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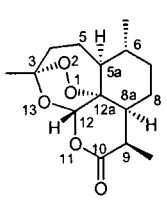
COMMENTARY

How Might Qinghaosu (Artemisinin) and Related Compounds Kill the Intraerythrocytic Malaria Parasite? A Chemist's View

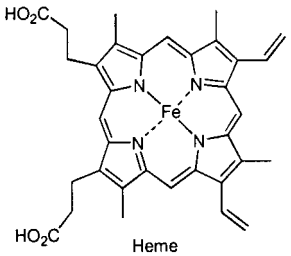
ABSTRACT

The antimalarial mechanism of qinghaosu (artemisinin) has been a problem since the late 1970s. During the past decade, several molecular level theories were postulated. However, their further development has been very difficult. By looking into the QHS cleavage process and all possible reaction paths available to the resulting transient radicals, the present commentary reveals those major hidden problems with the existing theories and tries to identify some essential features of the parasiticidal events that may take place within the intraerythrocytic malaria parasite. A seemingly more reasonable theory is also introduced.

Qinghaosu[1] (QHS) and its derivatives/analogues are currently regarded as the most promising weapons against malaria, an ancient disease that once was thought to be near eradication in the 1950−60s but that later has become a serious threat[2] to humankind again as a result of the appearance of multi-drug-resistant variants. The unique 1,2,4-trioxane structure of QHS is entirely incompatible with the traditional antimalarial structure−activity theory. Therefore, its antimalarial mechanism is of great interest to the scientific community.

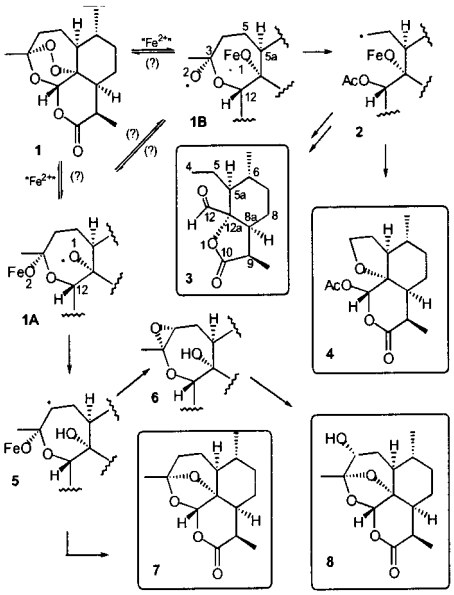


QHS, **1**



Heme

Scheme 1



It was proposed[3] from the beginning and still remains unchallenged that these types of agents have a mode of action entirely different from those of traditional alkaloidal antimalarial agents.

Efforts to elucidate QHS's antimalarial mechanism started in the 1970s. Over the past 30 years, a tremendous number of papers have been published. Progress in understanding, however, lags far behind. One of the main reasons is that QHS does not directly exert its lethal effects on the malaria parasite through the whole intact molecule, but rather, through some transient species generated after cleavage of the peroxy bond. Such cleavages are chemical processes unfamiliar to most biomedical scientists; therefore, involvement of chemists in the investigations on the antimalarial mechanism is essential.

Since the early 1990s, when Posner and Oh[4] showed for the first time the potential importance of chemistry in the antimalarial mechanism study, many chemists have joined the investigation into this novel type of compound and gained much detailed knowledge of the cleavage (Scheme 1) process.[5−12] On the basis of the chemical studies, several

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different opinions[13] (theories) about the *molecular* level antimalarial mechanism have appeared in the literature, with the lethal effects being attributed to (1) direct oxygen transfer from a QHS-derived open hydroperoxide ROOH (formed through ring-opening of QHS) to an *aliphatic* primary or tertiary amine;[7c,13a] (2) reactions with the oxygen-centered radicals derived from the ROOH;[7c,13a] (3) alkylation[13b] of the intraparasitic free heme; or (4) attacks of an epoxide,[5,13c,d] a high-valent iron species,[5,13c,d] or carbon-centered radicals[5,13c,d] produced by the cleavage of QHS. However, up to now, none of these theories appeared substantial enough to be recognized as a working hypothesis that is fully compatible with the available knowledge of chemistry and biology. In fact, despite the broad influence and frequent citation, the deeper one looks into these theories (opinions), the more doubtful about them he/she will be. To prompt the appearance of more plausible hypotheses and, thus, advance the study in this field, it appears to be imperative now to clarify the situation and unveil the hidden problems with the various theories.

