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Sensor array detection of malaria volatile signature in a murine model

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ABSTRACT

The relationship between malaria infection and volatile compounds has been claimed mainly on the basis that they are believed to be an attractant for mosquitoes. However, since the association of emitted molecules with diseases has been observed for many pathologies, malaria-related volatile compounds are a potential diagnostic tool. The recent confirms of this hypothesis prompts the development of sensors for an effective exploitation of these potentialities.

On these bases, we investigated the alteration of volatile compounds in a malaria murine model. For the scope, the total "volatilome" of Plasmodium berghei-infected mice was compared with that of non-infected animals. Gas chromatographic analysis of the sampled air reveals the existence of a pattern of compounds that, collectively considered, detects malaria infection. Finally, an array of porphyrins functionalized quartz microbalance gas sensors was applied to sort non-infected from infected mice. The application of a classification model to the sensor data provided more than 80% of correct identification with errors confined to mice with a low parasitemia level. Noteworthy, the sensor array was trained on data collected months before to run the tests. These results provide, although limited to a murine model, a first evidence of the potentialities of gas sensor technology for malaria diagnosis.

1. Introduction

Malaria remains a worldwide health problem, with 214 million new cases in 2015 and 438000 deaths, most of which in Africa. During the past 15 years, malaria case incidence and mortality rate considerably decreased thanks to vector control, chemoprevention and early diagnosis and treatment of infections [1]. However, the decline of malaria prevalence is leading to a significant increase of low density and subclinical infections that require detection tools more sensitive and accurate than those currently available. Malaria is conventionally diagnosed by microscopic examination of stained blood samples or rapid diagnostic tests (RDTs) based on parasite antigen/enzyme identification. However, these gold standard methods have a limited sensitivity. Alternative molecular diagnostic methods, based on nucleic acid detection, are highly sensitive but expensive, and require trained personnel [2]. In any case, malaria diagnostic tools need collection of blood samples with several disadvantages: poor compliance of patients, due to fear or blood taboos of some communities and risk increase of accidental infections [3]. Therefore, the development of sensitive, non-invasive, easy-to-use and low-cost diagnostic devices is essential to improve malaria control.

Plasmodium parasite is transmitted through the bite of an infected mosquito. Several studies on human malaria and rodent models, showed that infection increases attractiveness to mosquitoes, probably due to the emission of volatile organic compounds (VOCs) [4,5]. The source of these VOCs should be traced back to the parasite itself, or to metabolic interaction between parasite and host.

The detection of volatile metabolites as indicators of diseases is becoming a popular research theme [6]. In general, pathological states that influence the metabolism elicits an alteration (quantitative and qualitative) of VOCs.

Analysis of VOCs emitted by the human malaria parasite Plasmodium falciparum in culture produced divergent results with

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negative [7] and positive [8] detection of malaria-related VOCs. In vivo studies, were conducted on global emission of VOCs by Plasmodium chabaudi infected mice [5]. Gas chromatography (GC) results showed that alteration of VOC profile due to the disease progression led to increased attractiveness to mosquitoes. Altered VOC profiles were also detected by GC analysis of breath samples collected on volunteers, before and after malaria infection [9].

Collectively these results suggest that host response is involved in the production of disease-specific VOCs. All these findings form the ground for the application of gas sensor arrays. Indeed, arrays of partially selective sensors can brilliantly discriminate among samples characterized by different profiles of VOCs [10]. Such sensor arrays are inspired by the natural olfaction where millions of odors can be distinguished by few hundred of kinds of olfactory receptors, even if their differences are tiny [11,12].

In this paper, we applied GC and sensor array technologies to study the VOCs released by healthy mice and mice infected by the rodent malaria parasite Plasmodium berghei. The gas sensor array was composed of metalloporphyrins-coated Quartz Microbalances (QMB) of the same kind of those previously used in diverse medical applications [13], among them lung cancer by breath analysis [14,15].

In line with previous experiments, GC identified a subset of infection-related compounds. Interestingly, the sensor array was able to detect malaria infection in mice over a wide range of parasitemia. These experiments open the perspective use of sensor technology for malaria diagnosis.

2. Experimental

2.1. Animals and parasites

Female CD1 mice (6–8 weeks old) were kept in the animal facility of the Istituto Superiore di Sanità (National Institute of Health). The animal work has been approved by the Service for Biotechnology and Animal Welfare of the Istituto Superiore di Sanita and has been authorised by the Italian Ministry of Health, according to the Legislative Decree 116/92, which implemented in Italy the European Directive 86/609/EEC on laboratory animal protection.

