulation of its biological activity. The drug is used for treatment of different viral infections (influenza, herpesviral infections, gastrointestinal viral infections). The aim of this work is to present a summary of anaferon's anti-influenza efficacy.
Methods: Analysis of preclinical and clinical studies of anti-influenza activity of anaferon conducted in Russia, Europe and USA for the last 10 years. Preclinical studies were conducted in Balb/c mice inoculated with lethal and non-lethal doses of influenza A/H3N8, A/H3N2, A/H5N1, A/H1N1v strains. Oseltamivir (4–25 mg/kg/day) was used as reference, anaferon's vehicle - as control. Survival rate and virus titers in lungs were assessed. Double-blind randomized placebo controlled clinical studies were conducted in children and adults (more than 1000 patients in total) with laboratory confirmed influenza A or B. Duration of clinical signs (fever, catarrhal and intoxication symptoms) and adverse events were assessed.
Results: It was shown that treatment of mice with anaferon resulted in a statistically significant later disease onset (1–2 days vs. control), reduction in mortality (2–4 times vs. control) and virus titres in lungs with the efficacy comparable to oseltamivir. In clinical studies anaferon ameliorated significantly signs of influenza and reduced their duration. Compared to placebo, duration of fever was one-two days shorter and duration of catarrhal and intoxication symptoms were more than two days shorter. The number of complications was considerably lower in patients receiving anaferon. No adverse events were reported.
Conclusions: Anaferon is an antiviral drug that is effective against many strains of influenza, including pandemic ones. Its excellent safety profile together with high antiviral activity allows us to consider it as a first-line option candidate in prophylaxis and treatment of influenza in adults and children.

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ONGOING EDUCATIONAL ACTIVITIES TO ENHANCE THE APPROPRIATE USE OF VANCOMYCIN IN A LEADING HOSPITAL IN VIETNAM; FINDINGS AND FUTURE DIRECTION

Godman B., Gam T., Anh L., Huong N., Gustafsson L.

Introduction: The authorities in Vietnam have instigated a number of reforms to enhance universal coverage. Medicines are a key area since they constitute an appreciable proportion of total health care expenditure in Vietnam, with appreciable consumption in hospitals. Currently there are limited activities to enhance the rational use of medicines in hospitals despite government circulars to instigate Drug and Therapeutic Committees. The dosing of vancomycin is a concern. Even in a leading tertiary hospital in Hanoi (Bach Mai hospital), patients appeared to have the same dose despite different creatinine clearance (ClCr) rates. Under-dosing can increase MRSA development and over dosing increase side-effects. This situation was not helped by lack of guidelines. In May, 2013, new protocols were launched for vancomycin for the treatment of serious infections.
Aim: We undertook an analysis of vancomycin use in Bach Mai hospital to (i) Understand the extent of inappropriate dosing, (ii) implement extensive educational and other activities in ICU with limited activities in other wards (iii) Monitor the % change in appropriate vancomycin usage, (iv) Suggest future steps.
Methodology: Baseline data collected on the extent of rational use of vancomycin (dose and blood sampling) in 3 wards (ICU, ID and Respiratory wards - with vancomycin dosing especially important in respiratory wards). Intervention for 3 months - Clinical Pharmacist visiting ICU every morning to monitor vancomycin use, give advice to nurses on how to dilute and administer vancomycin with flow charts placed on several locations around the ward; activities in the other 2 wards included physicians invited to a meeting to hear about the new protocols and discuss it, guidelines for vancomycin distributed on the wards, some follow-up by clinical pharmacists. Post intervention: Prospective 2 week sampling of vancomycin use in the three wards. The principal outcome measure is % change in appropriate vancomycin usage in each ward.

Results: Preliminary results suggest a positive effect in the ICU and concerns about reduced adherence to guidelines in the ID ward. Further analysis is ongoing and will be reported to give guidance to Bach Mai hospital as well as other hospitals in Vietnam.

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PATTERN AND DETERMINANTS OF ANTIMICROBIALS USE AMONG IN-PATIENTS AT A TERTIARY HEALTH CENTRE IN A DEVELOPING COUNTRY- A CROSS-SECTIONAL REVIEW

Ogundele S., Ogunleye O., Mutiu B.

