

An Orchestrator on Xenobiotic Metabolism is the Human Intestine Biota

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Abstract

One of the most impact issues in recent years refers to the COVID-19 pandemic, the consequences of which thousands of deaths recorded worldwide, are still inferior understood. Its impacts on the environment and aquatic biota constitute a fertile field of investigation. Thus, to predict the impact of the indiscriminate use of azithromycin (AZT) and hydroxychloroquine (HCQ) in this pandemic context, we aim to assess their toxicological risks when isolated or in combination, using zebrafish as a model system. In summary, we observed that 72 h of exposure to AZT and HCQ (alone or in binary combination, both at 2.5 µg/L) induced the reduction of total protein levels, accompanied by increased levels of thiobarbituric acid reactive substances, hydrogen peroxide, reactive oxygen species and nitrite, suggesting a REDOX imbalance and possible oxidative stress. Molecular docking analysis further supported this data by demonstrating a strong affinity of AZT and HCQ with their potential antioxidant targets.

Introduction

The human digestive tract is a location for xenobiotic metabolism, and microbes that live there play a role. The gut microbiome, which is a collection of microorganisms found in the gastrointestinal system, can change the pharmacokinetics of medications, environmental toxins, and heavy metals by altering their metabolic result. Depending on the enzymatic activity within the microbial niche, direct chemical alteration of xenobiotic by the gut microbiome, either through the intestinal tract or via enter hepatic circulation, might result in enhanced metabolism or bioactivation. [1-15] Those that reverse the alterations given by host detoxification pathways are among the unique enzymes encoded in the microbiome.

Disruptions to the composition and activity of the gut microbiome contribute to a variety of human diseases. An indicator of microbiome health is community diversity, as redundancy in functional pathways supports the maintenance of essential functions upon perturbation. Such imbalances can contribute to a variety of conditions throughout the body, including inflammation, muscle mass, depression, and blood pressure in the elderly, suppressed infant weight gain, perturbed immune and endocrine system development, increased allergic responses and behavioral and neurochemical alterations. However, the most notable and well-understood examples are in relation to metabolism. Disruptions to the generally consistent metabolic activity of the microbiome can contribute to obesity and metabolic disease through the dysregulation of lipid and carbohydrate metabolism.

Subjective heading

The AZT uptake by zebrafish was assessed according to the methodology adopted by Keskar and Jugade with little modifications. It was used 8 animals/group, weighing approximately 350 mg/animal, which were euthanized (immersion in ice-slurry) and subsequently macerated in 1 mL of phosphate buffered saline (PBS), and centrifuged at 13,000 rpm for 5 min (at 4°C). Aliquots of 30 µL of the sample supernatant were transferred to test tubes (previously sanitized) and mixed with 470 µL of acetonitrile solution (0.01 M), 500 µL of bromocresol green solution

Discussion

The gut microbiome directly metabolizes xenobiotics. Inhaled xenobiotics interact with the many microbial populations that populate the small intestine and colon, which can often modify them

in ways that are unique or complimentary to the host. While human metabolism consists primarily of oxidation, hydrolysis, and chemical conjugation with tiny molecules such as glucuronide or glutathione, enteric bacteria have a far broader metabolic repertoire. The gut microbiome mostly relies on reduction, acetyl and methyl group addition, and radical production for modification. The procedures used for the quantification of HCQ followed the recommendations of Bergqvist, with some modifications. The supernatant of the same 8 animals/group mentioned above was used. In that case, 200 µL aliquot of supernatant from each sample was transferred to previously cleaned hygienic conical bottom microtubes and, sequentially, 400 µL of the bromothymol blue solution (0.65 mmol/L) and 600 µL of dichloromethane P.A. were added sequentially. Then, the solutions were homogenized in a vortex mixer (for 30 s) and centrifuged at 1500 rpm, for 5 min, at 23°C. Subsequently, the aqueous phase of the mixture was discarded and 200 µL of the organic phase was transferred to a 96-well microplate, for later reading at 405 nm, in an ELISA reader. The concentrations of HCQ in the samples were determined from the equation of the straight line obtained by making a standard curve, using known concentrations of HCQ (0, 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/mL). The background fluorescence of the control samples was also determined and subtracted from the samples from zebrafish exposed to HCQ.

