Use of a Mouse Model to Evaluate the Effect of Statins on the Immune Response to an Influenza Virus Vaccine

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ABSTRACT

Statins are cholesterol lowering drugs that have shown a profound effect on human health by reducing cardiovascular disease. However, two recent publications in the Journal of Infectious Diseases showed that statin use had a negative impact on the influenza vaccine response in elderly individuals [1, 2]. We propose to evaluate the immune response following vaccination in mice undergoing statin treatment. An initial study will determine the therapeutic window for statin treatment of mice fed a lipid-enriched diet. From the literature this window is expected to be between 21-28 days. That therapeutic window will be used for evaluation of the immune response following vaccination with an inactivated influenza vaccine. After treating the mice with statins for the optimum time to see an effect, we will vaccinate mice and evaluate the immune response to vaccination. Current lab activities are focused on developing assays to determine cholesterol, trigyceride, and HDL-cholesterol levels in mice.

INTRODUCTION

In a recent review of the literature for appropriate animal models to study the efficacy of statins, Pecoraro, et al stated that “Given the complexity of cardiovascular diseases and its multifactorial nature, it is unlikely that a single model will perfectly represent human physiology…” [3]. The main difficulty with the use of mice for the study of statins is that they lack the plasma cholestereryl ester transfer protein that facilitates the transport of cholesteryl esters and triglycerides between lipoproteins. Therefore, mice are not the best model of atherogenesis in humans. However, these authors also suggest the use of Apo E-/- transgenic mice in studies to model severe dyslipidemia [3]. When fed a lipid-enriched diet, the total cholesterol in Apo E-/- knockout mice can reach five times the level in wild-type C57BL/6J mice, and statins have been shown to significantly reduce the total cholesterol [4]. In addition, studies in Apo E-/- mice have shown the anti-atherosclerotic effect of statins through an anti-inflammatory mechanism on heart vessels in addition to their cholesterol-lowering activity [4,5].

We propose to evaluate the immune response following vaccination in Apo E-/- transgenic mice undergoing statin treatment. An initial study will determine the therapeutic window for statin treatment of transgenic mice fed a lipid-enriched diet. From the literature this window is expected to be from 21-28 days. That therapeutic window will be used for evaluation of the immune response following vaccination with an inactivated influenza vaccine. After treating the Apo E-/- transgenic mice with statin for the optimum time to see an effect, we will vaccinate mice and evaluate the immune response to vaccination. A group of wild-type mice will also be evaluated in parallel to determine if the gene knockout is required to show the effect on the immune response, or if simply the duration of treatment will produce the effect.

METHODS

Study 1: Determination of therapeutic treatment time:

No./ Cage	Group No.	Treatment	Dose (mg/kg/day)	Mouse	Observations / Testing
10	1	Simvasatin	10	Apo E-/-	Blood collected prior to treatment, then 2x per week for cholesterol determination
10	2	Simvasatin	30		
10	3	Simvasatin	100		
10	4	Placebo	NA		
10	5	Simvasatin	10	Wild-type	
10	6	Simvasatin	30		
10	7	Simvasatin	100		
10	8	Placebo	NA		

METHODS

Study 2: Evaluation of immune response following vaccination:

No./ Cage	Group No.	Vaccine	Statin	Dose (mg/kg/day)	Mouse	Observations / Testing
20	1	IIV*	Simvasatin	TBD	Apo E-/-	Blood collected prior to treatment, then 2x per week for cholesterol determination. Blood collected pre and post vaccination for serological evaluation.
20	2	IIV	Placebo	NA		
3	3	Controls observed for normal weight gain				
20	4	IIV	Simvasatin	TBD	Wild-type	
20	5	IIV	Placebo	NA		
3	6	Controls observed for normal weight gain				

IIV* = inactivated influenza vaccine

Figure 3: Cholesterol and Triglyceride Reagent Sets. Used to quantitatively determine cholesterol and triglyceride amounts in serum and plasma.

Cholesterol assay

Pipette 1.0 ml of reagent, pre-warm, then add .01 mL (10ul) of the sample into each tube. Incubate at 37°C for five minutes. Insert into zero spectrophotometer with reagent blank at 520nm, and record absorbances of all test tubes.

Triglyceride assay

Pipette 1.0 ml of reagent into each cuvette. Place all tubes in incubator and bring reagent up to 37°C. Pipette 0.01 ml (10ul) of sample into respective tubes. Incubate all tubes for five minutes at 37°C. Zero spectrophotometer at 540nm with reagent blank, and record absorbances of all test tubes.

FUTURE STUDIES

This study has a high potential for human benefit. Dr. Tarbet hopes to ultimately determine whether statin use by humans can be temporarily discontinued in order to allow for a greater response to the influenza vaccine. It is assumed that this window would be only a few weeks or less in duration. According to guidelines set by the AHA (American Heart Association), the number of U.S. adults receiving or eligible for statin therapy is approximately 56.0 million, with 87.4% of men and 53.6% of women between the ages of 60-75 eligible for the drug [6]. The results of this study could help millions to more effectively receive an important vaccination.

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