Introduction: Antimicrobials agents are among the most commonly prescribed medications. Frequent and irrational use of antimicrobials is associated with many problems, such as; increase incidence of adverse drug reaction, increase emergence of drug resistant organisms and increase in the overall cost of health care. The study aimed to audit the current pattern and appropriateness of antimicrobials agents' prescription at our centre.
Methods: A retrospective cross-sectional review of patients' record. A random sample of records of patients who were recently discharged from the medical wards of the hospital was reviewed and relevant information extracted using a structured questionnaire, the review was over a week period. Information extracted from the case note of the patients included the following: sex, indications for antibiotic use, investigations requested and completed, name of antimicrobials during inpatient care, dosages of antimicrobials, duration of use of antimicrobials and outcome of patient management. The prescribed antimicrobials were reviewed by a microbiologist independent of the management team to determine its appropriateness. Extraction of data from the patients' case record was done by the researchers assisted by other doctors in the team. Appropriateness of antimicrobial prescribed was determined by the use of classification by *Gyssens et al, 1992*.
Results: Total of 56 case notes were reviewed, 31 (55.4%) males and 25 (44.6%) females. Indication for use of antimicrobial agents was therapeutic in 45 (80.4%) and prophylactic in 11 (19.6%). The commonest class of antimicrobial prescribed was cephalosporins (66%) this was followed by metronidazole (39.3%) and co-amoxiclav (23.4%) respectively. These drugs were used either alone or in combination. Most of the cases reviewed (38, 67.9%) had complete blood count as investigation of choice for infection and only 18 (32.1%) had a culture to isolate the infecting organisms. Use of antimicrobials were judged to be appropriate in 39.3% of the cases reviewed, in 44.7% the use were found to be inappropriate for various reasons and 16.1% could not be classified due to insufficient data.
Conclusion: There is a need for guidelines for the use of antimicrobials in our centres to prevent inappropriate use of these agents and problem associated with it.

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POPULATION PHARMACOKINETICS OF BENZNIDAZOLE IN A PEDIATRIC COHORT OF PATIENTS 0–12 YEARS OLD

Garcia Bournissen F., Ribeiro I., Caruso A., Moscatelli G., Moroni S., Moran L., Monla C., Bisio M., Mastrantonio G., Altcheh J.

Background: Chagas disease, caused by the parasite Trypanosoma cruzi, can lead to long term cardiac morbidity. Treatment of children with benznidazole is effective, but no pediatric pharmacokinetics data are available and clinical pharmacology information on the drug is scarce. No information is available for children under 2 years of age.
Methods: Prospective population pharmacokinetics (PopPK) cohort study in children between 0 and 12 years of age with Chagas disease (clinicaltrials.gov #NCT01549236) enrolled between 2011 and 2012 at five recruitment centers in Argentina (PEDCHAGAS Network). Patients were treated with benznidazole (Lafepe, Brazil) 12.5 or 100 mg tablets, dose: 5–8 mg/kg/d bid p.o. for 60 days. Treatment response was evaluated by T. cruzi specific PCRq at the end of treatment, and anti-T cruzi antibody titers. Five blood samples per child were obtained on Whatman 903 paper for measurement of benznidazole for PopPK analysis by HPLC-MS/MS.
Results: A total of 81 patients were enrolled. Median age: 12 months (IQR 6–72 months). Five patients discontinued treatment, 3 due to adverse drug reactions, and 2 due to lack of adherence. A total of 387 blood benznidazole measurements were obtained. Median observed Cmax was 8.32 mg/L (range 1.79–19.38). Median trough was 2 mg/L (range 0.14–7.08).
A one compartment model best fit the data. Weight-corrected clearance rate (CL/F) showed a good correlation with age, (younger patients had significantly higher CL/F than older children and adults). Simulated steady-state benznidazole concentrations were lower for children in our study than for adults and lowest for children under 7 years of age. Treatment was efficacious in all patients who completed the treatment course, and well tolerated with few, and mild, adverse drug reactions (ADRs).
Discussion: Observed benznidazole plasma concentrations in children, and particularly in infants, were markedly lower than those previously reported in adults (treated with comparable mg/kg doses), but nevertheless associated to a high therapeutic response in our cohort. Unlike adults, children have few adverse reactions to the drug, suggesting that there may be a correlation between drug concentrations and ADRs. Our results suggest that studies with lower doses in adults may be important

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RIMONABANT HAS PARADOXICAL EFFECTS ON CART ASSOCIATED METABOLIC DISEASE

Makamu B., Mwangi P., Bukachi F.