Syphilis is an infection caused by *Treponema pallidum*. Usually, *T. pallidum* is transmitted through sexual intercourse. In addition, syphilis greatly increases the risk of infection and transmission of acquired immune deficiency syndrome. In recent years, the global incidence of syphilis has increased because of the ability of *T. pallidum* to evade host immune defenses and spread from the initial site of infection to

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other organs and tissues. Hence, it is also termed a “stealth pathogen.” How *T. pallidum* overcomes the immune response and damages tissue is incompletely understood. Explaining the pathogenesis and immune mechanism of action of *T. pallidum* has become a key link to controlling syphilis.

Biochemical analyzes

Sample preparation

Prior to biochemical assessments, the samples to be analyzed were prepared, similarly to Guimarães. Eight fish/group were also weighed (approximately 350 mg/animal), euthanized (immersion in ice-slurry), macerated in 1 mL of phosphate buffered saline (PBS), and centrifuged at 13,000 rpm for 5 min (at 4 °C). The supernatant was separated into aliquots to be used in different biochemical evaluations. Entire bodies were used in the experiment due to difficulties on isolating certain organs from small animals. Organ “contamination” by organic matter and/or by other particles consumed by zebrafish can be bias at biochemical analysis applied to organs at dissection time. Samples were stored in sterile conical bottom microtubes at 80 °C for a maximum of 7 days.

Assessment of nutritional status

Previous reports on the exposure of different aquatic organisms to different drugs can affect animals’ feeding behavior and change their energy metabolism. Thus, the influence of treatments on total proteins, triglycerides, and total soluble carbohydrate levels was herein assessed. Total proteins and triglycerides concentrations were determined by using commercial kits, based on the Lowry method (Ref. BT1000900; BioTécnica, Varginha, MG, Brazil) and on the enzymatic colorimetric method by using glycerol-3-phosphate oxidase (GPO) (Ref. BT1001000; BioTécnica, Varginha, MG, Brazil), respectively. Total soluble carbohydrate levels were performed based on the methodology proposed by Dubois.

The possible neurotoxic effects induced by AZT and HCQ (alone and in combination) were evaluated by determining the activity of acetylcholinesterase (AChE) enzymes, according to the method of In addition, to assess whether these drugs were able to alter the mechanosensory system of the fish, we performed the count of superficial neuromasts in exposed individuals. For this, we adopted the procedures described in [1], in which, briefly, the live animals (n = 8/each group) were placed (for 30 min) in a beaker containing 400 mL of water (with constant aeration) reconstituted with 5 mM of the fluorescent dye 4-(4-Diethylaminostyryl)-1-methylpyridinium iodide (4-Di-2-ASP), from stock solution (40 mg of 4-Di-2-ASP) diluted in 10 mL of dimethyl sulfoxide P.A. Then, the animals were carefully removed and transferred to a beaker containing dechlorinated water (without dye), and remained for 30 min, to remove excess of dye in the body. After that, the animals were euthanized (immersion in ice-slurry) and positioned horizontally on glass slides for later observation under a fluorescence microscope. To assess the effects of potential interactions between AZT and HCQ and their possible targets in animals, we used a chemogenomics-based system called ChemDIS-Mixture, which is built using the previously introduced ChemDIS and statistical p-tests combined with tools available by using the STITCH database. To enable the inference of chemical-induced effects, ChemDIS-Mixture several databases are downloaded and integrated into a MongoDB database including STITCH 5, Reactome, SMPDB, miRTarBase, Ensemble, DOSE, DO.db, KEGG.db and org.Hs.eg.db. Currently, >430,000 chemicals with >15 million chemical-protein interactions can be

analyzed using ChemDIS-Mixture. For each drug (AZT and HCQ) the possible interacting proteins were extracted, and the enrichment analysis was conducted based on a hypergeometric test for identifying the enriched GO (Gene Ontology) terms with an adjusted p-value < 0.05 using Benjamini-Hochberg multiple test correction.

