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DEPARTMENT OF BIOLOGY

IMPACT OF DRUG DOSE AND TREATMENT TIME ON THE SYNERGISM BETWEEN PYRIMETHAMINE AND SULFADOXINE IN THE MOUSE MALARIA MODEL

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ABSTRACT

One of the most threatening and quickly growing health issues is drug resistance, and malaria is a disease with a rampant history of it. A common strategy to battle resistant parasites is to apply another drug aggressively; however, doing so selects for resistance to the whole combination. This is especially true in synergistic drugs, where the primary and partner drugs potentiate each other to be more effective as a combination than either drug is on its own. There is evidence suggesting that different partner drug ratios and treatment timings at different points in immune response development could alter resistant parasite growth. This project examines changes in these factors in a classic failed antimalarial combination sulfadoxine-pyrimethamine (S/P), which synergistically impairs the parasite folate synthesis pathway that is necessary for growth. Different levels of sulfadoxine were tested in combination with a constant high dose of pyrimethamine on pyrimethamine-resistant parasites, first 1 day post-infection and in another experiment 6 days post-infection.

In early treatment, higher doses of sulfadoxine increased the synergism and delayed growth rather than killing the parasites, which suggests that these doses may be optimal for inhibition without killing and as a result selecting for resistance to S/P. This could mean that S/P failed due to the ratio of partner to primary drug being too high. In later treatment, the effect of synergism changed to clearing infection, with even small doses of sulfadoxine clearing parasites immediately. However, sulfadoxine in later treatment also increased the probability of recrudescence, suggesting that no sulfadoxine treatment is actually better when pyrimethamine-resistant parasites are at their peak. Host health measured by anemia did not differ much by timing of treatment.

Ultimately, these results lead to better understanding of how best to use antimicrobial drugs in combination and when to prescribe treatment, and they will contribute to the goal of developing treatment strategies that avoid inadvertently promoting the growth of drug-resistant pathogens. Future experiments are planned to test high, medium, and low S/P ratios in mixed infections to explore the chance of preventing S/P resistance development by competitive suppression and to determine the optimal dose.

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Chapter 1
Partner drug dose and treatment timing significantly alter synergism and resistant parasite growth in single infections

1.1 Introduction

As one of the top public health crises facing modern medicine, antimicrobial drug resistance kills hundreds of thousands worldwide each year [1]. Drug resistance is the heritable reduction in a pathogen's sensitivity to a drug [2]. Due to the fitness costs that often come with resistance mutations, susceptible pathogens normally suppress the growth of resistant pathogens by outcompeting them for resources and space in the host [3]. Upon aggressive drug treatment, the host is cleared of susceptible pathogens and therefore the competitive suppression, and resistant pathogens rapidly occupy the newly available niche, another phenomenon called competitive release [4-8]. Because it is impossible to create drugs as quickly as parasites evolve or to depend on an infinite number of drug-producing compounds [9, 10], we clearly need to instead develop new treatment strategies that prevent resistance growth in the first place. Evolutionary biology may present the most sustainable solution [11, 12].

One popular strategy to thwart resistance evolution is combination therapy, or applying two drugs instead of one to reduce the probability of resistance mutations. However, aggressive combination therapy imposes strong selection for resistance to the combination [13]. This effect is especially interesting in synergistic drugs, a type of combination therapy in which the drugs enhance each other to be much more effective than either is on its own [14]. The synergism itself

selects even more strongly for resistance to the combination [15], and resistance to one drug also provides resistance to the combination due to inhibition of the synergistic effect [16]. Efficacy of synergistic combination therapy could be improved by changing the dose of the partner drug in respect to the primary drug, which would alter the strength of synergism.

Another important determinant in infection dynamics is drug treatment timing, which alters the point at which pathogens are targeted with respect to factors in the host, such as red blood cell (RBC) status, host nutrient availability, and immune response development. Most interesting is immune response, which has been shown to reduce the emergence of drug resistance when it remains strong during drug treatment [17]. In malaria, cytophilic antibodies can target multiple strains of parasites [18], and the non-specificity could intensify competitive suppression by targeting both susceptible and resistant parasites. Because the immune system is already clearing pathogens, higher drug doses increase the probability of significant resistance growth when resistant pathogens evolve before or during treatment [19]. Additionally, models of malaria infection dynamics fail after day 10 post-infection, indicating that varying immune responses influence variability in post-peak parasite levels [20]. Indeed, evidence of immunity's importance in malaria is shown in that immunodeficient patients suffer the greatest malaria burden [21].

Malaria is one disease majorly potentiated by drug resistance. In 2015 alone, malaria was responsible for 212 million cases and 429,000 deaths [22], and resistance has already developed in 5 countries for the current recommended treatment [23]. Malaria parasites need folate for DNA replication, but because mature RBCs do not uptake it [24], parasites can only obtain it by (1) utilizing folate salvaged from the host serum and/or (2) synthesizing it themselves from p-

aminobenzoic acid (pABA). The failed antimalarial combination sulfadoxine-pyrimethamine (S/P) is a very effective synergistic pair [25] that reduces parasite folate synthesis through inhibited enzyme activity. Pyrimethamine (the primary drug) inhibits the first pathway at dihydrofolate reductase (DHFR) [26], and sulfadoxine (the partner drug) inhibits the second pathway at dihydropteroate synthase (DHPS) [27]. Pyrimethamine resistance develops before S/P resistance [27], and it comes at the fitness cost of impaired DHFR exogenous folate acquisition [28] and elevated requirement of exogenous pABA [29]. Although sulfadoxine has little effect on parasite survival on its own [30], its combination with pyrimethamine inhibits the growth of pyrimethamine-resistant parasites by inhibiting both pathways [31, 32]. However, high sulfadoxine dose increases the synergism [15] and thus selection for S/P resistance.