Background: Treatment of HIV infection with cART often results in development of a lipodystrophic syndrome whose etiology, phenotype and metabolic derangements resemble that of metabolic syndromes. Overactivity of the endogenous cannabinoid system is a feature of metabolic syndrome and its antagonism improves physical and biochemical profiles. This study looked at the effects of rimonabant on cART induced lipodystrophy.
Objective: To test the hypothesis that use of rimonabant with cART prevents the metabolic and phenotypic derangements associated with chronic cART in Sprague-Dawley rats.
Materials and methods: Thirty (30) adult male Sprague-Dawley rats housed under standard conditions were randomized to control, positive control (LPV/r + AZT) and test (LPV/r + AZT + rimonabant) groups. The rats received drugs or vehicle by oral gavage daily for 4 weeks. Thereafter insulin tolerance test (ITT), visceral and subcutaneous fat depot weight, serum lipids and serum fatty acid binding protein type 4 were assessed.
Results: The test group had better insulin tolerance than either positive controls or controls. Fasting blood sugar in the test group was 49.7% and 13.8% lower at 120 min ($P = 0.002$) than positive controls and controls respectively. Rimonabant treated rats had 39% less visceral adiposity ($P = 0.05$) than positive controls. Serum triglycerides were 57% and 33% higher in the test group vs. the positive controls and controls ($P = 0.002$). LDL cholesterol levels were 102% and 22% higher in the test group vs. positive controls and control rats respectively ($P = 0.001$). Rimonabant co-administration was associated with 60% lower HDL cholesterol levels vs. positive controls ($P = 0.009$). Serum FABP4 levels were increased by 80% and 75% in the test group vs. positive controls and control groups respectively but this was not statistically significant ($P = 0.23$).
Conclusions and relevance: Concurrent treatment with the cannabinoid receptor type 1 antagonist rimonabant causes an increase in insulin sensitivity and reduces visceral fat accumulation. It paradoxically increases serum triglycerides and serum LDL cholesterol levels. These findings may limit the utility of this approach in addressing cART associated lipodystrophy. Further research is necessary to characterize the precise mechanisms that produced the dyslipidemia observed.

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SENTINEL STUDY ON RESISTANCE TO ARTEMISININ BASED ANTIMALARIAL COMBINATION THERAPY IN KADUNA NORTHWEST – NIGERIA

Umaru L., Abdu-Aguye I., Zezi U., Danjuma Mohammed N., Uyaiabasi G.

Malaria resistance still remains the major obstacle in the fight against malaria. The World Health Organisation adopted Artemisinin Combination Therapy (ACT) as first-line drug treatment in 2001. Recent reports of resistance to ACTs in Southeast Asia has again brought to fore the issue of resistance. The study was an open-label randomized prospective evaluation of clinical and parasitological responses to directly observed treatment (DOT) for uncomplicated *falciparum* malaria in Kaduna State, North - Western Nigeria for 2011, using artemether-lumefantrine (AL), artesunate-mefloquine (AM) and dihydroartemisinin-piperaquine (DP).

The study subjects consisted of patients, age from 6 months to 60 years who were confirmed to be malaria positive and satisfied the inclusion and exclusion criteria for the study. Patients were randomly placed in one of the three treatment groups and given either AL, AM or DP standard doses. Patients treated with AL were followed-up for 28 days, while those treated with either AM &DP were followed up for 42 days. The fever and parasite clearance times were determined and therapeutic efficacy of study drugs assessed with WHO approved specialised Excel software using Kaplan - Meier.

A total of 324 patients satisfied the study criteria and were enrolled. All study drugs had excellent fever and parasite clearance times. 81% of the 176 patients with fever at enrolment had their fever cleared after 24 h, with average fever clearance time of 1.44 days. 62.1% of patients had complete parasite clearance after 24 h, while mean parasite time was 1.41 days. A total of 28 (9.2%) patients had re-appearance of parasites during the study. The cumulative PCR-Uncorrected success rates for study drugs were 96.3% and 90.6% after 28 and 42 days follow-up respectively, which are lower than previously reported rates. AL, AM and DP had 91.1%, 86.2%, 94.9% individual rates respectively. It was observed that 90% of all failures were recorded in patients aged between 5 and 14 years.

In conclusion, the artemisinin based drugs are still very effective for the treatment of malaria in Nigeria, however with declining rate. Further and continuous therapeutic monitoring of the ACTs is therefore very important.

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SPUTUM VOLUME AND MYCOBACTERIAL LOAD AS CANDIDATE BIO MARKERS FOR TREATMENT EFFECTIVENESS IN EARLY BACTERICIDAL ACTIVITY STUDIES ON PATIENTS WITH PULMONARY TUBERCULOSIS

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Background: The quantification of mycobacterial load in expectorated sputum samples from patients with pulmonary tuberculosis is important for determining severity of the disease and for monitoring treatment effectiveness of anti-tuberculosis drugs. So far, the conversion of culture for *Mycobacterium tuberculosis* after 8 weeks of treatment is the only established biomarker in treatment success and the need for faster and more reliable biomarkers is obvious. In this study we investigated the hypothesis that sputum volume influences mycobacterial load.

Method: Extracted data from six early bactericidal activity (EBA) studies were obtained from a laboratory in Cape Town and retrospectively analyzed. The data for each sample included sputum volume, duration on anti-tuberculosis treatment, time to positivity (TTP) of liquid culture, and colony forming unit counts (CFU/ml) of solid culture. Multiple linear regression models were used to estimate the association of sputum volume with TTP and CFU/ml and were adjusted for the effect of repeated measures. The predictor variables used were sputum volume, duration on treatment (days), EBA study participated, and volume treatment duration interaction.

