Analysis of unique and specific genetic markers for diagnosis of antibiotic resistant, pathogenic escherichia coli (E. coli) encoding resistance to the third generation antibiotic, cefotaxime

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Abstract

Among the wide range of antibiotic available, beta-lactam antibiotics are used widely in bacterial pathogenic infections. The most common cause of bacterial resistance against beta-lactam antibiotics is the production of beta-lactamases by many members of Enterobacteriaceae family, including especially, E. col. (Harwalkar, Sataraddi et al. 2013). Extended-spectrum Î²-lactamases (ESBLs) are resistant to the third generation antibiotics, cephaloosporins and monobactum, additionally, these ESBL producing organismsâ™ exibit co-resistant to many other classes of antibiotics resulting limited treatment alternatives (Paterson, Bonomo 2005, Lartigue, Zinsius et al. 2007, Lewis, Herrera et al. 2007). Over the past decade, it has been observed dramatic increase of these ESBL producing E. coli strains, especially, CTX-M family of ESBL producers, to the third-generation cephalosporin antibiotic, Cefotaxime (Bonnet 2004, Lartigue, Zinsius et al. 2007, Paterson, Hujer et al. 2003, Paterson, Bonomo 2005, Ruppe, Lixandru et al. 2013). Although studies have published the diagnosis of various ESBL producers in worldwide, only few reports of molecular identification of CTX-M family of genes have been published (Al-Mayahie 2013, Harwalkar, Sataraddi et al. 2013, Literacka, Bedenic et al. 2009). This study aims to identify the DNA segments of CTX-M genes unique to E. coli, and analyse the surrounding genetic makeup of CTX-M genes. Collectively, understanding the genetic makeup of these resistance genes will shed a light on their mechanistic of antimicrobial resistance. Unlike other conventional clinical diagnostic methods, molecular diagnostics seeks evidence of a disease at the very basic causative level by detecting the nucleic acid identity. The main advantage of this diagnostic method is that the diagnosis carried out in the molecular level, and the method will identify the microbial pathogens at the early stages of the infectious diseases (Versalovic, Lupski 2002). Different types of rapid detection systems such as Real-Time PCR have been used to identify the different CTX-M family target genes (Birkett, Ludlam et al. 2007, Li, Chen 2012, Versalovic, Lupski 2002, Woodford, Fagan et al. 2006, Woodford, Sundsfjord 2005). The identified genes so far, has some degree of potency for identification, but the majority of these genes lacks the characteristics of a strong and unique genetic marker that can used as a specific target solely for identification of any CTX-M family of ESBL producing E. coli strains. Therefore this study analyse short DNA segments and develop a sensitive, specific and reliable method that can be used for rapid selective detection of ESBL producers resistant to Cefotaxime in antibiotic resistant E. coli strains.

Luminescent dual sensors reveal extracellular pH-gradients and hypoxia on chronic wounds that disrupt epidermal repair

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Abstract

Wound repair is a quiescent mechanism to restore barriers in multicellular organisms upon injury. In chronic wounds, however, this program prematurely stalls. It is known that patterns of extracellular signals within the wound fluid are crucial to healing. Extracellular pH (pHe) is precisely regulated and potentially important in signaling within wounds due to its diverse cellular effects. Additionally, sufficient oxygenation is a prerequisite for cell proliferation and protein synthesis during tissue repair. It was, however, impossible to study these parameters in vivo due to the lack of imaging tools. Here, we present luminescent biocompatible sensor foils for dual imaging of pHe and oxygenation in vivo. To visualize pHe and oxygen, we used time-domain dual lifetime referencing (tdDLR) and luminescence lifetime imaging (LLI), respectively. With these dual sensors, we discovered centripetally increasing pHe-gradients on human chronic wound surfaces. In a therapeutic approach, we identify pHe-gradients as pivotal governors of cell proliferation and migration, and show that these pHe-gradients disrupt epidermal barrier repair, thus wound closure. Parallel oxygen imaging also revealed marked hypoxia, albeit with no correlating oxygen partial pressure (pO2)-gradient. This highlights the distinct role of pHe-gradients in perturbed healing. We also found that pHe-gradients on chronic wounds of humans are predominantly generated via centrifugally increasing pHe-regulatory Na+/H+-exchanger-1 (NHE1)-expression. We show that the modification of pHe on chronic wound surfaces poses a promising strategy to improve healing. The study has broad implications for cell science where spatial pHe-variations play key roles, e.g. in tumor growth. Furthermore, the novel dual sensors presented herein can be used to visualize pHe and oxygenation in various biomedical fields.