Mice were inoculated by intraperitoneal injection of erythrocytes infected by the wild type Plasmodium berghei cloned line 8417HP or the isogenic transgenic line constitutively expressing the green fluorescent protein, under the control of P. berghei HSP70 promoter (plasmid pBAT-SIL6, kind gift of dr. Taco Kooij). In all experiment a group of non-infected mice was used as a control.

2.2. Determination of parasitemia

Parasitemia was evaluated by microscopic inspection of Giemsa stained blood smears and by flow cytometry (FACS calibur cytometer) when fluorescent parasites were used. Cells were gated according to FSC-H/SSC-H parameters to exclude debris and 106 events were acquired.

2.3. Volatile compounds sampling

In order to sample the VOCs released by mice, each animal was kept in a stainless steel box (30 cm × 30 cm × 35 cm) during the measurement. The box was closed with a Poly(methyl methacrylate) lid equipped with a connector allowing for the insertion of either a Solid Phase Micro-Extraction (SPME) sampler or the gas sensor array sampling tubes.

Mice were individually kept in the box for 15 min to establish a constant headspace. The box was designed to collect the total mouse volatilome integrating the different sources such as skin, breath, urine, and faeces.

The sampling box was wide enough to allow the animal a normal physical activity.

2.4. Gas chromatography–mass spectrometry analysis

SPME has been used to preconcentrate the VOCs released by the mouse in the box headspace. The SPME fibre was 50/30 μm Divinylbenzene/Carboxen/PDMS (SUPELCO, Bellefonte, PA, USA). The fibre was kept in the sampling box for 1 h.

The sampled SPMEs were measured over three hours following the collection with a GC–MS instrument (Shimadzu GCMS-QP2010, Kyoto, Japan).

From SPME fibre VOCs have been desorbed into GC injection port at 250 °C for 3 min, in splitless mode. The VOCs were separated on EQUITY-5 capillary column poly (5% diphenyl/95% dimethyl siloxane) phase –(30 m length × 0.25 mm I.D. × 0.25 μm thickness, SUPELCO, Bellefonte, PA, USA) using an initial oven temperature of 40 °C for 5 min, first increased by 7 °C/min to 220 °C, afterwards ramped by 15 °C/min to 300 °C, then held for 3 min (total runtime: 39 min).

Ultra-high purity helium has been used as carrier gas, working in linear velocity constant mode, with a pressure of 25 kPa, total flow of 5.9 mL/min, column flow of 0.7 mL/min and linear velocity of 30.2 cm/s. The GC–MS interface and the ion source were kept at a constant temperature of 250 °C.

Mass spectrometer was a single quadrupole mass analyzer operating in electron ionization mode. Full scan spectra were recorded in a mass range of 40–450 amu. The detector voltage was set at 0.7 kV.

GC–MS data were analyzed using the section GCMS post-run analysis of the GCMS solutions software (version 2.4, Shimadzu Corporation), peaks were aligned with a matlabe routine in-house developed. The compounds identification was carried out using NIST 127 and NIST 147 mass spectral libraries.

2.5. Gas sensor array

The sensor array was an ensemble of eleven quartz microbalance (QMB) gas sensors. In these sensors, a slight mass change (Δm) on the quartz surface results in frequency changes (Δf) of the electrical output signal of an oscillator circuit at which each sensor is connected. The quantities Δm and Δf are linearly proportional in the low-perturbation regime [16]. QMBs had a fundamental frequency of 20 MHz, corresponding to a mass resolution of the order of a few nanograms.

The sensing materials were solid-state layers of porphyrins a corroles. Table 1 lists the name of the molecules, the acronyms used in the text, and references to the preparation literature methods.

Thin films of the sensing materials were deposited by spray coating on both the sides of quartz disks, from 10^{-3} M solutions of porphyrins in $CHCl_3$. For each sensor, the total coating resulted in a frequency shift of approximately 30 KHz.

The sensor system used in these experiments was the last version of a series of instruments designed since 1996 at the University of Rome Tor Vergata. It may accommodate up to twelve Quartz Microbalances (QMBs) in a measurement cell whose volume is approximately 8 cm^3. The gas sensors are complemented by temperature and relative humidity sensors. Each QMB is connected to an oscillator circuit, the frequencies of the oscillators outputs are measured taking advantage of a temperature compensated reference quartz that allows for a frequency resolution of 0.1 Hz. Electronics is implemented in a FPGA. Gaseous samples delivery is controlled by a miniature diaphragm pump (0–200 sccm).The instrument is connected and powered via a single USB connection. Functions and the data acquisition are controlled with a in-house software running in Matlab.