Optimal drug doses and treatment timing would reduce infection and prevent resistance by imposing exactly enough selection and not more than is necessary [33]. In this thesis, efficacy of the synergistic pair is studied with different doses of partner drug sulfadoxine and different timings of treatment.

1.2 Methods

Hosts. In both experiments, inbred C57BL/6 mice were divided into groups of 5 mice per treatment group (35 mice in 7 groups for Experiment 1 and 30 mice in 6 groups for Experiment 2). They were fed 5001 Laboratory Rodent Diet (LabDiet, USA). A week before infection, water with 0.05% pABA was administered by standard practice in malaria mouse experiments for optimal parasite growth [34-36]. Experiments were conducted by the protocol approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University (Permit Number 44512-1).

Parasites. *Plasmodium chabaudi* parasites were originally isolated from *Thamnomys rutilans*, or thicket rats. Mice were inoculated with 10^5 pyrimethamine-resistant parasites (AS_{123p} clone) on day 0 in both experiments. This number reflects the approximately 10^4 to 10^5 resistant parasites that burst from the liver in a person who is already infected by another strain [37], accounting for the fact that resistant parasites are rarely seen alone in nature [42-43].

Drug treatments. Mice were treated in 11 doses over 7 days (twice daily on days 1-4 and once daily on days 5-7 in Experiment 1, and the same regimen but on days 6-12 in Experiment 2) to reflect the treatment strategy in humans [38]. All mice were given the same dose of pyrimethamine at 8 mg/kg. Each group of 5 mice received a different dose of sulfadoxine (0, 0.1625, 0.3125, 0.625, 1.25, 10, and 20 mg/kg in Experiment 1, and 1.25, 2.5, 5, 10, and 20 mg/kg in Experiment 2). Both drugs were dissolved in DMSO. Because DMSO has toxic side

effects, control mice with no sulfadoxine treatment were injected with the same amount of DMSO. Each mouse received 0.03 mL solution.

Monitoring infection dynamics.

Parasite density. In Experiment 1, 5 µL of blood was taken from the tail of each mouse daily, and DNA was extracted with the Qiagen DNeasy kit. Parasite density was quantified by quantitative real-time PCR (qPCR). In Experiment 2, the proportion of RBCs infected was determined daily from Giemsa's stained thin blood smears by the CDC protocol [39]. Parasite density was calculated by multiplying the proportion RBCs infected by the RBC count.

Red blood cell count. RBC counts were obtained daily from 2uL of blood taken from the tail analyzed through flow cytometry (Beckman Coulter).

Statistical Analysis. R (version 3.3.1) was used to perform statistical analysis, and mice that died were excluded. In both experiments, total parasite density (the sum of all parasite densities throughout the infection) was calculated and log transformed for analysis. The effects of sulfadoxine dose change on total parasite density and RBC density were analyzed by ANOVA. For Experiment 1, time to continuous growth was calculated by finding the first day post-infection at which parasite density increased for two days consecutively. The effect of sulfadoxine dose on time to continuous growth was analyzed using ANOVA. For Experiment 2, time to recrudescence (recurrent infection) was calculated by finding the first day post-treatment at which parasites were observed. The probability of recrudescence was analyzed with binomial generalized linear models. Total recrudescence density (the sum of all parasite densities post-treatment) was calculated and log transformed for analysis by ANOVA.

