BMSS summer studentship report

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Project title: Interrogation of drug receptor interactions using Mass Spectrometry: Can quinoline, acridine and benzophthrydine-haem adducts in the gas phase reveal antimalarial mechanism of action?

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Abstract

This abstract should be about 120 words in length and describe, to a general scientific audience, the outcomes of the project. Text should be in Times new Roman, font size 9. Use (1) to refer to reference number 1 in the text.

Pyronaridine, a Chinese antimalarial drug developed in the 1970's, has been resurrected by the Medicines for Malaria Venture (MMV) for world-wide prophylactic use to replace currently used drugs with diminished effectiveness due to parasites developing drug resistance. This study found a 1:1 stoichiometry of drug interaction between the drug pyronaridine and haem target receptor using positive ion electrospray measurements. Subsequently, using collision induced dissociation (CID) experiments; functional groups which preferentially interact with one another, allowed affirmation of a novel, lowest energy form of adduct geometry from Density Functional Theory experiments and vibrational studies. Knowledge of mass spectrometry and thermodynamic interactions between haem and pyronaridine continue to unravel the mechanism of antimalarial action and ultimately aid in designing more potent drugs.

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Report

1750 - 2250 words in length. The report should include an Introduction, Materials and Methods, Results and Discussion, Conclusion and References. Acknowledgements may also be included. Please use the notation (number) to refer to the reference number in the text, i.e. (1) for reference 1.

Illustrations, including legends, should be sent separately, ideally as high resolution .jpg, .eps or .tif files using the file naming system ‘SS_your initials_Your University_Fg1.jpg’

Introduction

Invasion of host erythrocytes by Plasmodia during a malarial infection prompts deconstruction of haemoglobin within acidic vacuolar parasite compartments, providing a nutrient source and space for apicoplast growth and replication (1). Successive release of four, redox active, toxic porphyrins per molecule of haemoglobin, induces a complex detoxifying biomineralization process eventually producing a malarial pigment (haemozoin). The mechanisms of anti-malarial action of 4-aminoquinolines (such as chloroquine; CQ, Resochin, Figure 1) and 9-amino-1-acracidines (such as pyronaridine, PA) remain imperfectly understood, despite considerable effort using a variety of biochemical, pharmacological and spectroscopic approaches. However, most investigators agree that antimalarial action involves inhibition of the biomineralization of haem or the release of toxic haem (Hm) through drug action (1).

Figure 1

Spectrometric methods have generally been underutilised in understanding antimalarial drug action (1,5). Consequently, interrogating the bonding interaction and geometry between an anti-malarial drug and its receptor (Hm) in the gas phase may provide additional insight unattainable by spectroscopic methods, since it allows speciation of selected ions and charged fragments. We have already used this information in improving rational drug design by synthesising molecules that both modulate suspected interactions and testing these in silico, in vitro and in vivo. Specifically, interactions between Hm and various drugs are commonly and persistently assumed to involve π-π interactions or to be dispersive in nature, a view tentatively supported by assigning bands in spectroscopic studies (4). Such weak interactions are unlikely to persist under conventional electrospray conditions during either nominal or accurate mass spectrometry. Notably, a lack of crystal structure investigations involving non-covalent drug receptor complexes between Hm and compounds in current use, relates to the deposition of amorphous adducts. However, single crystal x-ray diffraction studies have been recently used to establish that halofantrine (6) and quinine (7) form a covalent adduct with haemin chloride.

A variety of binding modes involving CQ have been assigned to each of the different forms of Hm found in solution which include haematin (where the axial coordination site on the metal is occupied by OH rather than Cl) (1,2), a Hm μ-oxo dimer ([Hm-O·Hm]) (8), and binding to the haemozoin biomineral, either on its growing face (9) or upon the side of the structure (10).

One popular geometrical structure is composed of a sandwich of μ-oxo dimer: CQ:μ-oxo dimer derived isothermal titration calorimetry experiments (8). Our previous molecular mechanics modelling and high resolution electrospray study involving monomeric haematins cast doubt that drugs interacted with Hm via μ-oxo dimer or by long range π-π interactions (11). That study revealed that a number of competing geometries could exist (spanning around 50 kCalMol\(^{-1}\) above the lowest energy conformer) and, importantly, that interaction could occur not only on the face obverse to the axial coordination site (bottom) but the energy of interaction on the axial side (top) was consistently lower than with the bottom face (1). We did not detect evidence of any μ-oxo dimer formation in any of our electrospray investigation in either positive or negative mode. Unsurprisingly, under our conditions, binding of CQ to μ-oxo dimer in methanol could also not be detected using conventional positive ion electrospray conditions.

