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Defective DNA Repair as a Potential Mechanism for the Rapid Development of Drug Resistance in *Plasmodium falciparum*†

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ABSTRACT: The development and spread of highly drug-resistant parasites pose a central problem in the control of malaria.Understanding mechanisms that regulate genomic stability, such as DNA repair, in drug-resistant parasites and during drug treatment may help determine whether this rapid onset of resistance is due to an increase in the rate at which resistance-causing mutations are generated. This is the first report to demonstrate DNA repair activities from the malaria-causing parasite *Plasmodium falciparum* that are specific for ultraviolet light-induced DNA damage. The efficiency of DNA repair differs dramatically among *P. falciparum* strains with varying drug sensitivities. Most notable is the markedly reduced level of repair in the highly drug-resistant W2 isolate, which has been shown to develop resistance to novel drugs at an increased rate when compared to drug-sensitive strains. Additionally, the antimalarial drug chloroquine and other quinoline-like compounds interfered with the DNA synthesis step of the repair process, most likely a result of direct binding to repair substrates. We propose that altered DNA repair, either through defective repair mechanisms or drug-mediated inhibition, may contribute to the accelerated development of drug resistance in the parasite.

Malaria continues to be a major healthcare problem with approximately 300−500 million cases each year and well over 1.5 million deaths annually (*1*, *2*). Efforts to control the disease are hindered by rapidly emerging parasite resistance to antimalarial drugs. To date, drug resistance has only been well characterized in two of the four species of human malaria, *Plasmodium falciparum* and *P. vivax* (*2*). Of particular concern in antimalarial drug development is the relative speed at which *P. falciparum* drug resistance has developed against newer antimalarials such as mefloquine (∼5 years) and Malarone (∼1 year) compared to older drugs such as chloroquine (∼15 years) (*2*, *3*). Because the development of new drugs takes much longer than the development of drug resistance (12−17 years to develop a drug and as little as 1 year for resistance to emerge), new drug development strategies that focus on improved drug durability must be devised (*2*). Antimalarial drug resistance appears to occur through single point mutations or multiple mutations that confer reduced sensitivity to a given drug or class of drugs (*2*). Many mutations have been identified that correlate with drug-resistant phenotypes (*4*−*7*). Organisms are known to utilize a variety of mechanisms that regulate

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the stability of the genome and generally operate to ensure that the introduction of mutations is minimized. One mechanism central to genomic stability and the control of mutagenesis is DNA repair. The primary function of DNA repair is to remove potentially deleterious lesions through either damage reversal or damage excision. Damage excision processes are characterized by the mechanism of excision employed. Nucleotide excision repair (NER),[1] for example, is characterized by dual incisions in the phosphodiester backbone both 5′ and 3′ to the damaged site, and the resulting repair patch ranges from approximately 12−29 nucleotides (*8*). Alternatively, during the base excision repair (BER) process, repair is initiated and damaged bases are excised by cleavage of the N-glycosidic bond and repair patches range from 1 to 6 nucleotides (*9*). To date, the only characterized repair pathway in the major malaria-causing parasite *P. falciparum* is the BER pathway (*10*, *11*).

In general, the effect of antimalarial drugs on mutagenesis in the parasitic genome has not been characterized. There is evidence that certain antimalarial drugs interfere with DNA repair. Chloroquine, one of the most widely used antimalarials, has been shown to inhibit DNA excision repair processes (*12*−*14*) and increase mutagenesis in a variety of organisms (*15*, *16*). While the exact mechanism by which chloroquine inhibits DNA repair is unknown, this antimalarial is known to directly bind to DNA, and disruption is thought to be due to altered DNA polymerase activity on chloroquine-complexed substrate (*17*, *18*). Organisms with defective DNA repair are often referred to as "mutators" because the rate at which mutations are generated in their genome is elevated (*19*, *20*). The ARMD phenotype (accelerated resistance to multiple drugs), described for *P. falciparum* strains that have elevated rates of acquired drug resistance, is suggestive of a mutator phenotype although the underlying mechanisms are unknown (*21*).