1.3 Results

Parasite density

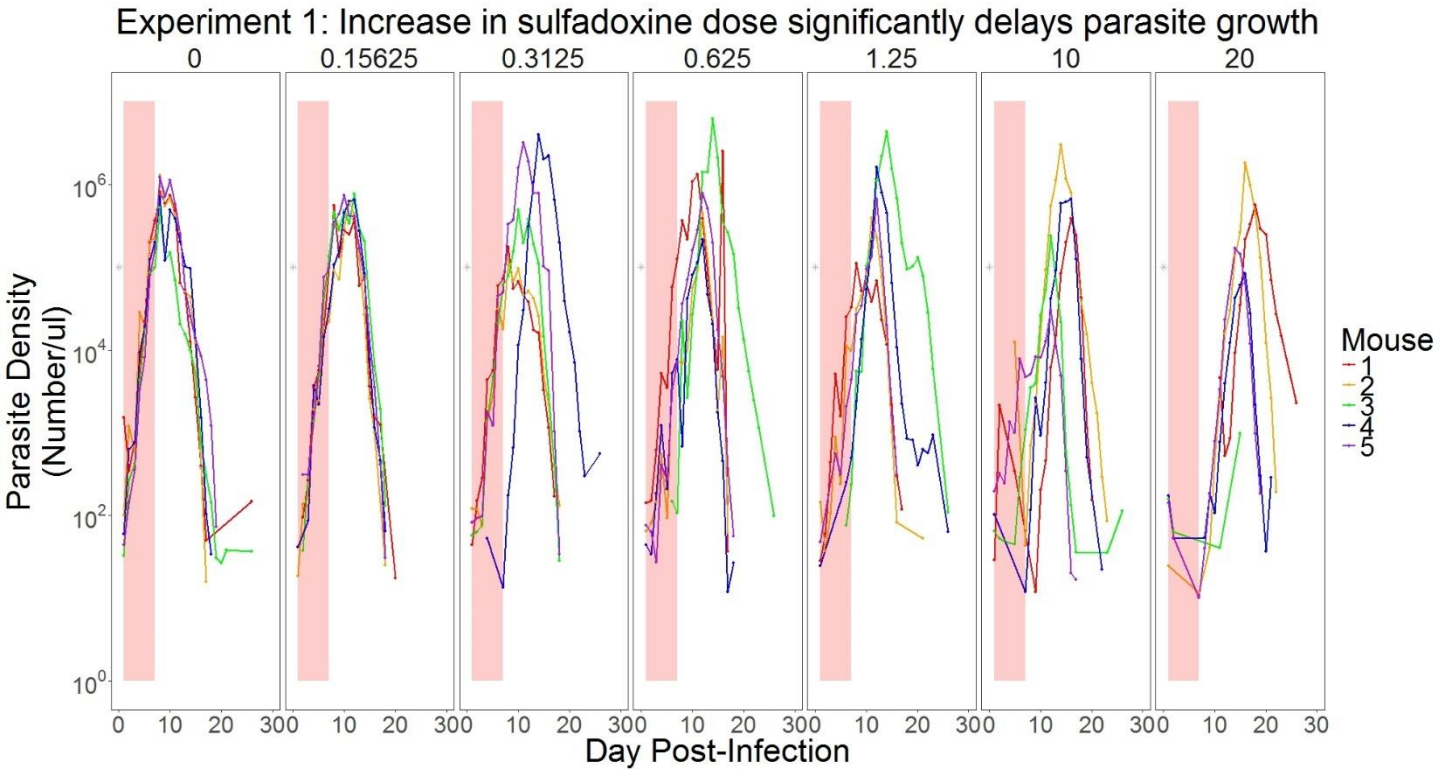

Figure 1. Increasing the dose of sulfadoxine in days 1-7 post-infection significantly delays parasite growth. Each box is a different dose ratio. Pyrimethamine was held constant at 8 mg/kg while sulfadoxine was varied, starting on the left from 0 mg/kg (the control) to 20 mg/kg. The colored lines display parasite density (number/µL blood) over time in days post-infection (PI), with five mice in each treatment group. The grey star at day 0 in each box represents the inoculum time and density, which was constant across groups. Drug treatment, indicated by the red bars, also was constant for all groups: twice daily on days 1-4 and once daily on days 5-7.

Experiment 1

Increasing sulfadoxine dose increased the time until continuous growth of AS_{123} parasites, most significantly at 10 mg/kg and 20 mg/kg (*Figure 1*, $F_{6,28} = 7$, $p < 0.001$). The most marked delay in growth was seen in mice that received the highest sulfadoxine dose. Increasing the sulfadoxine dose did not change the total infection size (*Figure 1*, $F_{6,28} = 1.371$, $p = 0.261$), but it did increase the variance in total parasite density between mice in each group.