Results: Out of the 5552 sputum samples which were obtained from 439 patients,5237 CFU/ml values (converted into logCFU) and 5372 TTP values (converted into hours) were included in the analysis. The findings show that sputum specimens with a volume greater than 10 ml had an average increase in logCFU of 0.644 ($P < 0.001$) and a decrease in TTP of 1.297 h ($P < 0.001$) compared to volumes of ≤5 ml. Furthermore, TTP and logCFU values from sputum specimens with a volume of ≥10 ml had less variability compared to those with volumes of ≤5 ml.

Conclusion: Both, sputum specimen volume and duration on treatment influence the mycobacterial load quantified. We observed that participants with higher sputum volume had a higher mycobacterial load and both variables decrease with treatment duration. This suggests that sputum volume has the potential to serve as a biomarker for treatment effectiveness in patients with pulmonary tuberculosis.

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SUB-LETHAL TIGECYCLINE TREATMENTS INDUCE SOS RESPONSE AND ADEABC EFFLUX PUMP IN *ACINETOBACTER BAUMANNII*

Ling B.

Background: The growing incidence of multidrug resistance in Acinetobacter baumannii is an emerging challenge in the treatment of nosocomial infections, particularly in severely ill patients. Tigecycline, the first antibiotic of glycylcyclines, has favorable activity against Acinetobacter baumannii, but isolates showing reduced susceptibility have emerged in many countries. In this study, we performed research on Tigecycline activity against clinical isolates of Acinetobacter baumannii and we analyzed the expression profiles of AdeABC efflux pump genes after Tigecycline treatment, to elucidate the drug resistant genes related to the SOS response.

Methods: Minimal inhibitory concentrations (MICs) of clinical isolates were determined using Clinical Laboratory and Standards Institute (CLSI) methodologies. Antimicrobial susceptibility was ascertained according to FDA interpretive criteria. The expression of SOS genes and AdeABC efflux pump genes was determined by using a quantitative reverse-transcription polymerase chain reaction technique before and after sub-lethal tigecycline treatments.

Results: Tigecycline was active against more than 90% of 32 *Acinetobacter baumannii* isolates. Significant ($P < 0.05$) increases in SOS genes were found after tigecycline treatments. The SOS response related gene changes showed a significant correlation with the expression profiles of AdeABC efflux pump genes.

Conclusions: Tigecycline can induce *Acinetobacter baumannii* SOS response, which stimulates the expression of AdeABC efflux pump genes.

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TB-HIV CO-INFECTION AND CONCOMITANT ANTI-TB THERAPY AND HAART INCREASES THE RISK FOR DRUG INDUCED LIVER INJURY: A PROSPECTIVE FOUR-ARM COHORT STUDY

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Objectives: To evaluate the incidence, patterns, severity and predictors of antiretroviral and/or anti-tuberculosis drugs induced liver injury (DILI) among HIV, tuberculosis or TB-HIV co-infected patients.

Methods: A total of 1060 treatment naive patients were prospectively enrolled into four treatment groups: HIV patients receiving efavirenz based HAART alone (Arm-1); TB-HIV co-infected patients with CD4 < 200 cells/μl receiving concomitant rifampicin based anti-TB and efavirenz based HAART (Arm-2); TB-HIV co-infected patients with

CD4 >200 cells/μl receiving anti-TB alone (Arm-3); TB patients taking rifampicin based anti-TB alone (Arm-4). The incidence and predictors of DILI were analyzed using Cox Proportional Hazards Model

Results: A total of 159 patients (15%) developed DILI with grades 1, 2, 3 and 4 being 53.5%, 32.7%, 11.3% and 2.5%, respectively; cholestatic, hepatocellular or mixed pattern being 61%, 15% and 24%, respectively. Incidence, pattern and severity of DILI were significantly associated with disease status and type of treatment received. Incidence of DILI was highest in Arm-2 (24.2%) >Arm-3 (10.8%) >Arm-1 (8.8%) >Arm-4 (2.9%). Concomitant anti-TB-HIV therapy increased the risk of DILI by 10-fold than anti-TB alone ($P < 0.0001$, OR = 10.8). HIV co-infection increased the risk of anti-TB DILI by 4-fold ($P = 0.004$, OR = 4.2). HAART associated DILI was 3-fold higher than anti-TB alone, ($P = 0.02$, OR = 3.0). HAART was associated with cholestatic and grade 1 DILI where as anti-TB therapy was associated with hepatocellular and grade ≥2 DILI. Treatment type, lower CD4, platelet, hemoglobin and higher AST and direct bilirubin at baseline were significant DILI predictors.