Previously, using Density Functional Theory (DFT) we showed that the endocyclic heterocyclic quinoline nitrogen covalently coordinates to iron in a porphyrin model, similar in geometry to that observed between Hm and histidine within haemoglobin during oxygen binding in vivo. Evidence for such interactions is robust since it has been quantified not only by spectroscopy but single crystal x-ray diffraction studies. Dascombe et al. (1) have found that an axial (orthogonal) covalent interaction between quinoline and a porphyrin model system was shown to be

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This evidence supports the hypothesis that the acceptor–donor bond is consistent with DFT calculations in the solid state, FTIR-IR showing agreement with DFT with the LANL2DZ basis set for Fe and 6-aminocaridine (CID fragmentation was changed mainly in the presence of drugs and covalent bond marker bands).

Using collision induced dissociation (CID)-MS studies it is possible for the first time to test predictions from density functional theory (DFT) which define the geometry of CQ bound to its receptor in the gas phase as “edge on” and involving hydrogen bonding between acceptor–donor atoms and propionic acids donors within Hm. Recently, the Ismail group has clarified the geometry within CQ-Hm adducts by the use of ion mobility electrospray mass spectrometry (14).

Since 4-aminoquinoline and 9-aminoacridine antimalarials share a common mode of action, i.e. binding to Hm, cross correlating electrospray mass spectral data with biological activity, especially in a high throughput automated mode, may also minimise not only the use of cultured cells but that of animal models, which will alleviate ethical and moral concerns whilst accelerating the drug discovery process.

Materials & Methods

Compounds were purchased from Sigma-Aldrich Co. or withdrawn from the Medicinal Chemistry Research Group drug repository. Haemin chloride is commonly referred to as Hm in this and other studies. Cryptolepis Sanginolenta was obtained from West Africa (15) and a voucher specimen deposited in our Pharmacognosy archive. Pyronaridine tetraphosphate was a gift from Professor Wallace Peters (London School of Tropical medicine and Hygiene). Hydrangea was sourced from a garden in Urmston Manchester. All masses were detected in positive ion mode using a Waters Quattro Premier or LCT for accurate MS. Drug-Haem (Dg-Hm) complexes in the solid state were produced by co-grinding (agate mortar (5 min) then application of hydrostatic pressure (886,610 kNm⁻²), M-30 Press, Research & Industrial Instrument Co., London, U.K. (15 min) and re-grinding the thin film 5 min); then re-pressurisation (15 min). A fragment was then dissolved in HPLC grade methanol, filtered, and subjected to Electrospray MS in positive and negative ion modes. In the solid state, FT-IR spectroscopy using a diamond ATR accessory was used to quantify hydrogen and salt bridges. Complexes between Hm and various antimalarial drugs (1) were also studied using 1H-NMR (300 MHz, Brucker, in DMSO-d₆) and UV spectrometry. Ultra-pure metoquine, cryptolepine, chloroquine, pyronaridine and methylene blue were all converted when required as free bases using ammonia saturated methanol and complexed with haemin chloride using the techniques described above. Resonance Raman (RR) spectra between various drugs, including 4-aminoquinolines and their putative receptor, haemin, were recorded (using 457.9 and 514.5 nm outputs of the argon-ion laser) by probing into the absorption bands of the quinoline ring that interacts with the Hm receptor. Structures were optimised using DFT with the LANL2DZ basis set for Fe and 6-31G for remaining atoms. Spin state 6 was used for Fe(III).