To better understand the molecular mechanisms of mutation induction and drug resistance, we designed a system to monitor DNA repair in *P. falciparum*. We present data that demonstrate the presence of repair pathways specific for ultraviolet (UV) light-induced DNA damage in *P. falciparum* extracts, show differences in the levels of repair among various parasite strains, and reveal that certain antimalarial drugs interfere with parasitic DNA repair activities.

EXPERIMENTAL PROCEDURES

Proteins, Enzymes, and Reagents. Purified Uve1p from *Schizosaccharomyces pombe* was kindly provided by Dr. Paul Doetsch (Emory University). XL10-Gold ultracompetent cells were purchased from Stratagene. [α^{32}P]-dATP (3000 Ci/mmol) was purchased from Amersham.

Parasite Culture. Parasite cultures (5% hematocrit) were grown at 37 °C under standard culture conditions in RPMI 1640 supplemented with 10% human plasma, 2 mg/mL hypoxanthine, 25 mM Hepes, and 28 mM sodium bicarbonate. Media was replaced daily, and cultures were gassed with 5% O_2, 5% CO_2, and 90% N_2. Morphology and parasitemia were assessed by microscopic examination of thin blood smears stained with Giemsa stain. Parasitized red blood cells were harvested when cultures were between 7% and 10% parasitemia and were in the trophozoite stage.

Parasite Extract Preparation. Parasitized red blood cells were isolated by centrifugation at 5000 × *g*, washed once with 2 volumes of RPMI and twice with 1× PBS, pH 7.4, and lysed by the addition of 1× PBS/0.15% saponin. Isolated parasites were further washed with 1× PBS until all red blood cell debris was removed. Parasite pellets were resuspended in lysis buffer (50 mM Tris-Cl, pH 7.4, 250 mM NaCl, 0.5 mM EDTA, 0.1% NP-40, 40 mM NaF, 1 mM PMSF, 2 μg/mL aprotinin, and 2 μg/mL leupeptin) and were lysed by vortexing eight times for 45 s with 5 min on ice between bursts. Insoluble protein was removed by centrifugation at 20 000 × *g* for 30 min at 4 °C. Protein concentrations were determined by Bradford analysis (Biorad).

DNA Substrates. pUC18 was transformed into XL10-Gold cells and purified from large cultures using the Qiagen Maxiprep kit according to the manufacturer's instructions. Plasmid DNA was diluted to 400 ng/μL in TE buffer, pH 8.0, and, when indicated, subjected to 80 J/m^2 of UV light using a Stratagene Stratalinker.

Incision Assay. Four hundred nanograms of irradiated or unirradiated pUC18 was incubated with varying amounts of *P. falciparum* extract for 60 min at 37 °C in a buffer containing 40 mM Tris-Cl, pH 7.4, 10 mM $MgCl_2$, and 1 mM DTT (50 μL final volume). The 3D7 parasite clone was used in all assays unless otherwise indicated. Reactions were terminated by the addition of EDTA, pH 8.0, to a final concentration of 10 mM, SDS to 0.2%, and proteinase K to 200 μg/mL followed by an additional incubation for 60 min at 55 °C and phenol/chloroform/isoamyl alcohol (PCIA) extraction. Reaction products were separated by electrophoresis on a 0.8% agarose gel containing 1.5 μg/mL ethidium bromide and were visualized by exposure to UV light. As a control for the presence of UV damage in the repair substrates, 400 ng of UV-damaged pUC18 was incubated with 170 ng of Uve1p in a 10 μL reaction containing 40 mM Tris-Cl, pH 7.4, 10 mM $MgCl_2$, and 1 mM DTT for 1 h at 37 °C.

Complete Repair Reactions. Complete repair reactions were carried out under the same conditions as the incision assays using 25 μg of *P. falciparum* extract with the addition of 5 μCi of [α^{32}P]-dATP (3000 Ci/mmol), 2.5 μg of creatine kinase, 40 mM phosphocreatine, and dATP (8 μM), dCTP, dGTP, and dTTP (50 μM each) to each reaction, and the incubation time was extended to 360 min unless otherwise indicated. Following PCIA extraction, unincorporated radiolabeled nucleotides were removed using ProbeQuant G-50 Micro Columns (Amersham), and the reaction products were separated by agarose gel electrophoresis. DNA incision was visualized as described above. The gel was then dried, and subsequent reaction steps were visualized by autoradiography. Repair assays that included antimalarial drugs were carried out exactly as described above, except damaged substrates were incubated for 30 min on ice with or without various compounds dissolved in water or DMSO at indicated concentrations prior to the addition of any other reaction components. Final DMSO concentrations never exceeded 6%, and the addition of DMSO did not change the level of repair activity (data not shown).

