Sustained Release of the Anti-Malarial Artemether via Polymer Coating of Drug Particles

Summary

The goal of our research is to develop a sustained release subcutaneous formulation of the anti-malarial drug Artemether. This paper clarifies the procedures used in forming, testing, and detecting drug particles, and in effect, introducing a new alternative to modern medical delivery methods.

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Submitted August 5, 2007
Revised August 29, 2007
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Sustained Release of the Anti-Malarial Artemether via Polymer Coating of Drug Particles

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Abstract: Malaria is responsible for over one million deaths every year and is the leading cause of death in children under five in Africa. Treatments against the malaria parasite include multiple oral doses or continuous intravenous infusion. The goal of our research is to develop a sustained release subcutaneous formulation of the anti-malarial drug Artemether. Potential advantages include increased efficacy and a decrease in the development of drug resistance. Formulation was achieved via polymer coating of drug particles. In vitro testing of coated drug particles showed prolonged release of drug content into solution with a slow, continuous rise at start and an eventual plateau.
Introduction

According to the World Health Organization, about 300-500 million people are infected worldwide by malaria and about 1 million people die annually from it. It is the leading cause of mortality among African children under five years of age. *Plasmodium falciparum* is one of the most deadly forms of the parasite, rapidly spreading through the bite of a mosquito and capable of killing its host in a matter of hours. It is growing more and more resistant to commonly used drugs such as Chloroquine and Mefloquine. Especially in developing countries, Resistance can be caused by the lack of medical supplies or the lack of access to a clinic. And when treatment requires multiple doses over several days, resistance becomes an even bigger problem due to non-adherence to therapy and lack of medical infrastructure. A single dose therapy could improve outcomes significantly.

Artemisinin is one of the more popular herbal remedies used in China since the third century. It contains a unique endo-peroxide bridge, its primary mode of attack. Artemisinins’ semi-synthetic derivatives have already been put into use in regions where multidrug resistance has arisen. One disadvantage of these anti-malarial is the relatively short half-life of the peroxide, which necessitates multi-dose administration.

The goal of this program is to sustain the release of the drug to prolong its effects. This will allow longer intervals between dosages and may reduce the chances of the parasite growing resistant to the treatment. In addition to prolonging drug release, the concentration of the drug may also be controlled to remain above therapeutic levels while staying below toxicity levels, thus reducing potentially hazardous side-effects.
Methods and Materials

Drug Particle Formation

Artemether (ARM), the semi-synthetic derivative of Artemisinin (ART), was supplied by Auritec Pharmaceuticals, Inc., purchased from LTK Laboratories. ARM came in crystalline form, making it easy to produce particles from. These ARM crystals are somewhat platey with round edges and range between 100-325\(\mu\)m in diameter.

Particles were made from these crystals through a process of sieving. ARM crystals were placed into known sieve grades, generally 106-125\(\mu\)m in size. At times, the crystals required crushing to increase yield of desired sizes. It was found that light crushing using a mortar and pestle worked best, though another technique exists.

A process of tumble polishing using glass and stainless steel beads (3-6mm in diameter). These beads are placed in the sieves with the crystals and the sieves are capped and placed in a shaker. The vibrations of the shaker allow the beads to jump around and crush the crystals.

After sieving the drug crystals, the desired sized particles are isolated and stored for coating and later dissolution studies.

Coating of Particles

Two polymers are used in the coating process; polyvinyl alcohol (PVA, hydrophilic) and poly lactic acid (PLA, hydrophobic). Since ARM is lipid soluble, the particles were first coated using PVA using a method of dip coating, then coated with PLA using the spray coating technique.

Particles were placed in a pool of PVA on top of black filter paper over a Hirsch funnel. PVA was added drop-wise with a Pasteur pipette over the particles. The polymer was then vacuum-sucked through the filter. The particles were then placed in the oven at 70°C until the filter paper appeared dried from excess polymer. This process was repeated 4 times to achieve the required layers of coating.