The potential doubts with the QHS-derived open hydroperoxide-ROOH-based theories include (1) Could the ring-opening[7c] of QHS to the ROOH possibly be fast enough to compete with the parallel radical reactions? If this reaction is much slower than the radical-mediated cleavage[9a] (Scheme 1), the resulting ROOH would be present in only negligible amounts. (2) The existing version of oxygen transfer does not encapsulate any selectivity for a specific aliphatic tertiary or primary amine. A vital enzyme with an *aliphatic tertiary* amine at the active center does not seem to be known yet, and there are numerous primary amines competing for the ROOH. How might the traces of ROOH lead to fatal effects through direct oxygen transfer to a specific aliphatic amine? (3) In principle, the oxygen-centered radicals generated from ROOH are similar to those derived from molecular oxygen and, therefore, cannot possibly be responsible for the antimalarial activity unless there is an explanation for the reason they are not eliminated by the intraparasitic system against oxidative stress[14] (oxidative damage inflicted by oxygen species).

Alkylation of the free heme as a mode of action may make sense only because the alkylated heme probably cannot polymerize to give nontoxic hemozoin[15] (malaria pigment) and, thus, lead to accumulation of the redox-active alkylated free heme, which may raise the levels of molecular oxygen-derived radicals etc. In this case, the real killers should be the active oxygen species, not the alkylated heme itself. Consequently, whether this theory may hold entirely depends on the fate of the molecular oxygen-derived active species (vide infra).

Among the theories based on the QHS-derived transient species, the problems with those involving the high-valent iron and the epoxide have already been discussed.[9a] Therefore, only the "radical-killing" one is dealt with here. A fundamental problem with this theory is the implication that in the lethal events, QHS-derived radicals attack vital targets *intermolecularly* (behaving like those radicals generated from simple peroxides). The unique structure of QHS decides that the radical level available for intermolecular attacking at other molecules is much lower than that with simple organic peroxides. Regardless of the Fe(II) species, Path a (Figure 1) always exists, which intramolecularly eliminates substantial amounts of radicals by forming **4**. Additional paths are also present when the reducing species is, for example, cysteine–

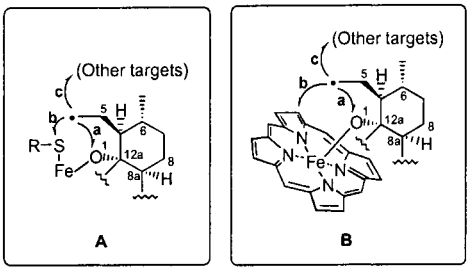


FIGURE 1. Schematic representation of some possible reaction paths available for the primary radical **2** generated by Fe–S-type chelates (A), and heme (B). For clarity, some substituents are not shown.

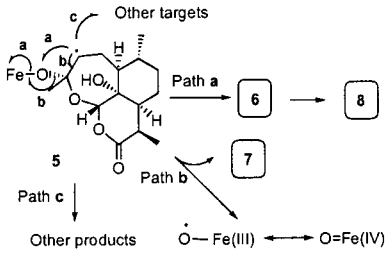
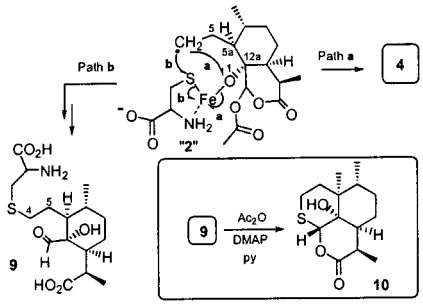


FIGURE 2. Some possible reaction paths available for secondary radical **5**. Although unlike the primary radical **2**, **5** can neither attack the sulfur ligand to yield corresponding S-alkylated product nor add to free heme to afford a QHS–heme adduct because of steric crowding and conformational restriction, the unpaired electron at C-4 may attack the O-2 to give **8** via **6**. Alternatively, a β-scission might take place to finally form **7** and a priori a high-valent iron species. Thus, as a result of the co-presence of the kinetically favored intramolecular reactions, only a small fraction of **5** can possibly undergo path c, acting like a simple radical to attack other molecules.