Integrated testing strategy of pharmaceutical intermediates for occupational health

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Abstract

In order to assess the occupational health hazard of intermediates (IM), hazard data were traditionally generated through experimental animal testing, which are more and more replaced by alternative methods considering animal welfare and costs. Indeed, there are a number of other ways to assess the health hazards: comparing substances with similar structures, grouping them together into logical categories, doing specialized computer modelling, using weight of evidence approach (WoE) and integrated testing strategy (ITS). We have evaluated the current state of scientific knowledge from the literature and from in silico evaluation tools. We have proposed new ITS for Novartis Pharma own pharmaceutical IM for occupational health purposes which defines the tiered approach in performing in silico, in vitro and in vivo studies which support risk management to prevent workplace diseases that could be triggered by IM and reduces the number of in vivo tests required.

Bilateral wrist robot-assisted training in chronic hemiplegic stroke: A pilot study

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Abstract

Background: The stroke population needing physical rehabilitation of the upper extremity is constantly increasing. To solve this problem, robot devices for improving motor performance are developed to assist physical rehabilitation in Korea. The aim of this study was to develop the robot-assisted wrist training system and to apply this to the chronic hemiplegic stroke participants.
Methods: Bilateral wrist robotic device was developed and we applied this to four chronic hemiplegic stroke participants. Robot-assisted wrist

training system was assessed by Hand grip force, Medical Research Council, Range of motion, Upper extremity Fugl-Meyer Assessment, and Motor Activity Log. In addition, we measured functional magnetic Resonance Imaging (fMRI) analysis for cortical reorganization during both hand movement. Participants received 60 min, 20 sessions, five days a week, for four consecutive weeks. The assessments were done before and after 20 training sessions

Results: Robot-assisted wrist training system was built with the bilateral wrist flexion/extension and pronation/supination performance that provides a repetitive active-active, active-passive, and passive-passive mode. Muscle strength, Motion of wrist, and motor function were enhanced after 4-week training of wrist. And the cortical activity change was associated with inducing reorganization of motor cortex networks

Conclusion: We developed bilateral wrist robotic device and robot-assisted wrist training system. Robot-assisted wrist training system showed improvement of upper limb function after training of wrist. Our system might be efficient robot-assisted wrist training for improving muscle strength, motor performance, and cortical reorganization in patients with chronic hemiplegic stroke and contribute to the development of translational research in rehabilitation robot.

Development of an "In Situ" renal perfusion system to study the origin of urinary biomarkers in a nephrotoxicity model induced by gentamicin

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Abstract

Background and Aims: Gentamicin is an aminoglycoside antibiotic widely used for the treatment of many infectious diseases. Its main side effect is its nephrotoxicity, which occurs in 10-25% of therapeutic courses, despite proper monitoring and hydration of patients, can lead to acute kidney injury (AKI). We have previously demonstrated that urinary damage markers like albuminuria increased as a consequence of the addition of gentamicin. In the present work we aimed at specifically studying the renal handling of albuminuria in a nephrotoxicity model, through in situ renal perfusion experiments.

Methods: Male Wistar rats were administrated by a single dose of gentamicin (150 mg/kg), or not. After 5 days, rats were anesthetized and an extracorporeal circuit for kidney perfusion was set up. The renal artery, vein and ureter of the right kidney were ligated. The renal artery of the left kidney and the urinary bladder were canulated. A catheter was placed in the right carotid artery and connected directly to the renal artery. Urine was continuously collected from a catheter placed in the urinary bladder at 10 min intervals. After 1 h of renal perfusion with blood from the carotid artery, oxygenated and warm (37 °C) Krebs-dextran (40 g/L of dextran) was perfused through the renal artery at 3 mL/min, and was discarded through the renal vein. Albuminuria was measured in the different urine fractions.

Results: From the second day after gentamicin administration, albuminuria was significantly increased, as compared to control rats, in which urinary markers were undetectable. When exogenous Krebs solution prefunding the kidney, neither gentamicin rats nor control rats excreted albuminuria. As a control of the perfusion experiments, urinary markers still appeared in the urine in gentamicin rats whose kidney was perfused with its own blood. However, albuminuria was undetectable in control rats.

Conclusions: Our results support the idea that excess albumin found in the urine (albuminuria) as a consequence of treatment with gentamicin comes from the blood ultrafiltrate reaching Bowman's capsule and not from the renal parenchyma. More interestingly, our results provide and in situ method to test the origin of urinary biomarkers in different conditions.