[1] Abbreviations: NER, nucleotide excision repair; BER, base excision repair; ARMD, accelerated resistance to multiple drugs; UV, ultraviolet; CPD, cyclobutane pyrimidine dimer; 6−4 PP, 6−4 photoproduct.

ELISA Assay. Parasitized red blood cells (7−10% parasitemia) were exposed to 254 nm UV light at 90 J/m^2. The parasites were returned to 37 °C under standard culture conditions and allowed to recover for 0, 30, 60, or 120 min. The cells were harvested, and DNA was extracted using an EasyDNA kit (Invitrogen). The amount of DNA damage was then quantified by means of an indirect ELISA assay. Briefly, 96-well microtiter plates were coated in triplicate with 100 ng of sample DNA. The plates were placed in an incubator at 34 °C and allowed to dry for several days. Two hundred microliters of blocking solution (0.2× PBS, 1% BSA, 1% goat serum) was added to each well, and plates were incubated at 37 °C for 60 min and washed four times in 0.2× PBS. One hundred microliters of 1:5000 diluted anti-UVssDNA primary antibody (Trevigen) was added, and the plates were incubated overnight at 4 °C, followed by four washes in 0.2× PBS. One hundred microliters of 1:10 000 diluted goat anti-mouse horse radish peroxidase (HRP) (Sigma) was added, and plates were incubated at 37 °C for 60 min and washed four times in 0.2× PBS, and 100 μL of TMB peroxidase substrate solution was added. After 20 min incubation at 37 °C, the absorbance from each well was read at 655 nm in a SpectroMax Plus microplate reader (Molecular Devices).

RESULTS

To evaluate DNA repair activities in *P. falciparum*, we have designed an assay that allows us to analyze the repair of a damage-containing plasmid substrate using parasite extracts. Initial experiments were designed to assess the recognition and cleavage of DNA containing UV light-induced DNA damage. Such DNA lesions consist mainly of thymine−thymine cyclobutane pyrimidine dimers (CPD) or thymine−cytosine 6−4 photoproducts (6−4 PP) and are generally repaired by the nucleotide excision repair pathway (*8*). In the assay, DNA incision is noted by the shift from fast-migrating supercoiled species to more slowly migrating nicked species during agarose gel electrophoresis. The presence of these lesions in the repair substrate was confirmed throughout our analysis using UV damage endonuclease (Uve1p) from *S. pombe*, which recognizes and incises DNA immediately 5′ to a variety of DNA lesions including CPDs and 6−4 PPs (*22*).

Recognition of UV light-induced DNA damage by *P. falciparum* extracts is shown in Figure 1A. There is no difference in the electrophoretic migration of unirradiated and irradiated DNA, indicating that the UV dose used is not sufficient to induce strand breaks (data not shown). Any change in migration, therefore, is due to nuclease activity present in the extract. Inclusion of parasite extract in the reactions demonstrated evidence of DNA incision, as indicated by the shift in electrophoretic mobility (Figure 1A, lanes 4−6). The incision was absolutely dependent on the exposure of the DNA substrate to UV light as illustrated by the absence of DNA incision when unirradiated plasmid was incubated with the highest amount of *P. falciparum* extract (Figure 1A, lane 3). These experiments demonstrated that proteins were present in *P. falciparum* that recognized DNA containing lesions induced by UV light. Figure 1B shows the effect of varying the dose of UV light on the ability of *P. falciparum* extract to recognize damage-containing DNA and to initiate repair. Increasing doses of UV light (10, 40, and 80 J/m^2) resulted in an increased amount of DNA incision.