Conclusion: HAART associated DILI is mainly cholestatic and mild while hepatocellular or mixed pattern with high severity grade is more common in anti-tuberculosis DILI. TB-HIV co-infection, disease severity and concomitant treatment exacerbates the risk of DILI.

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THE ANTIBIOTIC RESISTANCE PATTERN IN THE PAEDIATRIC INTENSIVE CARE UNIT OF UNIVERSITAS ACADEMIC HOSPITAL IN SOUTH AFRICA

Van Wyk R., Walubo A.

Background: Antibiotic resistance is a major problem in all settings where antibiotics are regularly used, especially in intensive care units. Therefore, the aim of this study was to describe the pattern of antibiotic resistance in the Paediatric Intensive Care Unit of Universitas Academic Hospital (PICU-UAH).

Methods: This was a retrospective study of patients admitted to the PICU-UAH from 1998 to 2007. Data collected included admission information, patient demography, problems on admission and during stay in the PICU-UAH, culture and sensitivity, and antibiotics used.

Results: The top ten bacteria accounted for 91.8%, viz; *Staphylococcus* (29.3%), *Klebsiella* (11.8%), *Acinetobacter* (11.7%), *Pseudomonas* (11.2%), *Escherichia* (8.5%), *Enterococcus* (5.9%), *Streptococcus* (4.1%), *Enterobacter* (4.1%), *Stenotrophomonas* (3.4%) and *Haemophilus* (2%). The majority (58.7%) of the bacteria cultured were Gram-negative and these were mainly from tracheal aspirates (90.1%), while Gram-positive bacteria were mainly from blood (73.2%). *Staphylococcus* exhibited high resistance to all penicillins with no resistance to vancomycin. *Klebsiella* and *Pseudomonas* exhibited resistance to some aminoglycosides, cephalosporins and penicillin, but *Klebsiella* remained sensitive to imipenem. *Acinetobacter* and *Stenotrophomonas* were highly resistant (>70%) to almost all antibiotics, except tobramycin for *Acinetobacter* and co-trimoxazole for *Stenotrophomonas*.

Conclusions: It has been shown that the pattern of bacterial resistance in the PICU-UAH depicts increasing resistance by well-known bacteria to the most commonly prescribed antibiotics, implying that acquired resistance is the most prevalent mode of resistance.

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THE ANTIRETROVIRAL AGENTS AND PRO-INFLAMMATORY ACTIVATION OF RAT MICROGLIA VIA AN ARGINASE-DEPENDENT MECHANISM

Lisi L.

Despite the introduction of the Highly Active Antiretroviral Therapy (HAART), has significantly increased life expectancy of HIV seropositive patients, approximately 50% of HIV infected patients show signs and symptoms of neurological complications. This may result from a combination of factors such as reduced effectiveness of HAART in the central nervous system reservoir, concurrent illnesses, adverse effects associated with treatments, including antiretrovirals (ARVs). With this in mind, in the present study we carried out a screening of different AVRs (Atazanavir, Darunavir, Lopinavir, Indinavir, Ritonavir, Efavirenz, Nevirapine, Abacavir and Tenofovir) for their potential pro-inflammatory effects on microglial cells. As a marker of microglial activation, we measured the expression and activity of NOS2, and analyzed in parallel the production of reactive free radicals. When microglia were activated with $Gp120_{CN54}$ and IFNγ, some drugs, namely Efavirenz, Neviparine, Darunavir and Atazanavir increased NO production. Interestingly, the drugs found able to increase NO production also reduced the intracellular levels of urea, taken as a marker of arginase activity. Take together these data suggest that ARG is an additional molecular target of different ARVs, whose inhibition can contribute to their pharmacological activity as well as explain the neurotoxic potential.

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THE COPPER CHELATING AND ANTIMALARIAL ACTIVITY OF 8-HYDROXYQUINOLINE DERIVATIVES

Jansen Van Vuuren N., van Zyl R.

Background: A growing resistance to antimalarials by *Plasmodium falciparum* demands the development of new classes or combinations of antimalarial agents. Copper is essential in the metabolism of malaria and infected individuals have a higher serum copper concentration than uninfected individuals. Depriving the parasite of this metal has the potential to result in cell death. In this study, the copper chelating properties of 8-hydroxyquinoline derivatives were correlated with their *in vitro* antimalarial activity.

Methods: Twenty-one 8-hydroxyquinoline derivatives were tested for their ability to chelate copper (I) chloride or copper (II) sulphate, with neocuproine and D-penicillamine, respectively used as the positive controls. The haemolytic protective properties of the compounds against high concentrations of coppers (I) and (II) was determined. The tritiated hypoxanthine incorporation assay was used to measure the sensitivity of the 3D7 parasite strain to the derivatives compared to chloroquine and quinine. To elucidate an additional mechanism of action, the compounds were evaluated for their ability to inhibit parasitic haemozoin formation using the ferriprotoporphyrin biomineralisation inhibition assay.