Another technique was developed for the application of PVA. Particles were placed in a small cylindrical flask (≈ 5 ml flask) filled with about 1.5 ml of PVA and the opening wrapped with par film after inserting the particles. This mixture was then vortexed to suspend the particles in the polymer and the mixture was immediately poured over a vacuum-filter. The particles were then dried in the oven at 70°C.
An airbrush was used to apply PLA via multiple cycles of spray coating over a Hirsch funnel. During each cycle, the particles were scraped and spread around to ensure complete coating. After a series of three to four scrapes, the particles were put in the oven at 70°C for about 5-7 min. the process was then repeated 3 times. The airbrush was cleaned with the PLA solvent stock (40.7% DCM in ethyl acetate) between each cycle to prevent clogging of the spray components. All spraying and cleaning procedures occurred in the hood. Particle preparation for the GC/MS/SIM was coated with 3% PLA instead of 5% PLA.

A final layer of PVA was applied to the particles to end the coating process.

**Detection of Artemether**

*Literature cites only few detection methods for ARM, only three were used and tested for their capacity to work.*

The initial method of detection was GC/FID (HP 5890 Series II), however, this method was found to be unsatisfactory as it resulted in thermal decomposition of the drug. (figure 1)

A method described by Thomas *et al*, 1992 involved acid decomposition of the endo-peroxide bridge into an α, β keto-ester which has a distinct UV peak at 254 nm (Cary 50 Bio, by Varian). These procedures were adapted for detection in water for use with the dissolution tests. 5M HCl was used as the proton source for decomposition and was added in a ratio of 2-3 (sample: acid).

The time necessary for the complete acidic decomposition was determined by monitoring the reaction of different concentration stocks of ARM at 254 nm for 24 hours, using the UV160U spectrophotometer, by SHIMADZU. It was determined that 6 hours was the minimum wait time before samples can be read. However, it was later found that the decomposition was not complete and that there were other decomposition products, one which read at UV-242 nm.

The procedures for the GC/MS/SIM where provided in the literature by Mohamed S.S. *et al*, detailing which ion spectrum to look for in determining ARM concentration. The method was applied to our GC/MS (GC: HP5890 Series II Gas Chromatogram, Column DB5-MS: 30m, I.D. 0.250mm; MS: HP 5972 Series Mass Selective Detector) and the peak with the described ion spectrum was found to come off at around 12.27min. A standard curve was produced from various concentrations of ARM in EtOAc, based on the height of the peak and an $R^2$ value of 0.9961 was achieved.

**Dissolution Tests**

Dissolution tests were carried out in a USP type II dissolution apparatus. The first test involved a constant volume at 500 ml Di-H$_2$O, paddles set to 30 rpm and approximately 20 mg of ARM
particles, coated and uncoated, in each dissolution well. Only two wells were reserved for each type of particle, total of four wells. Samples were taken at known time intervals and treated with acid 6 hours before taking a reading.

The second dissolution test consisted of a constant volume of 500 ml Di-H$_2$O, approximately 20 mg, and a constant temperature of 38°C. Paddles were set to 30 rpm and three wells were reserved for each type of particles in order to generate standard deviations from the data, total of six wells. Samples were treated with the 5M HCl in a 2-3 ratio and incubated in an oven kept at 40°C till read with the UV-Vis.

The third dissolution test consisted of a constant volume of 500 ml Di-H$_2$O, approximately 30 mg of particles in each well, and a constant temperature of 38°C. Paddles were set to 30 rpm and three wells were reserved for each type of particles (coated and uncoated). 1ml of sample was taken at known time intervals and extracted with 1ml EtOAc, dried with sodium sulfate (anhydrous) and read with the GC/MS/SIM.

**Results and Discussions**

**Figures are provided in appendix A.**

The concern with the particle formation method using the tumble polishing technique is the high loss of particles to the surface of the beads, over crushing of the drug to a powder and the decrease in the life of the sieves. With a mortar and pestle, there allows for more control of crushing and a lot less sample loss.

With regards to the polymer coating, the pool method for PVA application spreads the particles unevenly over the filter paper, increasing amount of particles sticking to the filter. Because of this, another technique was developed using the cylindrical flask. This provides a quicker and practically mess free technique compared to the pool technique by allowing even spread of the particles over the filter paper when poured, thus allowing more retrieval of the particles after drying.

