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Peripheral fibroblast metabolic pathway alterations in juvenile rhesus monkeys undergoing long-term fluoxetine administration

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Abstract
We report on biochemical pathways perturbed upon chronic fluoxetine administration to juvenile macaques using global metabolomics analyses of fibroblasts derived from skin biopsies. After exposure to tissue culture conditions confounding environmental factors are eliminated and identification of metabolites whose levels are affected by the drug become apparent with a better signal-to-noise ratio compared to data obtained from plasma and cerebrospinal fluid (CSF). Levels of more than 200 metabolites were analyzed to interrogate affected molecular pathways and identify biomarkers of drug response. In addition, we have correlated the metabolomics results with monoamine oxidase (MAOA) genotype and impulsivity behavioral data. Affected pathways include Purine and Pyrimidine metabolisms that have been previously implicated to contribute to neuropsychiatric disorders.

Abbreviations: 5HTTLPR, Serotonin transporter length polymorphic region; AICAR, 5-Aminoimidazole-4-carboxamide ribonucleotide; ASD, Autism spectrum disorders; CSF, Cerebrospinal fluid; DMEM, Dulbecco's Modified Eagle's Medium; FBS, Fetal bovine serum; FST, Forced swim test; KNN, *k* nearest neighbors; MAOA, Monoamine oxidase A; MDD, Major depressive disorder; NHP, Non-human primates; SSRI, Selective serotonin reuptake inhibitor; WGTA, Wisconsin general test apparatus

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1. Introduction

Research on the molecular mechanisms that govern higher brain function, disease and drug action in the CNS requires non-human primates (NHP) which exhibit similar brain function and structure as humans. The long postnatal period during which the primate brain matures provides an appropriate animal model for investigating juvenile brain development and its response to drugs. Juvenile macaque monkeys, the most common laboratory NHP, are becoming a preferred model for studying short- and long-term effects of psychoactive agents used in children (Popke et al. 2001; Patterson et al., 2010; Rodriguez et al., 2010; Soto et al., 2012; Shrestha et al., 2014; Golub et al., 2015).

Several clinical studies have found a wide individual variability in children's response to fluoxetine and other psychopharmacologic agents. These drugs are commonly prescribed for a variety of childhood diseases with neurobehavioral symptoms including major depressive disorder (MDD) and autism spectrum disorders (ASD) (Henry et al., 2012; Geller et al., 2001; Birmaher et al., 2003; Nilsson et al., 2004; Hollander et al., 2005; Hetrick et al., 2007, 2010; Quintana et al., 2007; Strawn et al., 2015). As in other areas of medicine the prediction of response to treatment in childhood pharmacotherapy is critical for an optimal therapeutic response with minimal adverse side effects. In the current study we have attempted to address this issue by examining peripheral metabolome biosignatures in response to fluoxetine administration. In other studies focused on the molecular pathology of mental disorders cultured fibroblast cells obtained from patient skin biopsies have already been successfully used (Gassó et al., 2014; Kálmán et al., 2014). Following up on a previous study where we analyzed plasma and cerebrospinal fluid (CSF) specimens, the focus of the current investigation was fibroblasts obtained from rhesus monkey's skin biopsies as a novel source for the identification of metabolomic markers and perturbed molecular pathways associated with juvenile fluoxetine administration.

Blood and CSF are the most common sample types for biomarker development for psychoactive agents since they are readily available and used in the clinical laboratory. However, both fluids contain metabolites that have been produced and processed in multiple tissues and do not reflect the response of primary cellular targets of the drug. Hence they are prone to reflecting many life style and environmental influences on the metabolome.

We hypothesized that brain metabolic pathways affected by fluoxetine treatment will also reveal themselves through metabolomics analyses in other cells of the body. Skin fibroblasts can be retrieved by punch biopsy, which is a less invasive procedure and carries a much lower risk of infection compared to lumbar puncture. After replication, fibroblast cells established in tissue culture are removed from other environmental influences that are confounding metabolomics analyses in blood and CSF. We therefore reasoned that drug primary induced pathway activities that persists in cellular metabolism are reflected more accurately in cultured fibroblasts than in blood and CSF body fluids. In addition, metabolite signature variations in the fibroblasts may also serve as candidates for predicting individual differences in therapeutic response by associating them with behavioral phenotypes of the rhesus monkeys. We report on our metabolomics analyses of fibroblasts from skin biopsies and compare the results with those obtained from CSF and plasma. Our data implicate Purine metabolism to be affected in response to long-term fluoxetine administration and to correlate with impulsive behavior of the rhesus monkeys. Purinergic signaling and mitochondrial energy homeostasis have been implicated in psychiatric disorder pathology. Our results support the notion that these molecular pathways are also relevant in current psychopharmacology (Lindberg et al., 2015).

2. Experimental procedures

2.1. Assurance of compliance with animal codes

All procedures followed the Guide for the Care and Use of Laboratory Animals of the National Research Council. The California National Primate Research Center (CNPRC) is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. Protocols for this project were approved prior to implementation by the UC Davis Institutional Animal Care and Use Committee.

2.2. Animals and animal care

The cohort of male rhesus monkeys (*Macaca mulatta*) that were sampled for this study has been described previously (He et al., 2014; Golub et al., 2015). At approximately 1 year of age animals were selected from the outdoor colony at the CNPRC, relocated to indoor caging, and assigned to treatment (fluoxetine) or control (vehicle) groups. There were two transfer groups relocated two weeks apart based on birth dates and sampling at necropsy for metabolomics studies was conducted separately on the two groups after one-year of dosing. The impulsivity test reported here was also conducted after one-year of dosing. Dosing continued for another year, followed by a one-year post dosing evaluation, sacrifice and necropsy.

In addition to fluoxetine dosing, genetic polymorphisms of MAOA, which affect serotonin mediated brain functions, were identified by genotyping and included as a variable in the study. The entire cohort was housed in the same cage room in double cages that allowed socialization in pairs. All subjects received identical, standardized husbandry and enrichment according to CNPRC protocols. Animal health was evaluated daily. No conditions resulting in veterinary diagnosis were reported, with the exception of episodes of diarrhea treated with Tylosin®. Linear and ponderal growth were measured at intervals throughout the study without indication of fluoxetine effects during the dosing period (Supplementary Table 1). Based on experience, cage location variables and transfer group were screened as potential covariates in data analyses.

2.3. Fluoxetine dosing

Fluoxetine dose selection was based on information in the human and non-human primate literature and on a preliminary pharmacokinetic/pharmacodynamic study to provide steady-state circulating levels of fluoxetine/norfluoxetine in the range reported for therapeutic use of fluoxetine in children (Golub and Hogrefe, 2014). Dosing was initiated at one-year of age at 1.6 mg/kg/day and adjusted to 2.4 mg/kg/day after 11 months, one month before the one-year sampling reported here. The initial dose for the present study was set lower (1.6 mg/kg) while the monkeys were being adapted to indoor housing, daily dosing and behavioral testing