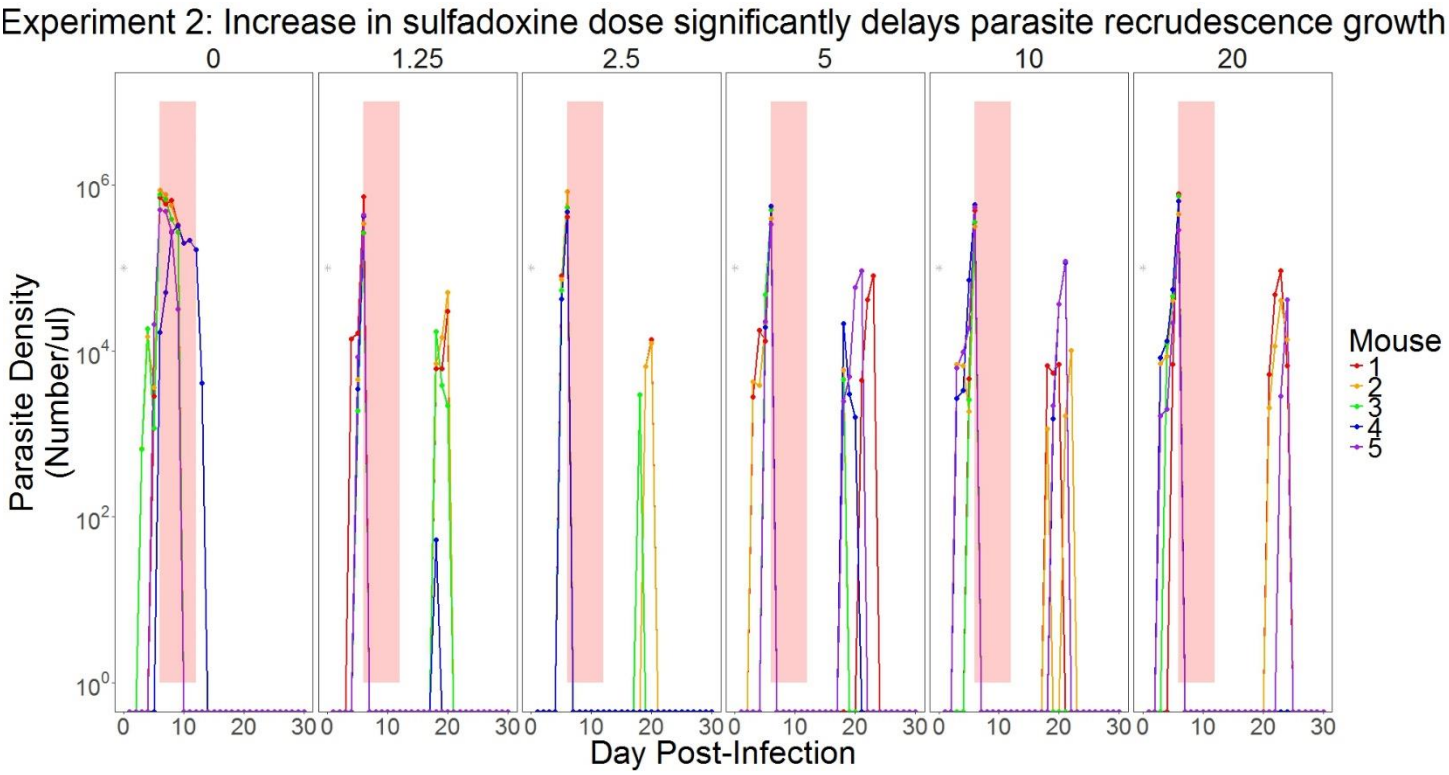

Figure 2. Increasing the dose of sulfadoxine in days 6-12 post-infection significantly delays parasite recrudescence growth. Each box is a different dose ratio. Pyrimethamine was held constant at 8 mg/kg while sulfadoxine was varied, starting on the left from 0 mg/kg (the control) to 20 mg/kg. The colored lines display parasite density (number/µL blood) over time in days post-infection (PI), with five mice in each treatment group. The grey star at day 0 in each box represents the inoculum time and density, which was constant across groups. Drug treatment, indicated by the red bars, also was constant for all groups: twice daily on days 6-9 and once daily on days 10-12.

Experiment 2

All treatments by sulfadoxine significantly decreased the total parasite density of the infection (*Figure 2*, $F_{5,23} = 11.83$, $p < 0.001$). This is most likely driven by the difference between the control (0 mg/kg S), where parasites grew through pyrimethamine treatment, and S/P treated groups, in which parasites were undetectable within 24 hours of treatment.

The frequency of recrudescence was significantly increased upon treatment by sulfadoxine (*Figure 2*, $\chi^2_5 = 16$, $p = 0.006$). Again, this is most likely because of the difference between the control which had no recrudescence and the treated groups which all had some mice

with recrudescence. Increasing sulfadoxine dose significantly increased the time of recrudescence (*Figure 2*, $F_{4,14} = 8.319$, $p = 0.001$). The delay was increased by an average of 5 days between the lowest sulfadoxine treatment group (day 18), to the highest sulfadoxine treatment group (day 22). There was no significant difference in total recrudescent infection size between the groups in mice that had recrudescence (*Figure 2*, $F_{4,14} = 0.872$, $p = 0.505$).