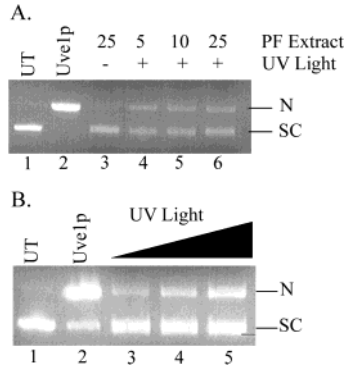

FIGURE 1: UV light-dependent DNA incision using *P. falciparum* extracts. In panel A, 400 ng of unirradiated or irradiated pUC18 was incubated with varying amounts of *P. falciparum* extract (5, 10, or 25 μg) for 60 min at 37 °C in a buffer containing 40 mM Tris-Cl, pH 7.4, 10 mM $MgCl_2$, and 1 mM DTT (50 μL final volume). Reaction products were separated by electrophoresis on a 0.8% agarose gel containing 1.5 μg/mL ethidium bromide. The migration of buffer-treated plasmid DNA that has been irradiated is indicated (UT). Incubation of the damage-containing plasmid with Uve1p serves as a control for the presence of UV light-induced damage. The migration of nicked (N) and supercoiled (SC) DNA species are indicated. In panel B, incision reactions were carried out as described for panel A using 25 μg of *P. falciparum* extract with varying doses of UV exposure (10, 40, and 80 J/m^2).

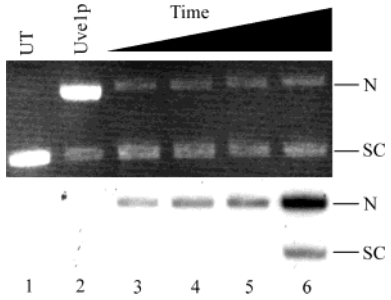

FIGURE 2: *P. falciparum*-mediated repair of DNA substrates containing UV light-induced damage. Complete repair reactions were carried out under the same conditions as the incision assays using 25 μg of *P. falciparum* extract with the addition of [α^{32}P]-labeled dATP, an ATP generating system, and additional unlabeled nucleotides. Reactions were terminated at 30, 60, 180, and 360 min. DNA incision was assessed as described in Figure 1, the gel was dried, and subsequent steps in the repair were evaluated by autoradiography. The photograph of the agarose gel is depicted on top with the corresponding autoradiograph shown below. Nicked (N) and supercoiled (SC) DNA species are noted.

To assess the ability of *P. falciparum* extracts to completely repair the UV damaged substrate, assays were designed such that subsequent repair events could be monitored. For these reactions, [α^{32}P]-labeled dATP was included in the reaction in addition to unlabeled nucleotides and an ATP generating system to support DNA synthesis and ligation. Figure 2 represents the results of this analysis (agarose gel, top; autoradiograph, bottom). Lanes 3−6 illustrate the results obtained when *P. falciparum* extracts were incubated with the damaged substrate for 30, 60, 180, or 360 min. As demonstrated earlier, DNA incision events were observed at all time points of the experiment. With increasing time, subsequent steps of the repair process were visualized on the autoradiograph as radiolabeled bands that

migrated as distinct species. The incorporation of radioactive nucleotides into the nicked and supercoiled DNA species indicated the occurrence of DNA repair synthesis and ligation, respectively. The presence of a radioactively labeled, supercoiled DNA species indicated that following incision, nucleotides were incorporated into the DNA scaffold and the integrity of the DNA backbone was restored, noting complete DNA repair.

Three *P. falciparum* strains were identified for further evaluation on the basis of their known drug susceptibilities: the pan-drug-sensitive clone, 3D7 ("Netherland"); the mefloquine-resistant clone, D6 (Sierra Leonne); and the multidrug-resistant clone, W2 (Indochina) (*21*, *23*). For this analysis, every effort was made to ensure that the parasites were harvested under similar conditions with respect to percent parasitemia and asexual, erythrocytic stage of the cell cycle. Equivalent amounts (20 μg) of *P. falciparum* extract were combined with UV light-treated pUC18 and analyzed as described above. Interestingly, this comparison revealed different capacities for DNA repair among these parasites (Figure 3A). Although the relative level of DNA incision observed on the agarose gel did not vary greatly among the different strains, subsequent DNA synthesis differed appreciably. Notably, the multidrug-resistant strain (W2) demonstrated limited evidence of complete DNA repair, while the drug-sensitive strain (3D7) demonstrated excellent repair activity.