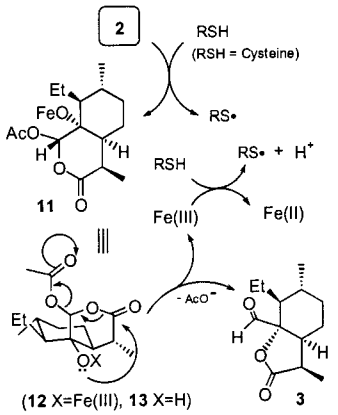
Scheme 2



iron chelates[16] (Scheme 2). Note that when the Fe(II) species is heme, formation of the QHS–heme adducts[17] may well proceed intramolecularly (B, Figure 1) instead of solely intermolecularly as strongly implied in the published[13b,17a,b] papers.

Similar elimination paths for the secondary radical **5** are also available (Figure 2). Thus, the levels of both **4** and **5** available for attacking other molecules (path c, Figures 1 and 2) must be much lower than otherwise due to the presence of the radical "self-quenching"[16b] pathways. If QHS killed the malaria parasite through intermolecular radical reactions as the existing radical-killing theory suggests, then one could never explain why QHS is a potent antimalarial, whereas most simple peroxides (which generate stoichiometric amounts of radicals for similar attacks) are inactive, because the efficiency of intermolecular radical attacks relies proportionally on the radical quantity generated in the cleavage (viz. the larger the

Scheme 3



number of radicals, the more serious the damage to the targets the radicals may cause).

Complete omission of the existence of the intracellular radical-scavenging system is another major problem with the radical-killing theory (and essentially all other existing ones). In actuality, radicals are generated in living cells all the time. However, because of the presence of GSH and the antioxidative stress enzyme system, essentially all of these radicals are eliminated rapidly without causing any discernible damage to the cell. If under normal physiological conditions those "endogenous" (i.e., not related to administered drugs) radicals can be scavenged efficiently inside the parasite, why could not the QHS-derived ones? Note that the same consideration is also applicable to the oxygen-centered radicals in other theories, including the high-valent iron O═Fe(IV) ↔ •O—Fe(III) and those radicals derived from the ROOH, which exert their lethal effects through random intermolecular attacks. These radicals are expected to be easily eliminated like the endogenous radicals without causing any fatal damage to the parasite unless they are present in such large quantities that the parasite's antioxidative stress system is overwhelmed.

The quenching of **2** (Scheme 3) to give **3** (never occurring without a thiol) by cysteine has been observed[16] experimentally. It is, therefore, reasonable to expect that not all of those QHS-derived radicals undergoing Path c (Figures 1 and 2) may necessarily cause damage to the vital target molecules, because at least a substantial portion of them will be eliminated in the encounter with GSH. Thus, after the "self-quenching" reactions and the intermolecular quenching by GSH, the radicals remaining available for attacking other molecules may account for only a very small fraction of the total number generated in the cleavage.

On the basis of the above reasoning, one may conclude that the radicals generated by simple iron species (either simple iron chelates or free heme,[18] which may be termed "nonessential" irons, because they alone do not play any essential physiological role in vivo, as compared with those iron-containing proteins/enzymes) are unlikely to cause fatal damage to the parasite, because all the radicals would be eliminated either by the "self-quenching" reactions or through the interaction with GSH. Thus, contrary to the current belief, at therapeutic[19] doses, those cleavages induced by the nonessential iron species probably have nothing to do with the parasiticidal action. Instead, they help the parasite to fend off the assaults on its vital molecules (by decomposing the trioxanes before they encounter vital redox centers), comprising part of the parasite's defense system against oxidative stress.

The preceding discussion, however, does not mean that radicals cannot be the lethal entities in QHS's antimalarial action and definitely does not imply that the knowledge gained from the cleavage by the nonessential irons is totally irrelevant to QHS's antimalarial mechanism. This is because apart from the nonessential iron species, there may well exist in the parasite some one-electron redox centers of catalytic importance, which can also cleave QHS. For instance, Fe—S-type redox species contain Fe—S bonds similar to that in the simple cysteine—iron chelates. So long as they are accessible to QHS, the same cleavage as observed in the cysteine model is expected to occur. In such cases, the S-alkylation would lead to irreversible damage of the redox center as a result of the covalent bonding to QHS. If the inactivated redox center is, for example, a critical enzyme/functional protein, lethal consequences may of course result. Since the S-alkylation now also occurs intramolecularly (i.e., the redox center and QHS are temporarily linked together in the transition state through an Fe—O bond), it would not only effectively compete with another intramolecular "self-quenching" path (i.e., formation of **4**) but also gain an upper hand in the confrontation with the intermolecular quenching by GSH.