Red blood cell density

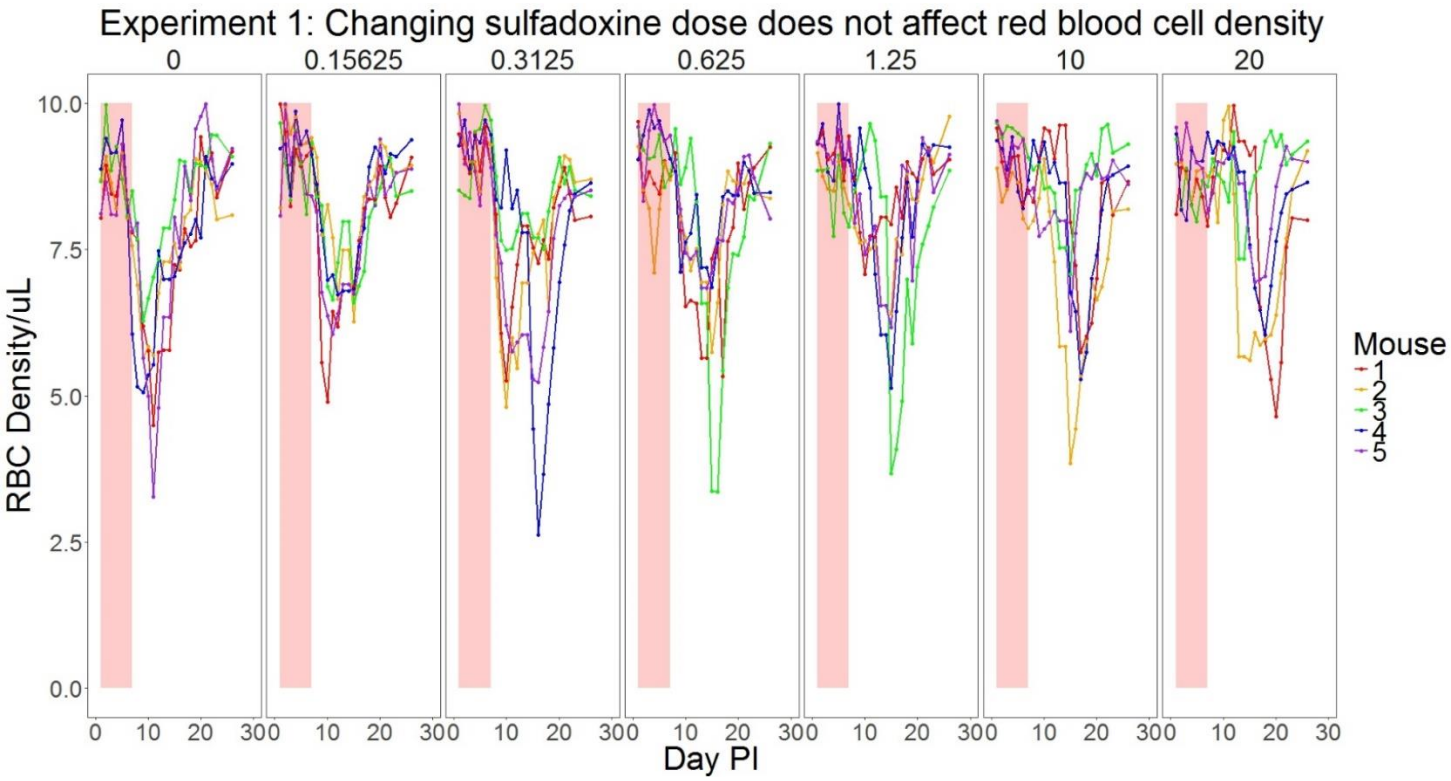

Figure 3. Increasing the dose of sulfadoxine in days 1-6 post-infection does not affect red blood cell density. Each box is a different dose ratio. Pyrimethamine was held constant at 8 mg/kg while sulfadoxine was varied, starting on the left from 0 mg/kg (the control) to 20 mg/kg. The colored lines display RBC density (number/µL blood) over time in days post-infection (PI), with five mice in each treatment group. The grey star at day 0 in each box represents the inoculum time and density, which was constant across groups. Drug treatment, indicated by the red bars, also was constant for all groups: twice daily on days 1-4 and once daily on days 5-7.

Experiment 1

Increasing sulfadoxine dose did not significantly change the total RBC density (*Figure 3*, $F_{6,28} = 1.152$, $p = 0.5391$). Increasing sulfadoxine dose also did not significantly change the minimum RBC density (*Figure 3*, $F_{6,28} = 0.64$, $p = 0.697$), although it did significantly increase the time of minimum RBC density (*Figure 3*, $F_{6,28} = 5.681$, $p < 0.001$).

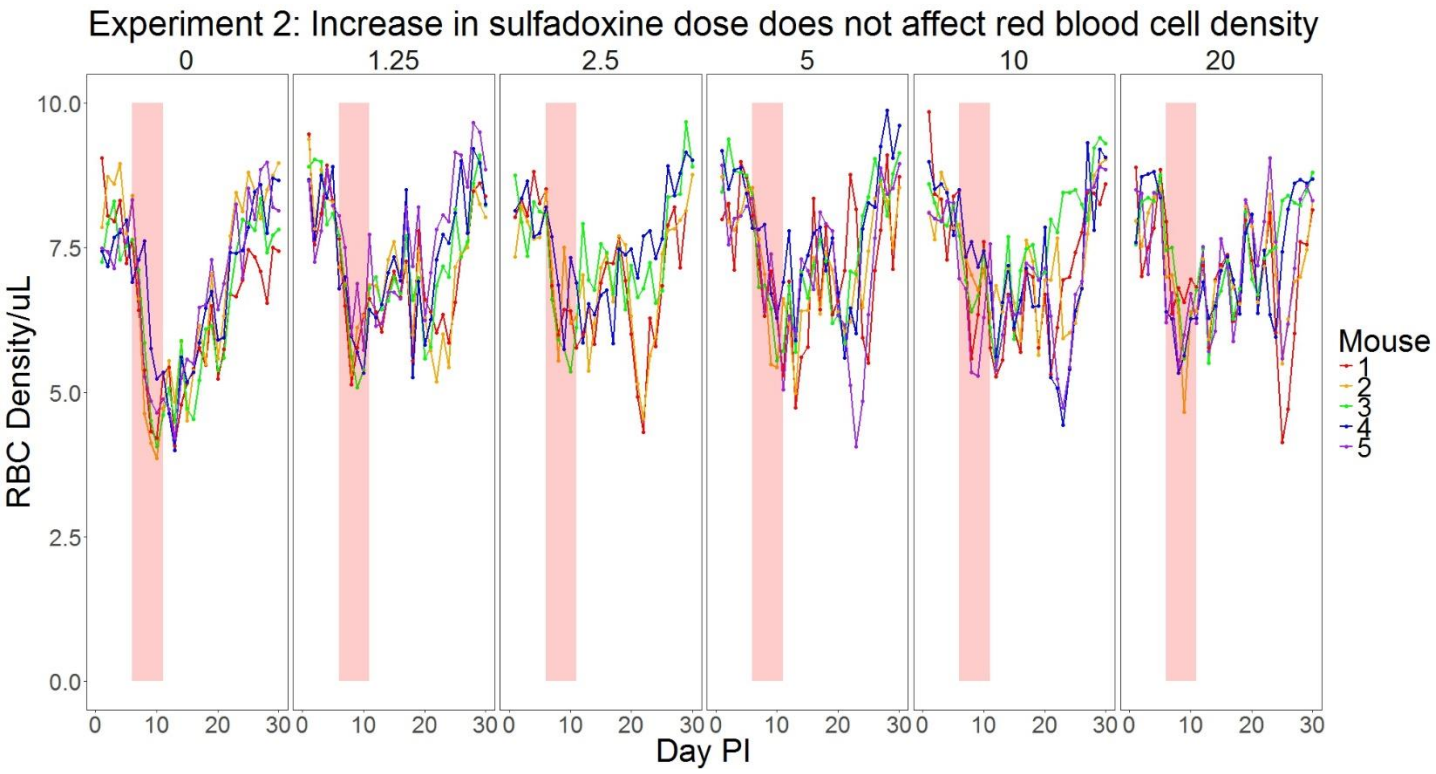

Figure 4. Increasing the dose of sulfadoxine in days 6-12 post-infection does not affect red blood cell density. Each box is a different dose ratio. Pyrimethamine was held constant at 8 mg/kg while sulfadoxine was varied, starting on the left from 0 mg/kg (the control) to 20 mg/kg. The colored lines display RBC density (number/µL blood) over time in days post-infection (PI), with five mice in each treatment group. The grey star at day 0 in each box represents the inoculum time and density, which was constant across groups. Drug treatment, indicated by the red bars, also was constant for all groups: twice daily on days 6-9 and once daily on days 10-12.