The application of PLA results in high sample loss and a low overall yield of coated particles in the end. The air pressure of the brush cannot be controlled and must be kept at a distance of at least a foot from the surface of the particles. In addition to this, the fan from the hood redirected the spray so the polymer would not fall on the particles while the fan was on. Also, it is believed that the PLA polymer dries in the air before contact with the particles so the particles may never actually get coated with sufficient PLA, which may explain the slight delay in behavior of the coated particle, as shown in figure 3.
Gas Chromatography resulted in multiple decomposition products, regardless of the method used. Because ARM has a low melting point (86.5-87.5°C), it is believed that it decomposes in the injection port. Figure 1 shows one of the more clear spectra retrieved from the GC analysis.

UV-spectroscopy seemed to be the more promising method of detection mainly because it was the only method at the time that was capable of reading ARM and was cited by others to have worked. It is possible though that since ARM is not entirely water soluble, acid decomposition in water may not be complete and maybe the reason for the chaotic data seen in the first dissolution test (figure 2). The first test was conducted without heat controlled wells and so it is possible that this allowed some of the drug to crystallize out during hotter time periods and re-dissolve during cooler times. However, the data from the second dissolution, where the wells were kept at a constant temperature throughout the testing phase, resembled the chaotic data of the first test. Another explanation was drafted that the possibility of sampling out nano-particles of the drug that get decomposed during treatment can read higher than what is representative of the dissolved concentration.

The issue with ARM not being water soluble and that there were more than one acid decomposition products (one peak at UV-242, another at UV-268), questioned the viability of the method. The paper by Mohamed et al detailed the parameters necessary for detecting ART derivatives using the GC/MS. It took some time to try this method of detection since the equipment was not readily available during the early phases of experimentation, yet has proven to be the most effective and reliable method of detection. The dissolution results of the GC/MS/SIM showed a clear separation of coated and uncoated particles (figure. 4). To better understand the relationship, the data was plotted cumulatively, where each data point is the sum of all previous data points, essentially showing the rate of concentration increase for each type of particle, as seen in figure 5. This figure shows that we have managed to prolong the release of the drug slightly.

Conclusion

The UV-Vis method was believed to be the right way to go with regards to the detection of ARM. Some reasons for the jaggedness of the concentration curves were that there may not be a complete decomposition or that there may be microscopic particles in the samples that are being decomposed which lead to the high peaks of concentration in the data curve. But in the end, the concern with the second peak visible in the spectrum at UV-242nm and the jaggedness of the concentration curves suggested that this still was not the method of choice.

Gas chromatography alone was thought to be the wrong method of detection. However, the GC/MS was used and found to be the most effective method for detecting ARM concentrations.
After perfecting this method and applying it to the dissolution system, the data retrieved showed that it is possible to delay the release of drug particles. Though it is shown here to have delayed it only slightly, it is believed that a longer delay in the drug particles’ release can be achieved with more polymer layers or a better coating system that is capable of reduce sample loss during coating procedures.

**Literature Cited**


3. Dr. H. Lai and Dr. NP Singh. 2002. *Dept. of Bioengineering, School of Medicine, University of Washington, USA.*


Appendix A
Figures and Diagrams

Figure 1: Thermal decomposition of Artemether. The decomposition products were never consistent, thus, GC/FID was abandoned as a detection method.

Figure 2: Results of the first dissolution test. It is believed that the jaggedness of the curves is a result of microscopic particles in the samples or a result of uncontrolled heating parameters.
**Figure 3:** Results of the second dissolution test with heat controlled wells. It is believed that the thin layer of PVA allowed for a short delay, hence the offset of the coated curve.

![Dissolution Test II Graph](image1)

**Figure 4:** Results of the third dissolution test using GC/MS/SIM as detection method.

![Dissolution Test III Graph](image2)
Figure 5: Cumulative data comparing the release rates of coated versus uncoated particles.

Appendix B

List of Abbreviations

ARM       Artemether
ART       Artemisinin
PVA       Polyvinyl alcohol
PLA       Poly lactic acid
Di-H$_2$O  De-ionized water
EtOAc     Ethyl acetate
DCM       Dichloromethane