The same considerations may also apply to heme-containing functional proteins/enzymes. Although the cleavage by the intraparasitic free heme is not likely to result in any fatal effects, the reactions of QHS with heme-containing functional proteins/enzymes are entirely different. In the latter cases, "intramolecular" alkylation of the prosthetic heme may change the accessibility of the active center of the functional protein/enzyme and, thus, interrupt its normal functioning.

Alternatively, the lethal effect may also result from radical attack through Path c (Figure 1) if the attacked point is somewhere in the peptide chains (of the Fe—S or heme-containing proteins) in close proximity (but may be rather remote in the first-order structure) to the metal center as a result of folding back of the peptide chains. It deserves mentioning here that the intramolecular alkylation of the sulfur ligands or prosthetic heme (or both) may provide potential explanations for the protein alkylation, which has long been regarded as an important clue[20] to the mode of action of QHS but suffers from the lack of detailed knowledge of the reaction involved.

Compared with the commonly met blockage of enzymes/receptors through reversible binding of an antagonist, the irreversible covalent bonding mode probably can work at lower drug concentrations, because the active center is irreversibly destroyed (cf., an antagonized active center may resume its activity with dissociation of the bound antagonist unless suppressed by a large enough concentration of antagonist). Note that the low therapeutic concentrations (nM levels) do not necessarily contradict the high concentrations (mM levels) used in chemical model studies. When measuring antimalarial potency, one observes the response of the parasite, not consumption of the dosed drug. Inactivation of an intraparasitic enzyme to give observable biological consequences may occur well before all the dosed QHS is cleaved. In chemical cleavage experiments, however, one has to isolate and characterize the products. Higher concentrations help to avoid side reactions and, thus, simplify the product composition, making the characterization of each component much more feasible.

It should also be noted that the above reasoning does not exclude the possibility that the intraparasitic free heme might be involved in the lethal actions in some way other than inducing cleavage of the peroxy bond. It is known, for example, that elevated levels of heme could cause lysis[21] of the malaria parasite through greatly enhanced peroxidation of the membrane lipids. The morphological changes[22] observed in the electron-microscopic studies of the QHS-treated parasite also suggest that damage to membranes might be the immediate cause for the death of the parasite. If the delicate balance between the generation of dioxygen-derived radicals as well as other forms of active oxygen species and the defense system against oxidative stress within the malaria parasite is broken (directly or indirectly) through interruption of, for example, the parasite's defense system due to inactivation of a critical enzyme, excessive peroxidation of the membranes may take place at much lower levels of heme (and, consequently, the resulting deleterious oxygen species).

When compared with the existing theories, the one introduced here appears superior in that (1) it has distinct structural requirements for the antimalarial activity of the trioxanes, which is consistent with the fact that the QHS's unique framework is closely related to its high activity; (2) it explains why the QHS-derived radicals could effectively confront the parasite's defense system against oxidative stress and result in the parasiticidal effect and how the high potency of QHS might result from traces of radicals; (3) it provides a possible clue to the reason for the QHS's extraordinarily low toxicity at therapeutic dosages; and (4) it also includes a potential explanation for the QHS's apparently selective toxicity to the intraerythrocytic parasite. Although the validity of the present theory is to be tested, judging from the observations[23] that QHS is easily metabolized in vivo and the reasoning that the intraparasitic nonessential iron species along with the self-quenching (forming **4**) in the lethal encountering of QHS with the genuine vital target molecules would consume large fractions of administered QHS, it appears certain that the radical really responsible for the antimalarial activity of QHS is rather small in quantity, probably only accounting for a tiny fraction of the administered dose. To ensure the efficiency of the disabling effect resulting from such small amounts of lethal radical, a feature (like intramolecularity) that secures kinetic advantages would be essential for any plausible hypothesis, and the genuine targets, which are vital to the parasite and can be destroyed by traces of radicals, would most likely be some species of catalytic importance.

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