Molecular docking

To predict the binding sites and affinity of the bonds among AZT, HCQ and the protein structures of the enzymes AChE, BChE, SOD and CAT, we performed docking and chemoinformatic screens. The ligands AZT (CSID: 10482163) and HCQ (CSID: 3526) were obtained from the virtual repository Chemspider and optimized with force field type MMFF94 in Avogadro software. The protein structures (targets) of the zebrafish were obtained by the homology construction technique by the SWISS-MODEL server) with structural similarity values between 87.14% and 99.8%. The validation of the structures was verified with the SAVES v.6.0 server. For molecular docking simulations, AutoDock tools (ADT) v4.2 were used to prepare binders and targets and AutoDock Vina 1.1.2, to perform the). The binding affinity and interactions between residues were used to determine the best molecular interactions. The results were visualized using ADT, Discovery Studio v4.5 and UCSF Chimera X.

Xenobiotic inactivation by the gut microbiome

Drug metabolism by gut bacteria is a source of concern for therapeutic efficacy and safety, and it’s difficult to predict in humans. The cardiac glycoside digoxin, a therapy for heart failure and arrhythmia, is an intriguing example of a medication being digested by a single bacterium. The action of digoxin is mediated by its unsaturated lactone ring, which binds to and inhibits Na⁺/K⁺ ATPases, lowering Ca²⁺ levels in cardiac myocytes.

Xenobiotic bioactivation by the gut microbiome

While bacterial metabolism inactivates some xenobiotics, others can be transformed from a precursor (prodrug) to an active metabolite. The large number of enzymes expressed in the gut microbiome can produce a variety of distinct active compounds that aren’t produced by the host. Drug efficacy can differ between animal research and human trials, as well as between individuals, because the microbiome of different animals and persons differs greatly.

Reactivation of host-detoxified compounds by the gut microbiome

The reactivation of compounds that have already been detoxified by host enzymes is a growing concern in the microbial metabolism of xenobiotics. Conjugation of xenobiotics or endobiotics with small molecules is used in human phase II metabolism to change their excretion. Acetylation, methylation, glucuronidation, sulfonation, and glutathione or amino acid conjugation are the most common modifications, all of which require cofactors and transferases to carry small molecules to recipient compounds. Conjugation limits uncontrolled passive passage of hydrophobic molecules through cell membranes by increasing their size and polarity, forcing retention in excretory channels. Although conjugation can bioactivate the changed molecule in a few circumstances, most xenobiotics are detoxified at this point. Because these reactions take place mostly in the liver, xenobiotics are called xenobiotics.

The possible neurotoxic effects induced by AZT and HCQ (alone and in combination) were evaluated by determining the activity of acetylcholinesterase (AChE) enzymes, according to the method

of In addition, to assess whether these drugs were able to alter the mechanosensory system of the fish, we performed the count of superficial neuromasts in exposed individuals. For this, we adopted the procedures described in, in which, briefly, the live animals (n = 8/each group) were placed (for 30 min) in a beaker containing 400 mL of water (with constant aeration) reconstituted with 5 mM of the fluorescent dye 4-(4-Diethylaminostyryl)-1-methylpyridinium iodide (4-Di-2-ASP), from stock solution (40 mg of 4-Di-2-ASP) diluted in 10 mL of dimethyl sulfoxide P.A. Then, the animals were carefully removed and transferred to a beaker containing dechlorinated water (without dye), and remained for 30 min, to remove excess of dye in the body. After that, the animals were euthanized (immersion in ice-slurry) and positioned horizontally on glass slides for later observation under a fluorescence microscope.