Results: All the derivatives displayed varying degrees of copper chelating ability which correlated with their *in vitro* antimalarial activity ($r^2 = 0.82$), but did not correlate with its ability to inhibit haemozoin formation ($r^2 = 0.46$). The derivatives preferentially chelated copper (I) than copper (II) ($r^2 = 0.98$). The most active derivative was N-butyl-2,2-imino-di-(8-OH) with an IC_{50} value of 34.81 ± 6.76 μM for copper (I) chelation; 23.24 ± 4.46 μM for the haemozoin formation inhibition (chloroquine control, IC_{50}: 22.91 ± 3.78 μM) and 5.54 ± 0.04 μM for the *in vitro* antimalarial assay and it decreased copper (I) haemolysis by 31.2%. 8-OH was preferentially a copper (II) chelator (IC_{50}: 28.38 ± 4.87 μM) in comparison to the D-penicillamine (IC_{50}: 7.16 ± 1.49 μM) and had comparable *in vitro* antimalarial activity (IC_{50}: 14.64 ± 2.55 μM) to chloroquine (IC_{50}: 4.82 ± 0.72 μM). It was protective against copper (I) haemolysis (decreased by 37.9%), but increased haemolysis of copper (II) by 30.0%.

Conclusions: The copper chelating properties of 8-hydroxyquinoline derivatives may be useful adjuncts in the treatment of this infectious disease.

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THE EFFECT OF QUININE AND ARTESUNATE CO-ADMINISTRATION ON LIVER FUNCTION TEST OF *PLASMODIUM BERHGEI* INFECTED AND HEALTHY SWISS ALBINO MICE

Shehu-bida R., Anuka J.

Background: Artemisinin-based combination therapy is currently the recommended treatment in malaria. Quinine and Artesunate, both effective anti-malarial agents have been co-administered and found to produce better efficacy than when administered singly. Hence, their effects on the liver when co-administered needs to be investigated.
Method: *1. Malaria Parasitized*: Twenty-five mice were grouped into five each. Groups II-V were infected with malaria parasite (*Plasmodium berghei* NK65 strain) as described by David *et al.,* (2004) and Peter and Anatoli (1998). Three days post inoculation of the malaria parasite; all the Groups (I-V) were treated orally for 7 days with Normal saline (10 ml/kg), Normal saline (10 ml/kg), Quinine (60 mg/kg), Artesunate (30 mg/kg) and Quinine-Artesunate respectively.
2. Non-Malaria Parasitized: Twenty eight mice were grouped into four of seven mice each; Group I-IV were treated orally for 7 days with Normal saline (10 ml/kg), Quinine (60 mg/kg), Artesunate (30 mg/kg) and Quinine-Artesunate (60 and 30 mg/kg) respectively.
On day 8, the animals were euthanized and their livers homogenized and centrifuged (3000 rpm for 15 min). The supernatants were assayed for liver function tests (Alanine transaminase-ALT, Aspartate transaminase-AST, and Alkaline phosphatase-ALP). Results were analyzed using ANOVA and Dunnett t-test.
Result: Quinine-Artesunate co-administration group (1440.00 ± 60.43) IU/L showed significant reduction ($P < 0.05$) in the ALT concentration level when compared to the quinine group (1652.80 ± 23.96) IU/L and an insignificant decrease when compared with the Artesunate and infected (untreated) group. ALP of Quinine-Artesunate co-administration didn't show any significant difference when compared with groups administered Normal saline, Quinine and Artesunate. However, Quinine-Artesunate co-administration showed a significant increase in AST when compared with normal control (group I).
In the second study, Quinine-Artesunate co-administration did not show any significant difference on the liver function test as compared with groups administered Normal saline, Quinine and Artesunate.
Conclusion: In this study, Quinine-Artesunate co-administration was found to show a potentially reduced ALT concentration level of the mice when compared with groups infected and administered Normal saline, Quinine and Artesunate. Quinine-Artesunate co-administration has almost the same relative liver-body weight with the normal control (group I). However, in the second study, the co-administration showed no significant effect on the liver function test of the mice.