An ELISA-based assay was also developed to further examine the observed variations in DNA repair. Parasites were irradiated with various amounts of UV light, and genomic DNA was isolated immediately or after a specific period of recovery time. The presence of DNA lesions was used as a marker for the efficiency of DNA repair and was detected using a monoclonal antibody specific for UV light-induced 6−4 photoproducts. The persistence of lesions in this assay is consistent with the repair profile observed in the plasmid repair assay (Figure 3B). Removal of the UV damage is markedly reduced in the W2 parasite when compared to 3D7. Repair in D6 is also significantly lower.

It has been shown that the commonly used antimalarial drug chloroquine inhibits DNA repair activity in a variety of organisms (*14*, *24*−*26*). We evaluated the effect of chloroquine, as well as various antimalarial drugs, on DNA repair in our system. Figure 4A illustrates the effect of chloroquine on the ability of *P. falciparum* extract to complete DNA repair. Lanes 3−7 represent the repair of UV light-damaged substrate by parasite extracts in the presence of increasing amounts of chloroquine (0, 0.97, 9.7, 970, 2910 μM, respectively). These results indicated that the presence of chloroquine resulted in a marked interference with DNA repair. The most appreciable inhibition of repair activity appears to involve DNA synthesis; however, at the highest concentrations of chloroquine, DNA incision is also inhibited. Our results demonstrate that chloroquine does inhibit *P. falciparum* DNA repair machinery, which is consistent with previous findings in other organisms. This inhibition most likely results from a physical block of the DNA polymerases due to the ability of chloroquine to bind to DNA (*12*, *17*, *27*).

To determine whether other antimalarial drugs inhibit DNA repair activities in a manner similar to chloroquine, several additional compounds were examined. Several drugs

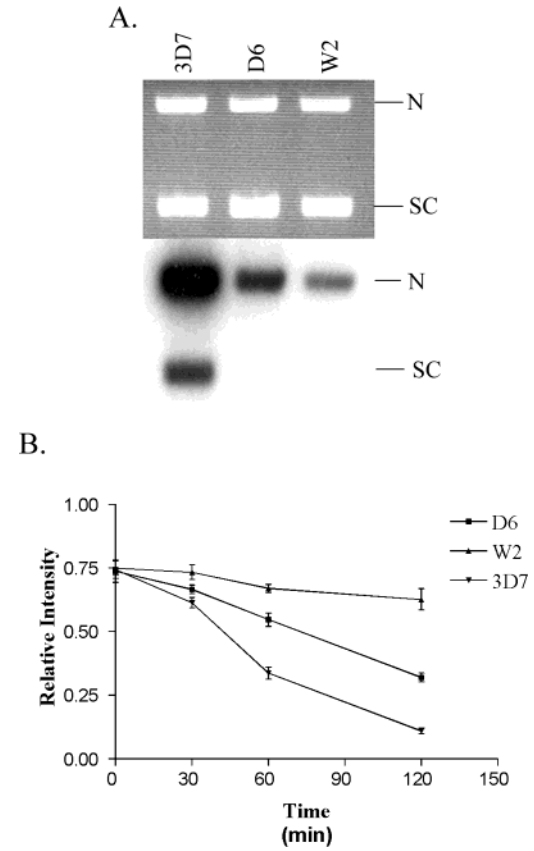

FIGURE 3: Comparison of DNA repair in drug-sensitive and drug-resistant parasites. Panel A displays results of the in vitro repair assay. Extracts (25 μg) from various parasite strains were incubated with irradiated plasmid (400 ng) in a complete repair reaction. The *P. falciparum* strain 3D7 is a representative drug-sensitive isolate, D6 is resistant to mefloquine, and W2 is resistant to chloroquine, quinine, pyrimethamine, and sulfanilamide. Reactions were performed as previously described. Data are representative of three independent experiments from three different protein isolations. Panel B displays the results of the ELISA assay. The presence of DNA lesions was analyzed using the anti-UVssDNA antibody. Parasite cultures were exposed to 254 nm UV light at 90 J/m^2 and allowed to recover for 0, 30, 60, or 120 min. Relative intensity is indicated for the D6 (■), W2 (▲), and 3D7 (▼) parasites, respectively.