Experiment 2

Treatment by sulfadoxine significantly increased the total RBC density (*Figure 4*, $F_{6,28}$ = 5.297, p = 0.00221). Sulfadoxine treatment also significantly increased minimum RBC density (*Figure 4*, $F_{5,23}$ = 3.835, p = 0.0113), especially at the lowest dose of 1.25 mg/kg. These effects are largely because the parasites in the control group grew through treatment, continuing the infection. Changing sulfadoxine dose did not affect time of minimum RBC density (*Figure 4*, $F_{5,23}$ = 0.779, p = 0.575).

1.4 Discussion

Experiment 1

Sulfadoxine treatment early in infection resulted in significant delays in pyrimethamine-resistant parasite growth. Once parasites began growing continuously after the delays, sulfadoxine did not inhibit or even change the overall magnitude of the infection even at the highest drug concentrations. Similarly, the time of minimum RBC density was increased by treatment, so the delay in parasite growth was reflected in delay of peak host illness. Because neither total nor minimum RBC density were affected, only time of illness and not disease severity was changed in respect to anemia (low RBC count). Therefore, it appears that timing of pyrimethamine-resistant parasite growth can be manipulated through changes in sulfadoxine dose without affecting infection size or compromising host health.

The mechanism of delay could involve the decrease of pABA availability resulting from sulfadoxine inhibiting DHPS. Interestingly, experiments with pABA depletion from the host diet in pyrimethamine-resistant single infections showed the same delaying effect, and in mixed infections, pABA depletion was shown to prevent resistance emergence entirely [40]. Based on the resemblance to the results in this study, we propose that sulfadoxine could also prevent resistance emergence in mixed infections. Further studies will be conducted to explore this idea, as discussed in Chapter 2.

Experiment 2

When treated a week into the infection, pyrimethamine-resistant parasites were made susceptible to pyrimethamine by combination with sulfadoxine treatment. This demonstrates that S/P has a massive synergism even with a 4-fold decrease in sulfadoxine dose to a very small concentration. In contrast and as expected, the pyrimethamine-resistant parasites in the control group with no sulfadoxine grew through pyrimethamine treatment, indicating that the clearance in the other groups was entirely due to synergism with sulfadoxine.

While S/P cleared parasites quickly, it also increased the probability of recrudescent parasite growth. All groups treated by sulfadoxine had recrudescent infections of similar size in several mice, while the control did not have any. Curiously, the time that parasites recrudesced was significantly delayed by as much as 5 days between the highest and lowest doses (appearing up to 10 days after treatment ended). This delay could also possibly be attributed to the pyrimethamine-resistant parasites' lowered ability to use pABA.

Host anemia was initially alleviated by sulfadoxine treatment in all dose groups compared to the control, by both measures of higher total RBC density and higher minimum point in RBC density. Although select mice in each group suffered from a low RBC density, overall the treatment groups fared better than the control, especially in the lowest dose group. Despite this, because sulfadoxine also increased the chance of recrudescence, the control treatment was actually better for the mouse as well as for overall clearance of drug-resistant infections. This indicates that we should be especially aware of drug synergism's effects when treating patients who are already sick.

Comparison of treatments

Difference in treatment timing changed the effect of synergism – in early treatment synergism delays growth, but in later treatment synergism clears infection. In fact, when treatment is begun immediately pyrimethamine-resistant parasites are only slightly susceptible to S/P treatment at the highest sulfadoxine doses, and when treatment is begun later, S/P treatment is effective at all studied concentrations even at very low sulfadoxine doses. Notably, the fact that sulfadoxine delays parasite growth in recrudescence with the later treatments indicates that they were still somewhat impeded after drug treatment.

It is uncertain why early S/P treatment does not kill the parasites. We hypothesize that DHPS is inhibited enough for pyrimethamine-resistant parasites to exhibit impairment from pABA deficiency, but not enough for growth to be completely halted. Higher doses of sulfadoxine in S/P treatment need to be studied in order to determine whether or not there is a dose at which pyrimethamine-resistant parasites can no longer survive.

Intriguingly, S/P is given at 20:1 mg/kg in the field [25], which equates to 160:8 mg/kg at the pyrimethamine dose used in these studies (8 times higher sulfadoxine dose than the highest given here). It could be that S/P has always been employed with too aggressive of a partner drug dose, selecting for resistance to the combination by killing the pyrimethamine-resistant parasites. Decrease of partner drug concentration may be optimal for slowing growth without imposing the aggressive selection that would drive S/P resistance evolution. On the other hand, decreasing sulfadoxine to levels too low could result in too little synergism and a complete loss of treatment efficacy. The highest doses in these experiments could be the intermediary between no selection and too much selection. The proposed experiment in Chapter 2 includes the 160:8 mg/kg dose in its design along with the 20:8 and 0.625:8 mg/kg doses in order to clarify these speculations.

Recrudescent infections appearing only when parasites were treated later could be associated with the difference in host status in many factors, but most interestingly in immune response development. When parasites are treated immediately, the immune system may have only barely detected their presence and may not have developed a response yet. At peak growth, the immune system has already had time to begin targeting parasites, and it is often able to clear the infection on its own without drug treatment, as seen in the control group in Experiment 2. The recrudescence exclusive to later treatment timing demonstrates that treating a resistant infection at this point may only be harmful to the host and potentiate resistance.