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THE METABOLISM OF 8-AMINOQUINOLINES IN RELATION TO THEIR EFFICACY AND SAFETY

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The 8-aminoquinoline (8-AQs) antimalarial drugs, of which primaquine (PQ) is the prototype, have been in use for 60 years. Their utility is unique because of the spectrum of activity, especially the efficacy against liver hypnozoites and late stage gametocytes. But the mechanism of action is still not understood, and to date the class is plagued with a peculiar safety issue: hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Both the efficacy and toxicity seem related to active metabolites, but in a fashion and by mechanisms that are not yet clear. Recent investigations at our laboratory and with our collaborators have shed some light on these issues. It is now known that the metabolism and transport/distribution of the 8-AQs behave in a stereo-selective fashion. PQ is used as a racemic mixture, and since the metabolic and pharmacokinetic profiles differ, this may have implications for the toxicity and therapeutic efficacy. However, the ultimate impacts of this for potency and safety of the drugs in humans are still not clear. Recent studies have highlighted the complexity of metabolism of the 8-AQs, which are substrates for amine oxidases and cytochromes P450 (CYPs). Metabolic activation by the CYP 2D family appears to be a requisite for efficacy of PQ and analogs. However, the active metabolites have not yet been identified. It still remains unclear what metabolites and pathways mediate the hemolytic toxicity of the 8-AQ drugs, and whether structural modifications or inhibition of certain CYPs can modulate metabolism to afford a better "therapeutic index" for the 8-AQs. Our findings to date on PQ metabolism suggest dramatic species differences, and have developed more relevant animal models for 8-AQ-associated hemolysis. Computational studies of 8-AQ/CYP interactions, and experiments on pathway inhibitors in human hepatocytes suggest strategies that may mitigate the toxicity of PQ or other 8-AQs. In addition, a number of insights from other newer structural analogs of PQ have been developed.

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THE PARENTERAL ARTESUNATE ACCESS INITIATIVE

Visser Kift E., Barnes K.

Background: Severe malaria is a medical emergency that requires prompt treatment to prevent death. A cochrane review showed that intravenous (IV) artesunate, compared to IV quinine, reduce in-hospital mortality by 39% in adults and 24% in children with severe malaria. Therefore, the World Health Organization strongly recommends intravenous (IV) artesunate as first line treatment for severe malaria. In South Africa (SA) high malaria case fatality rates persists despite the substantial reduction in malaria incidence. However, IV artesunate is not yet registered for use and accessible to severe malaria patients.
Methods: The Parenteral Artesunate Access Programme was approved by the SA Medicines Control Council (MCC) under Section 21 of the Medicines and Related Substances Act and launched in January 2010. The programme secretariat and central pharmacy is based at the University of Cape Town's Division of Clinical Pharmacology, Groote Schuur Hospital. Artesunate stock is procured from Guilin Pharmaceuticals (China), importation authorised and quality assurance performed. Sentinel hospitals are enrolled, trained and provided with stock. For each eligible patient, informed consent and MCC approval is obtained and case record forms is completed. Six-monthly programme progress reports are submitted to the MCC.
Results: To date, 462 patients (294 males) at 31 hospitals in seven provinces in SA have received IV artesunate. The mean (SD) age was 33.7 years (16.2). Malaria complications present at admission included impaired consciousness (n = 220), respiratory distress (n = 81), hyperparasitaemia (n = 238), hyperbilirubin (n = 148), renal impairment (n = 115), acidosis (n = 51), hyperlactataemia (n = 43), macroscopic haematuria (n = 24) and abnormal bleeding (n = 15). Of these acidosis, renal impairment, hyperlactataemia, impaired consciousness, macroscopic haematuria and abnormal bleeding were significantly associated with death ($P = 0.02$ to <0.0001). Of the 413 patients for whom we have received reports of outcome 321 (77%) were well on discharge, 59 (14%) not fully recovered, and there were 31 deaths (7.5%). Only two serious adverse events were reported.
Conclusion: The Parenteral Artesunate Access Initiative results describe the safe, effective use of parenteral artesunate for severe malaria patients in SA and supports continued access via the programme until its registration is completed.

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THE PATTERN OF ANTIBIOTIC USE IN THE PAEDIATRIC INTENSIVE CARE UNIT OF UNIVERSITAS ACADEMIC HOSPITAL (PICU-UAH) IN SOUTH AFRICA

Van Wyk R., Walubo A.

Background: Despite the existence of prescribing guidelines, appropriate antibiotic use remains a major challenge to all intensive care units. Knowledge of the pattern of antibiotic use is important to develop better strategies for rational antibiotic use in a particular setting. Therefore, the aim of this study was to describe the pattern of antibiotic use in the Paediatric Intensive Care Unit of Universitas Academic Hospital (PICU-UAH).
Methods: This was a retrospective study of patients admitted to the PICU-UAH from 1998 to 2007. Data collected included admission information, patient demography, problems on admission and during stay in the PICU-UAH, culture and sensitivity, and antibiotics used.
Results: There were 685 patients in whom 38 different antibiotics were prescribed at an average rate of 24.1 ± 2.5 per year. Broad-spectrum bactericidal antibiotics were more preferred and narrow-spectrum bactericidal antibiotics were used for specific indications. The top ten antibiotics accounted for 81.2% of antibiotic usage, of which 52.6% was for the top three (cefotaxime, amikacin and vancomycin). Regarding the phases of admission, 29 ± 5.8% patients were on antibiotics on admission, 79.9 ± 3.3% used antibiotics within the first 3 days, and 23.2 ± 4.6% had their antibiotics modified after 3 days. The top antibiotics used on admission and within the first 3 days were similar, but differed from those used after 3 days.
Conclusions: It has been shown that the pattern of antibiotic use in the PICU-UAH depicted a disproportionate utilization of a few older broad-spectrum and narrow-spectrum bactericidal antibiotics, mostly as combination regimens and according to the phase of admission.