were selected on the basis of structural similarities with chloroquine but had not been previously analyzed for DNA repair inhibitory activity. Figure 4B demonstrates the effects of the antimalarial drugs quinine, halofantrine, artemisinin, and mefloquine on DNA repair. These results indicated that there was no apparent inhibition of repair with artemisinin (lane 6) when compared to the control lane 3, while a substantial reduction in DNA synthesis was noted with mefloquine (lane 7). Results of experiments with quinine (lane 4) and halofantrine (lane 5) showed modest levels of inhibition, although not as dramatic as that demonstrated by mefloquine. Interestingly, the initial incision events are inhibited by the addition of mefloquine, which was not observed at comparable concentrations of chloroquine.

DISCUSSION

The underlying cause of drug resistance in malaria is the development of specific genetic mutations. Understanding the mechanisms by which these genetic alterations are induced may further our knowledge of how drug resistance

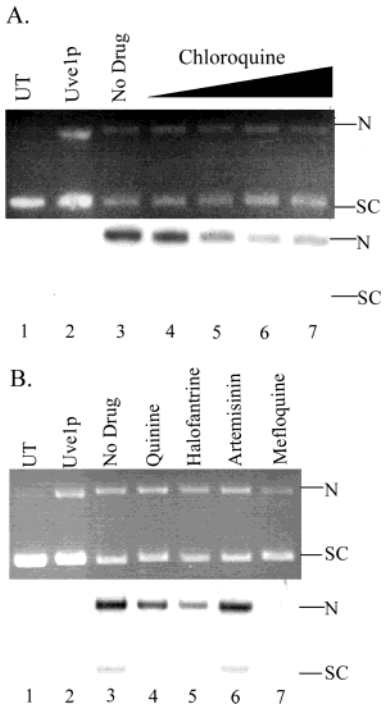

FIGURE 4: Antimalarial drugs inhibit the repair of UV light-induced DNA damage. In panel A, irradiated pUC18 was incubated with increasing amounts of chloroquine (0, 0.97, 9.7, 970, and 2910 μM) on ice for 30 min prior to the addition of the remaining reaction components. In panel B, inhibition of DNA repair was also assessed for quinine, halofantrine, artemisinin, and mefloquine. The drug concentration in these reactions was 1.5 μg/μL.

develops in the parasite. One explanation for the development of such mutations is the concept of a "rapid mutator" phenotype that results in a transient burst of mutagenic activity under drug pressure (*21*). A transient mutagenic event such as this could directly generate a resistance-causing mutation. Conversely, an alteration leading to a more permanent environment of mutagenesis may be generated initially, followed by the subsequent acquisition of a resistance-causing mutation. In either case, the endpoint is an increase in the number of DNA mutations generated and, consequently, an increase in the potential for acquiring drug resistance. The change in mutagenic potential could be the result of a variety of cellular defects, one of which is the inhibition of DNA repair.

These studies further characterize DNA repair in the malaria-causing parasite *P. falciparum.* This is the first report to demonstrate the repair of UV light-induced DNA damage in this organism. Although UV light-induced lesions may not be biologically relevant to the erythrocytic stage of the parasite, they represent only one class of a number of deleterious lesions that are repaired by the NER machinery (*8*). Thus, UV light serves as a convenient damage-inducing agent to facilitate the study of this pathway and DNA repair in general. Our results demonstrate that extracts from a variety of *P. falciparum* strains recognize and incise plasmid DNA containing UV light-induced DNA lesions (Figures 1 and 3). While the data presented does not allow for the determination of the specific DNA repair pathway involved in this analysis, evidence from other model systems implicates the NER pathway in the repair of UV light-induced DNA lesions, and this is most likely the mechanism employed in this assay (*2*). This hypothesis is supported by the observation that there are several sequences identified in the *P. falciparum* genome sequence database (PlasmoDB) that are homologous to genes involved in NER from other organisms, indicating that this pathway is likely present in the parasite (*28*). Sequences sharing homology to the ERCC1, ERCC4, and XPG nucleases as well as the XPD helicase, DNA ligase I and DNA polymerases have been identified.

