Drug Release Profile for an ETS-1 Inhibitor in ReGel

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ABSTRACT

Arteriovenous (AV) grafts made of expanded Teflon are a type of vascular access often used in hemodialysis. Stenosis often occurs in these grafts as a result of intimal hyperplasia at the anastomosis of the graft and vein. One approach to this problem is localized drug delivery to inhibit intimal hyperplasia, which is the focus of our research. The literature has shown that intimal hyperplasia of an AV graft usually happens at the graft-venous anastomosis, and also there is increased angiogenesis at the site of intimal hyperplasia. In this stage of the research project, we tried using an ETS-1 inhibitor as a potential drug to be delivered with ReGel. However, the drug profile has not produced the desired results, so in the next stage of our research, we plan to use an anti-angiogenic cancer drug, sunitinib.

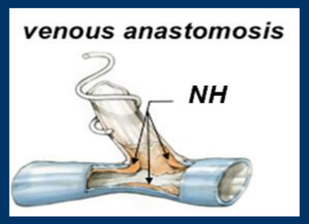

Figure 1. Stenosis at the venous anastomosis (the region between the graft and the vein) [3].

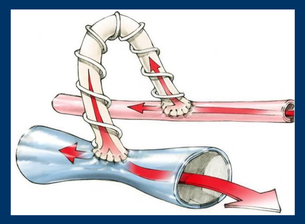

Figure 2. Diagram of the insertion of a graft and basic flow direction [4].

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INTRODUCTION

ReGel® is a drug delivery system discovered at the University of Utah which has thermally sensitive properties. This delivery system is liquid at or below room temperature, but when it is exposed to body temperatures, it solidifies into a biodegradable polymer that is able to distribute drugs at a controlled rate. One potential problem with ReGel is that it often has an initial period where a burst of drug is delivered [1]. However, one group of researchers was able to manipulate ReGel composition to achieve a continuous 35-day release [2].

Because ReGel is able to solidify at the specific site where the drug is needed, it presents a unique opportunity for drug delivery in our case. Figure 1 illustrates the main problem we are trying to address in our research, the narrowing (stenosis) that occurs in the region between the graft and the vein. Figure 2 demonstrates the basic flow direction of the graft in a simplified scheme.

The main goal of this portion of our experiment has been to obtain a drug release profile. This profile is important because it shows whether the drug is releasing at a steady rate, and which concentration of drug will release most steadily at a therapeutic level.

METHODS

1) Set up of the release experiments

ReGel was stored in the freezer. The gel was then thawed at room temperature, and while in liquid form, it was mixed with ETS-1 inhibitors at three different concentrations: 6.66, 66.6, and 250 µg/mL. Each ReGel+inhibitor sample was placed in a vial, where release medium was added. The release medium was collected daily and then stored in the freezer until the day of analysis, and new medium was added.

2) ELISA experiments and analysis

The basic methodology behind the drug release profile was to use a slightly modified indirect ELISA method (Figure 3) to obtain raw data. This data was then put through a series of equations in Excel to obtain drug release graphs, such as the graph shown in Figure 8.

In the conventional indirect ELISA method, the presence of an antibody is tested for by binding an antigen (blue) to the walls of a microtiter plate. If the antibody is present, as shown in Example 1 in Figure 3, serum (pink) added to the sample as well as a color-marked enzyme-antibody conjugate (green) will bind in a chain, producing a color change and verifying the presence of the antibody [3]. Our method (Figure 3) differed from conventional indirect ELISA in that we obtained plates that were already coated with streptavidin. In addition, there was no competitive binding in our samples, and the only biotins introduced to the streptavadin surface were our molecules of interest (i.e., biotin-labeled ETS-1 peptide).

RESULTS

The raw data obtained from this lab was used to plot the cumulative release of the ETS-1 inhibitor. (Figure 4). This graph demonstrated some concentration dependence on the release. In other words, higher concentrations of drug were releasing a greater amount of drug into their surroundings. There was a semi-linear trend line to the cumulative release, indicating that the release was fairly constant and steady. The ELISA showed us that the ETS-1 peptide was being released in the samples and that there was still biotin bound to the peptide.