Overall host health measured by anemia was not changed by different treatment timings. When treated earlier, hosts became sick later with increasing sulfadoxine dose but recovered quickly in most cases, as opposed to later treatment where some hosts were slower in recovery due to the recrudescent infections. From a clinical viewpoint of host health in respect to anemia, there is not a significant advantage to either timing of treatment. However, when the patient already has high levels of parasites resistant to the primary drug, no treatment by partner drug at all may be better.

Conclusions

Based on these results, it is clear that partner drug dose significantly alters the strength of synergism and that treatment timing changes the effect of the synergy. However, because pyrimethamine-resistant parasites rarely exist alone in nature, the effects of the delay on their ultimate fate in mixed infections with pyrimethamine-susceptible parasites remain unclear. We speculate that the delay in growth with the highest sulfadoxine doses in Experiment 1 would provide more time under competitive suppression by pyrimethamine-susceptible parasites, and this period of time could be used to prevent resistance emergence. The further studies designed in the next chapter will investigate the effects in mixed infections with both pyrimethamine-susceptible and pyrimethamine-resistant parasites.

Chapter 2
Partner drug dose and treatment timing in mixed infections

2.1 Introduction

In the dose study of Experiment 1, pyrimethamine-resistant malaria parasites were shown to have a significant delay in initial growth with increasing sulfadoxine dose in combination with a constant pyrimethamine dose. Curiously, the same delay effect has been observed in single infections of pyrimethamine-resistant parasites in pABA depletion experiments [40], which target the same part of the folate pathway as sulfadoxine does. Sulfadoxine, the synergistic partner drug in the sulfadoxine-pyrimethamine (S/P) combination therapy, inhibits DHPS, the enzyme that uses pABA (a folate synthesis precursor) as its substrate. Removal of pABA from the diet, which is possible because mammals do not require pABA [41], decreases substrate availability in the first place.

In single infections with only pyrimethamine-resistant parasites, pABA depletion had no influence on their overall growth as measured by total infection size, which is consistent with the results from the highest sulfadoxine treatments (and therefore strongest pABA depletion) in Experiment 1. However, single infections of pyrimethamine-resistant parasites rarely occur in the field [42-43]. In mixed infections, this delay by sulfadoxine could extend the period in which pyrimethamine-resistant parasites are under competitive suppression by pyrimethamine-susceptible parasites. In order to study the parasites in competition, the experimental design below tests different partner drug doses with both strains present.

In mixed infections, pABA depletion was shown to be lethal to pyrimethamine-resistant parasites under competition due to their original mutation causing impaired DHFR exogenous folate acquisition as well. Importantly, pABA depletion was only sublethal to pyrimethamine-susceptible parasites without the mutation, still permitting their growth although at a slightly depressed level. In fact, pABA depletion has been shown to prevent competitive release of pyrimethamine-resistant parasites entirely [40], most likely by sustaining susceptible parasite levels while delaying resistant parasite growth. The competition is key in this effect because the competitive suppression is created by the susceptible strain's presence, whereas in a single infection of pyrimethamine-resistant parasites, there is no competition for resources and ecological space.

Extending this knowledge, sulfadoxine treatment may imitate pABA depletion and specifically target pyrimethamine-resistant parasites while maintaining their competitive suppression under pyrimethamine-susceptible parasites, employing the drug as a resource-depleting agent that manipulates competition rather than as another treatment intended to kill. Drug treatment provides a more direct, measurable, and practical way to block pABA from parasites than by ensuring its removal from patients' diets.

2.2 Experimental Design

The experiment will be designed similarly to Experiment 1 with *Plasmodium chabaudi* parasites, but with both susceptible and resistant strains present. The susceptible strain will be inoculated at 10^6 per µL on day 0, and the resistant strain inoculated at 10^5 per µL on day 5. This will account for the fact that resistance mutations are most likely to evolve when the susceptible population is growing and of substantial size.

Mice will be treated in 11 doses over 7 days (twice daily on days 1-4 and once daily on days 5-7). All mice will be given the same dose of pyrimethamine at 8 mg/kg dissolved in DMSO. Inbred C57BL/6 mice will be divided into groups of 5 mice per treatment group, and each group will receive a different dose of sulfadoxine as detailed in the table below. Blood will be taken daily from the mouse tails for RBC counts and parasite density. R (version 3.3.1) will be used to perform statistical analysis.

		Treatment Type	Solo (P only) *Control*	Combination (S/P) *Low*	Combination (S/P) *Medium*	Combination (S/P) *High*
		Sulfadoxine Dose (mg/kg)	0	0.625	20	160
Infection Type	**Strains**					
Single	Resistant	5	5	5	5	5
Mixed	Susceptible + resistant	5	5	5	5	5

Table 1. Number of mice in each group of experimental design for mixed and single infections with varying doses of sulfadoxine in S/P treatment. There will be 5 mice in each group and 10 groups for a total of 50 mice. The sulfadoxine doses will be 0 mg/kg (control),0.625 mg/kg (low), 20 mg/kg (medium), and 160 mg/kg (high), while pyrimethamine will be constant across all treatments at 8 mg/kg. Pyrimethamine-susceptible parasites will be inoculated on day 0 at 10^6 per µL, and pyrimethamine-resistant parasites will be inoculated on day 5 at 10^5 per µL. Drug treatment will be given twice daily on days 6-9 and once daily on days 10-12.

2.3 Expected Results

The control as well as low and medium treatment groups with single infections are the same conditions as 3 groups in Experiment 1, so the results are expected to be the same – no effect in the control and low sulfadoxine treatment, and a delay in parasite growth in the medium sulfadoxine treatment. The high treatment group is expected to result in parasite clearance upon treatment but with a chance of S/P resistance growth later in the infection.