Results & Discussion

Haemin, used for complex formation generated non-covalent adducts of drugs with the cation [Haemin]⁺ at m/z 616.2 or with Hm reciprocal dimers at m/z 1232.3 Da [2 Hm]⁺ and m/z 1253.3 [2 Hm + Na]⁺ Da (1). Haemin chloride sprayed at various cone voltages and under CID conditions behaved differently in the presence of drugs such as CQ and metoquine. CID fragmentation was changed markedly in the presence of drugs and revealed interaction of the propionic acid within adduct decomposition. Previously, high resolution positive ion electrospray mass spectrometry indicated a 1:1 complex between drug and receptor in the gas phase that was consistent with DFT calculations and 1H NMR titrations. The latter revealed that the Hm propionic acid side chain peak diminishes upon successive addition of 4-amino-quinoline drug. High resolution positive ion electrospray mass spectrometry indicated the formation of a 1:1 complex in the gas phase that was consistent with DFT calculations in which one of the propionic acids bind to the endocyclic quinoline nitrogen in the bisquinolines meta-quine (1). This evidence supports the positive charge location upon the central iron atom (C₈H₈ClFeN₂O₆⁺) predicted mass: 1046.2525 Da; found mass 1046.2429 Da) and suggests

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that the drug binds “side on”. This hydrogen bonded, edge-type interaction is energetically more stable than previously suggested above face and below face interactions [1] and may be important for designing drugs more potent than CQ, as well as novel dosage formulations.

A feature almost universal to all known clinically useful antimalarials is the presence of a planar heterocycle that possesses a nitrogen containing side chain. Again, such compounds are commonly assumed to interact with Hm by π...π interactions and interpretations are often supported by a single modelled structure without examination of competing conformers. Cryptolepine lacks any flexible side chain (Figure 1) but is still an active antimalarial ingredient of the West African antimalarial shrub Cryptolepis Sanguinolenta (1,5,15). It associates with both the monomer cation and protonated reciprocal dimer of Hm. Under our conditions aerobic incubation lead to the formation of hydroxycryptolepine, probably through a single electron transfer process followed by addition of ground state oxygen and subsequent decomposition of the peroxyl radical to the phenolic species, in an autocatalytic free radical cascade [15b]. In a previous study, we have already established that cryptolepine also binds “edge on” to Hm by hydrogen bonding (5). Methylene blue is the only antimalarial that definitely associates with Hm by π...π interactions [15c] and, notably, did not show adducts with hemin chloride when examined under conditions that routinely showed adducts in positive ion electrospray experiments.

Mass spectral fragmentation of PA (16), was investigated using a variety of cone voltages and under CID conditions to determine its behaviour in the absence and presence of Hm (17). Ismail et al. already investigated its behaviour towards single electrons generated by pulsed radiolysis (18). Amodiaquine, which also contains an aromatic side chain, was used as a reference compound. Under positive ion electrospray conditions, both compounds ejected their tertiary amine side chains to produce quinone methides.

Examination of the CID breakdown behaviour for PA suggested that binding to Hm occurs differently to that adduced earlier for either CQ or any bisquinoline analysed so far. Analysis of various ions showed that the interaction with Hm suggested that the nitrogen at position 1, the methoxy group at position 2 as well as one of the pyrrolidine groups on the aromatic side chain was involved in binding to the receptor. This is in marked contrast to the original geometry considered where the endocyclic naphthyridine nitrogen at position 5 was involved in binding to a single propionic group. If Py interacts with Hm by π...π interactions we are unable to suggest any reasonable mechanism that would lead to the fragmentations observed in the CID spectra, since the hematin would be too remote to fragment via specific weak “through space” rather than strong “through bond” effects.

Figure 3

The suggested geometry was then modelled using DFT which is also consistent with a vibrational study, which shows strong quenching of the propionic peak around 1700 cm⁻¹ and are replaced by peaks of the corresponding anion. This finding explains why the endocyclic, aromatic heterocyclic nitrogen at position 1 is important in PA and is consistent with the presence of hydrogen bonding.

Conclusion

Pyronaridine interacts with Haem in the solid, solution and gas state by strong hydrogen bonding interactions rather than weak π...π interactions. Deduction of the binding geometry has led to the first rationally derived geometry using electrospray mass spectrometry for this important and upcoming antimalarial drug.

We gratefully acknowledge the financial support of the BMSS and the RSC NW AD Region to Sophie Rawthore.

References


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(17) Hieu van Truong. M.Pham thesis, Medicinal chemistry research Group School of Pharmacy & Biomolecular Sciences, LJMU, Liverpool L3 3AF, 2011.

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Figure 3: unpublished CID of pyroaridine Hm complex: Van Truong & Ismail

CID scan (blind scan, no tuning, ‘peak’ was at baseline).

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Figure 4 DFT Py Hm complex.

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Figure X: Vibrational spectra (Infra-red) showing from top to bottom respectively a) PA; b) Hms:PA complex and c)