As a check on our data, we are in the process of recording new data from the ETS-1 release and re-doing the Excel calculations (Charts 1 and 2). Our preliminary checks showed that a mistake had been made in copying down a value, so a concentration of 546 ng/mL rather than 500 ng/mL had been mistakenly recorded (Chart 2). We are still in the process of checking our calculations to see how much this will affect our results.

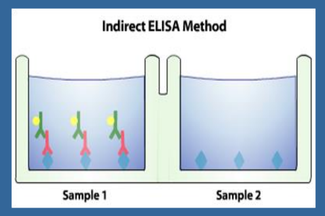

Figure 3. Indirect ELISA method [5].

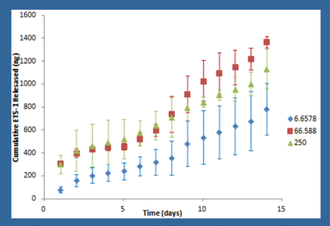

Figure 4. Cumulative Release of the ETS-1 Inhibitor.

Adjusted Absorbance

Initial ETS-1 Concentration in ReGel (ug/mL)	Days after Release	Vial A	Vial B	Vial C
6.6578	1	0.3743	0.5677	0.497
6.6578	2	0.3828	0.5459	0.5302
6.6578	3	0.2984	0.4087	0.3429
6.6578	4	0.2614	0.2968	0.3057
6.6578	5	0.1497	0.2472	0.1685
6.6578	6	0.1108	0.3275	0.4256
6.6578	7	0.2078	0.3362	0.4397
6.6578	8	0.2082	0.268	0.5222
6.6578	9	0.5523	0.422	0.8742
6.6578	10	0.2967	0.313	0.5237
6.6578	11	0.3824	0.3284	0.3523
6.6578	12	0.286	0.4378	0.4326
6.6578	13	0.2684	0.4552	0.3261
6.6578	14	0.5801	0.7112	0.3374

Chart 1. Adjusted absorbance graph (Absorbance at 450- Absorbance at 540)-(Blank at 450- Blank at 540)

Relevant ETS-1 Data

Calculated Values	Values Used in Calculation
Stock Concentration (in ng/mL)	Mass Concentration (in ng/mL)
546	1000
	ETS-1 purity
	54.6%
Stock Concentration Converted to nM	Stock Concentration (in ng/mL)
82.00907207	546
	Molecular Weight
	6667.8
Amount of Crude ETS-1 (in nmol)	Sample Volume (in L)
8.3394527	0.0015
	Final Concentration of ETS-1 (in nM)
	184.936
Amount of Crude ETS-1 in solution (in µL)	Amount of Crude ETS-1 (in µmol)
4.139243254	0.000339455
	Concentration of ETS-1 (in µmol/µL)
	8.24E-05
Amount of TBST in Solution (in µL)	Total Amount of Solution (in µL)
995.9607567746	996
	Amount of ETS-1 in solution (in µL)
	4.139243254

Chart 2. Relevant ETS-1 Data.

DISCUSSION

In examining the graphs that were calculated based on the raw data from ELISA (Figure 4), we found a continuous sustained release. However, we also observed that the highest concentration of drug (250 µg/mL) initially released slightly more drug over time, but at around the 6-day mark, the middle concentration (66.6 unit) actually started releasing more of the ETS-1 inhibitor. Our plan is to repeat the experiments and analysis to check our accuracy.

Although we plan on continuing to study ETS-1, as it has a unique mode of action, we are also planning on running a parallel study with sunitinib. Sunitinib is a cancer drug that inhibits the growth of capillaries. Because cells generally need to be within 100 µm of a capillary in order to survive, sunitinib's anti-angiogenic activity may work on the hyperplasia at the anastomosis of the graft and vein as well. Sunitinib is a potent drug that is FDA approved and has more data backing it up than the ETS-1 inhibitor. Thus, we believe we can get our product to market faster by using sunitinib. We will still continue to study the ETS-1 inhibitor because it potentially has fewer side effects and more study is needed to determine its efficacy.

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4. Provided by Dr. Alfred K. Cheung's research group in the Division of Nephrology, University of Utah.
5. Provided by Dr. Alfred K. Cheung's research group in the Division of Nephrology, University of Utah.