The effects of exposure to AZT e HCQ (alone or in combination) on oxidative stress reactions were evaluated based on (i) indirect nitric oxide (NO) (via nitrite measurement; NO_2^-) (ii) thiobarbituric acid reactive substances (TBARS) [predictive of lipid peroxidation]; (iii) production of reactive oxygen species (ROS), and (iv) hydrogen peroxide (H_2O_2) [which plays an essential role in responses to oxidative stress in different cell types]. The Griess colorimetric reaction [as described in was used to measure NO_2^- and the TBARS levels were determined based on procedures described by, respectively., with some modifications. The supernatant of the same 8 animals/group mentioned above was used. In that case, 200 μL aliquot of supernatant from each sample was transferred to previously cleaned hygienic conical bottom microtubes and, sequentially, 400 μL of the bromothymol blue solution (0.65 mmol/L) and 600 μL of dichloromethane P.A. were added sequentially. Then, the solutions were homogenized in a vortex mixer (for 30 s) and centrifuged at 1500 rpm, for 5 min, at 23°C. Subsequently, the aqueous phase of the mixture was discarded and 200 μL of the organic phase was transferred to a 96-well microplate, for later reading at 405 nm, in an ELISA reader. The concentrations of HCQ in the samples were determined from the equation of the straight line obtained by making a standard curve, using known concentrations of HCQ (0, 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/mL). The background fluorescence of the control samples was also determined and su

Conclusion

The complicated interplay between the gut microbiome, host factors, and xenobiotic metabolism is difficult to understand. The microbial world's variety of enzyme responses has broadened our understanding of how xenobiotics are digested. These microbial enzymes' products can perform new functions, whether they are distinct from host metabolites or complement those that are currently present. Microbial xenobiotic metabolism can result in bioactivation, detoxification, or even reverse host detoxification in some situations, such as with glucuronidases. By binding or importing xenobiotics or reinforcing the intestinal mucosal barrier, the microbiome atop

enterocytes can inhibit absorption. Finally, we're starting to understand how the microbiome affects the host's xenobiotic metabolism enzymes, changing the fate of endobiotics and xenobiotics.

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Conflict of Interest

The authors declare that they are no conflict of interest.

References

1. Qin J, Li R, Raes J (2010) A human gut microbial gene catalogue established by metagenomic sequencing *Nature*. 464: 59-65.
2. Abubucker S, Segata N, Goll J (2012) Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol* 8.
3. Hosokawa T, Kikuchi Y, Nikoh N (2006) Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol* 4.
4. Canfora E.E, Jocken J.W, Black E E (2015) Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* 11: 577-591.
5. Lynch SV, Pedersen (2016) The human intestinal microbiome in health and disease. *N Engl J Med* 375: 2369-2379.
6. Araújo A.P.C, Mesak C, Montalvao MF (2019) Anti-cancer drugs in aquatic environment can cause cancer insight about mutagenicity in tadpoles. *Sci Total Environ* 650: 2284-2293.
7. Barros S, Coimbra AM, Alves N (2020) Chronic exposure to environmentally relevant levels osimvastatin disrupts zebrafish brain gene signaling involved in energy metabolism. *J Toxic Environ Health A* 83: (3) 113-125.
8. Ben I,Zvi S, Kivity, Langevitz P (2019) Hydroxychloroquine from malaria to autoimmunity. *Clin Rev Allergy Immunol* 42 (2) : 145-153, 10.1007/s12016-010-8243.
9. Bergqvist Y, Hed C, Funding L (1985) Determination of chloroquine and its metabolites in urine a field method based on ion-pair. *Extraction Bull World Health Organ* 63 (5): 893.
10. Burkina V, Zlabek V, Zamarats G (2015) Effects of pharmaceuticals present in aquatic environment on Phase I metabolism in fish. *Environ Toxicol Pharmacol* 40 (2) : 430-444.
11. Cook JA, Randinitis EJ, Bramson CR (2006) Lack of a pharmacokinetic interaction between azithromycin and chloroquin. *Am J Trop Med Hyg* 74 (3): 407.
12. Davis SN, Wu P, Camci ED, Simon JA (2020) Chloroquine kills hair cells in zebrafish lateral line and murine cochlear cultures implications for ototoxicity. *Hear Res* 395: 108019.
13. De JAD Leon C (2020) Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *J Vis Exp* (159): Article e61122.
14. Dubois M, Gilles MA, Hamilton JK (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem* 28 (3): 350-356.
15. Ellman GL, Courtney KD, Andres V (1961) Featherston A new and rapid colorimetric determination of acetylcholinesterase activity *Biochem. Pharmacol* 7 (2): 88-95.