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THE USE OF THE 'QUEUING THEORY' AND PATIENT BASED CHARACTERISTICS TO ASSESS THE PERFORMANCE OF THE PAEDIATRIC INTENSIVE CARE UNIT (PICU) AT UNIVERSITAS ACADEMIC HOSPITAL IN SOUTH AFRICA

Van Wyk R., Walubo A.

Background: The performance of an intensive care unit (ICU) is the prompt admission of patients with specific conditions, and institution of appropriate management leading to expected outcomes within the expected time. Such performance is influenced by patient, institutional and environmental based characteristics. Patient based characteristics include the severity of the condition, complications developed, disease pattern, number of patients, and age, etc. Institutional based characteristics include human resources, structure of the health facility, equipment, supplies, and the nature of the ICU, while environmental based characteristics refer to the social-economic status and the health system of the country. Assessment of ICU performance generally involves selection of appropriate indicators in patient, institutional and environmental based characteristics, and their application in relevant mathematical models. Unfortunately, these models have tedious requirements and are more accurate in settings similar to those they were developed. Here, a simple method using the 'queuing theory' and patient based characteristics to gauge the performance of the Paediatric Intensive Care Unit (PICU) is described.
Methods: This was a 10 year retrospective study to determine the queuing nature and patient based characteristics in the PICU using records of patients who were prescribed antibiotics from January 1998 to December 2007. The daily arrival rates and length of stay were used in the queuing simulation formula to derive the appropriate parameters.
Results: Sixty-three percent of the PICU was utilised by patients who stayed for 7.48 ± 6.77 days. It admitted mainly children and infants who presented with low body weight, a variety of medical and surgical problems, and experienced many complications while in the PICU. These patients were successfully managed, whereby most patients improved, leading to a low mortality rate.
Conclusions: The queuing theory was successfully used to evaluate the performance of the PICU and to recommend appropriate remedial measures, and this should be applicable to other ICUs.

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THERAPEUTIC AND CHEMOPROPHYLACTIC POTENTIAL OF SOME MICRONUTRIENTS IN MALARIA

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Objectives: Although nutrition has been suspected to influence the course of malaria, the exact role of micronutrients in malaria therapeutics is still not clearly defined. In the present study, an *in vivo* evaluation of the role of some micronutrients in the treatment and chemoprophylaxis of malaria was elucidated using rodent malaria model of *Plasmodium berghei* NK-65 strain.
Methods: One hundred and sixty Swiss albino mice of either sex weighing 20.05 g were inoculated intraperitoneally with 10 million *Plasmodium berghei* infected erythrocyte. The timing of the inoculation varied depending on the stage of the study. The study involved a 4 day supressive test, a 4 day curative test and a repository test in which test agents (vitamin A 60 mg/kg, vitamin E 100 mg/kg, vitamin C 200 mg/kg, zinc 100 mg/kg and selenium 1 mg/kg) were administered daily.
Results: Micronutrients in this study exhibited significant schizonticidal activity in the early phase of *Plasmodium berghei* infection ($P < 0.05$) which was most marked in selenium treated group (82.01%) and insignificant in vitamin C treated group when compared with control ($P > 0.05$). Mean parasitemic levels after 4 days curative treatment was more significant in the selenium (5.82%) and vitamin A (9.95%) treated groups respectively when compared with the negative control group and between micronutrient treated groups ($F = 7.04$; $P < 0.05$). In the 4 day curative test, there was an initial decline in parasitemia after the first day of treatment in the selenium group. Significant repository activity ($P < 0.05$) occured in the vitamin A, E, zinc and selenium treated groups when compared with negative control. However, synergistic shizonticidal activity was more marked with the vitamin A + E combination therapy (94.52%) when compared with any other micronutrient combination after 4 days treatment of established infection. The decline in parasitemia was sustained in the vitamin A + E treated group till the 4th day post treatment when compared to the chloroquine treated group.
Conclusion: The study has demonstrated that vitamin A, E, selenium and zinc may be beneficial as adjuvants in malaria treatment particularly when used in combination.