In the mixed infection groups, the control and low sulfadoxine treatment groups are expected to have pyrimethamine-resistant growth upon pyrimethamine-susceptible parasite clearance. The medium treatment group is expected to be the optimal dose with no pyrimethamine-resistant parasites detected after treatment due to the delay in their growth. The high treatment group is expected to result in S/P-resistant parasites after drug treatment.

The results of this experiment will provide insight on whether or not an optimal dose of sulfadoxine in S/P combination therapy could prevent resistance emergence and if so, in what range that dose could lie. If the results confirm our hypothesis, they would suggest a better strategy in use of synergistic drugs and possibly explain through ecological concepts why S/P failed in the past.

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ACADEMIC VITA

Michelle S. Lai

Education

Medical Doctor
Icahn School of Medicine at Mount Sinai, New York, NY (expected) May 2021
- FlexMed Program – admitted as a college sophomore

Bachelor of Science with Honors
Biology (vertebrate physiology option), Minor in Health Policy and Administration
The Pennsylvania State University, Schreyer Honors College, University Park, PA May 2017

Experience

Research

Read Group, Center for Infectious Disease Dynamics, *Pennsylvania State University*, PA 2013-17
- Average of 12 hours/week during semesters; full-time position in summers 2014 & 2016
- Studied effects of resource depletion and varying multi-drug dose combinations on prevention of antimalarial drug resistance through manipulation of within-host population dynamics
- Independently designed, executed, and analyzed two large mouse experiments
- Thesis: *Impact of drug dose and treatment time on the synergism between pyrimethamine and sulfadoxine in the mouse malaria model*

Medical

PA Retina Specialists Internship, *Camp Hill*, PA: 90 hours Summer 2015
- Shadowed five ophthalmologists through their daily routines of exams and medical record entering
- Operated Optical Coherence Tomography machine

Operation Room Shadowing, *Harrisburg Hospital*, PA: 70 hours, volunteer 2013-15
- Assisted in setup and cleaning of operating rooms, observed surgeries

Post-Operation and Interventional Radiology, *Harrisburg Hospital*, PA: 100 hours, volunteer 2011-13
- Aided nurses making rounds, observed radiologists taking x-rays and studying images to make diagnoses

Music Therapy, *Community General Osteopathic Hospital*, Harrisburg, PA: 30 hours, volunteer 2011-13
- Played monthly concerts for rehabilitation patients in lounges and hospital hallways

Employment

Teaching Assistant: Freshman Research Seminar SC 297B Fall 2014
- Introduced faculty speakers, helped lead discussions, graded student writing

Model: *Bebe* (promotional), *Deb* (mannequin), *Blush Factor* (photo shoot) 2012-13

Babysitter 2008-13

Service and Leadership

Music Service Club 2014-17
Founder (2014), President (2014-15), Vice President (2015-16), Recruitment (2016-17)
- Created service organization with mission of performing music for elderly, disabled, ill, and youth
- Built the club to where it has twenty active members now and hundreds of student volunteers over the years
- Established connections with nursing homes, special education programs, daycares, and hospitals

- Designed, filled, and led executive board with twelve positions

Council of LionHearts

Member 2015-17

- Met with top service club leaders weekly to discuss advancing service at Penn State
- Collaborated with leaders of other clubs to coordinate joint events
- Organized first Service Organizations Fair where all clubs in Council recruited at the same time

Penn State Health Policy and Administration Department: Teaching Assistant Fall 2015

- Created lesson plans and quizzes, assisted in teaching lectures and organizing course, and graded papers for the course Comparative Health Systems (HPA 401)

GlobeMed: Grass Roots Onsite Work Coordinator (2014-15), member (2013-14) 2013-15

- Primary contact with partner organization, led re-partnering process, planned summer internship trip

MidState Literacy Council: English Tutor Fall 2014

- Taught conversational and medical language to immigrant with very little proficiency in English

Activities

Study Abroad:

Health Policy and Administration Foreign Study, embedded course (Costa Rica) Spring 2017

- Observed rural medical outreach and social health care system from hospital administration officials

Biology of EcoHealth, embedded course (Tanzania) Summer 2014

- Studied health care in Tanzania and how the ecosystem influences disease, medicine, and policy

Athletics:

Penn State Synchronized Swimming Team 2013-14

Honors and Awards

Smile for Sam Scholarship 2017

- Recognizes outstanding students at Penn State who have demonstrated past and continuing leadership

Council of LionHearts Howard Wu Publicity Award 2016

- For the selfless and constant promotion of service at Penn State

Eberly College of Science Undergraduate Research Poster Award 2016
Edward C. Hammond Jr. Memorial Scholarship in Biology 2016
Eberly College of Science Undergraduate Research Proposal Competition Grant 2015
Christopher R. Dyckman and Susan Scotto Scholarship in Biology 2015
Erickson Discovery Grant Program 2014
Pennsylvania State Employee Credit Union Scholarship 2013
Eberly College of Science Braddock Scholarship 2013
Pennsylvania State University Provost Award 2013
Schreyer Academic Excellence Scholarship 2013
National Merit Finalist 2013
AP Scholar with Distinction 2013
National AP Scholar Award 2013

Skills

Languages: Chinese (fluent), Spanish (proficient)
Lab Techniques: Collecting mouse blood samples, microscopy, DNA extraction, qPCR
Computer: Graphing and performing statistical data analysis with *R*; proficiency in MS Word, Excel, and PowerPoint
Medical: Optical Coherence Tomography machine, CPR certified (July 2015)