We further analyzed the steps following incision during our examination of DNA repair among various parasite strains and in the presence of antimalarial drugs. This comparison demonstrated a striking difference in the level of DNA repair, specifically DNA synthesis, among *P. falciparum* strains of varying drug susceptibilities (Figure 3). Defective DNA repair would suggest that the potential exists for an increase in the mutation rate that could eventually lead to the acquisition of resistance-causing mutations. It has been previously reported that the drug-resistant W2 strain used in this study developed resistance to novel drugs in the laboratory at significantly higher rates than a drug-sensitive counterpart (ARMD phenotype; *21*). Our results suggest that defective DNA repair may be one of the molecular mechanisms leading to this phenotype of rapid drug resistance. Interestingly, the D6 strain, which has reduced DNA repair although not as low as W2, does not possess the ARMD phenotype. Defective DNA repair may be the first of many steps required for the development of the ARMD phenotype and may be the mechanism by which subsequent mutations are acquired. D6 may be representative of an intermediate phenotype between a non-ARMD and an ARMD parasite strain. Additionally, other mechanisms that may lead to increased mutagenesis include defects in the fidelity in DNA polymerases, altered levels of recombination, or increased translesion synthesis. These mutagenic pathways have not been analyzed in parasites with the ARMD phenotype. Further analysis of the NER machinery in the parasite is critical and will help to determine the nature of the DNA repair defect and address the varying levels of repair among the different parasites.

Certain antimalarial compounds inhibit DNA synthesis during DNA repair (Figure 4). The ability of various antimalarial drugs to interfere with the parasite's capacity to repair damaged DNA supports the concept of drug-induced genetic variation as a potential mechanism for developing the mutations necessary for drug resistance. It is interesting to speculate that the mutations leading to the ARMD phenotype described above may be a result of a transient, drug-induced increase in the DNA mutation rate.

Although the drug concentrations at which significant inhibition of repair is observed may be above physiologically relevant levels, there are several noteworthy arguments that can be made that strengthen the significance of these observations. First, the drug-related effects observed in this assay represent considerable inhibition of DNA polymerase activity. Taking into account the essential roles of DNA polymerases, an inhibitory activity of this magnitude in vivo would be detrimental to any organism. However, moderate inhibition at physiologic concentrations of a drug could likely alter the overall mutation rate as has been shown for chloroquine (discussed below). Second, the local drug concentrations within the parasite may vary considerably from observed serum concentrations. Although the serum concentration for chloroquine has been estimated at ap-

proximately 15 μM, it is concentrated several thousand-fold in the parasitic food vacuole (*29*, *30*). It is likely that the concentration of other antimalarial drugs may also be elevated in the parasite. Finally, changes in the drug efflux pattern may alter local drug concentration significantly and may also change the subcellular localization of drugs.

The effects of chloroquine on a variety of mutagenic processes have been well documented (*12*−*16*). If continued chloroquine exposure did in fact play an active role in driving drug resistance, then the question is raised as to why so many years passed between the initial uses of the drug and the emergence of resistance. Mutations in the *pfcrt*, *pfmdr*, *cg1*, and *cg2* genes have been associated with resistance, but it is not entirely clear whether a single mutation alone is sufficient for high levels of chloroquine resistance (*31*−*34*). With respect to the number of required mutations, chloroquine resistance may be analogous to the development of other diseases such as cancer. A single mutation may be necessary for the initiation of the disease (or in this case, drug resistance) but may not be sufficient for progression in the absence of other critical genetic alterations (*35*). Interestingly, in many malignancies, where genomic instability and altered mutation rates are well documented, defective DNA repair has been correlated not only with the progression of the disease, but also with the development of drug resistance (*36*−*38*). The theory of multiple mutations and chloroquine resistance is further supported by studies that demonstrated that classes of compounds that mediate the reversal of chloroquine resistance, such as verapamil, only partially restore drug susceptibility in some drug-resistant parasites (*39*).

If the current trend in the development of antimalarial drug resistance continues, we can expect to see an increasing threat of malaria in the future. To avoid such an occurrence, the drug development industry must either produce new antimalarial drugs at an ever-increasing rate, which may be unlikely, or begin to develop new antimalarials with increased durability. This study suggests that one avenue to maximize the durability of future antimalarials may be to assess a candidate drug's ability to interfere with the parasites capacity to generate potentially resistance-causing genetic alterations.

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