



THE EFFECT OF TA1 ALPHA 1 AS AN ENHANCER OF ANTIBODY FORMATION IN INFLUENZA-VACCINATED CD-1 MICE

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ABSTRACT

A study was conducted to determine the potential of TA1 alpha 1 (thymalfasin; TA1) to enhance the formation of anti-influenza antibodies in CD-1 mice following different vaccination schedules with the seasonal influenza vaccine Fluvirin[®] 2008-2009. The mice received either control article or vaccine on Study Days (SDs) 1 and 10 or SDs 8 and 17. The mice also received different doses of TA1 at different times in relation to the vaccine administration. Both the control article and vaccine were administered via intramuscular injection to both the right and left hind limbs; TA1 was administered by the intraperitoneal route. All mice were given a fixed dose of control/vaccine regardless of the body weight. The mice were observed twice daily for mortality, moribundity, general health, and signs of toxicity; body weights were recorded prior to dosing. Blood samples were collected on either SD 20 or 27 (ten days after final vaccine administration) and these samples were analyzed for HAI antibody production. Following the blood collection, all animals were euthanized and discarded without necropsy. The results indicate that the HAI titer was generally greater in mice receiving both TA1 and Fluvirin vs. those receiving Fluvirin alone. In addition, the highest dose of TA1 utilized on this study (1.2 mg/kg) appeared to increase the titers more consistently when compared to the other doses utilized. Furthermore, the best dosing schedule appeared to be administration of TA1 seven days prior to and on the day of Fluvirin vaccination on SD 8 as all animals achieved desired anti-influenza antibodies in all tester strains. Overall, the results of this mouse study warranted additional studies in a ferret model; these subsequent studies have achieved similar results validating these results.



INTRODUCTION

There is an urgent need for novel vaccine enhancing compounds that can improve response in populations of individuals who are refractory or low responders to vaccination.

Thymosin alpha 1 (TA1; trade name ZADAXIN®) is approved and commercially available as a vaccine enhancer. TA1 is found naturally in the circulation and produced in the body's thymus gland. ZADAXIN® (a synthetic version of thymosin alpha 1) stimulates the immune system at least in part by affecting T cells and NK cells.

TA1 has an excellent safety record. In clinical studies to date, more than 3,000 patients, including adults, the elderly, and children, with viral hepatitis B and hepatitis C, primary immunodeficiency diseases, and numerous cancers have been treated with TA1 with virtually no drug-related side effects. Nor has there been any worsening of side effects when TA1 is combined with other agents such as interferon and chemotherapy. In animal studies, TA1 has been administered in doses as high as 800 times the recommended human dose with no evidence of adverse clinical signs.

Clinical trials have demonstrated that TA1 increased response to influenza and hepatitis B vaccines in the elderly and hemodialysis patients; however, the treatment regimen involves 8 injections of TA1 subsequent to vaccination. The current study was conducted to determine the potential of different doses and dosing regimens (primarily with fewer injections) of TA1 to enhance the formation of anti-influenza antibodies in CD-1 mice following two different vaccination schedules with the seasonal influenza vaccine Fluvirin® 2008-2009.



MATERIALS AND METHODS

Test System and Husbandry

Appropriate numbers of male CD-1 mice were purchased from Charles River Laboratories. The animals weighed 25 to 40 grams and were 7 to 9 weeks of age at the first dose. All animals received Certified Global Harlan Teklad Laboratory Diet 2018 (pellets) and water via an automatic watering system and/or water bottles. Animals were individually housed in polycarbonate cages with Certified SaniChip[®] hardwood bedding and suspended on stainless steel racks. Each cage was affixed with a cage card containing pertinent animal and study information. The temperature and humidity ranges were 18 to 26°C and 30 to 70%, respectively.

The Institutional Animal Care and Use Committee (IACUC) of Bridge approved this protocol and found it to be in accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals, and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.

Test and Control Articles

The control article was 0.9% Sodium Chloride for Injection, USP, and was stored at room temperature.

TA1 was diluted with phosphate buffered saline to the appropriate concentrations and stored at 2 to 8°C until used.

Fluvirin[®] 2008-2009 was diluted with 0.9% Sodium Chloride for Injection, USP, to the appropriate concentration and used on day of formulation.

Experimental Design

The study was divided into 2 cohorts, depending upon the vaccine dosing schedule; five mice/group were randomly assigned to each group. The first cohort of mice (20 groups) received control article or vaccine on Study Days (SD) 8 (Vaccine) and 17 (Boost) and the second cohort of mice (23 groups) received control article or vaccine on SDs 1 (Vaccine) and 10 (Boost). TA1 administration occurred as indicated in Tables 1 and 2.

The control article (0.9% Sodium Chloride for Injection, USP) and vaccine (9 µg/dose Fluvirin® 2008-2009) were both administered via intramuscular injection to both the right and left hind limbs at a fixed dose of 0.05 mL of control article/vaccine (regardless of the body weight).

TA1 (0.3, 0.6 or 1.2 mg/kg/dose) was administered by the intraperitoneal route at a dose volume of 1 mL/kg.

Animals were observed twice daily for mortality, moribundity, general health, and signs of toxicity. Animals were observed for skin and fur characteristics, injection sites, eye and mucous membranes, respiratory, circulatory, and autonomic and central nervous systems, somatomotor and behavior patterns. Body weights were recorded prior to dosing only.

Blood samples for analysis of influenza antibody titer (HAI analysis) were collected from all the animals via cardiac stick on SD 20 or SD 27 (ten days after final control article/vaccine administration). Following the blood collection, all animals were euthanized by CO₂ inhalation, exsanguinated and disposed of without necropsy.

HAI analysis was performed in triplicate against the 3 vaccine strains present in the Fluvirin® 2008-2009 vaccine (Florida [B], Brisbane 10 and Brisbane 59).



RESULTS

All animals survived until scheduled termination and there were no test article-related clinical/cageside observations or body weight effects noted in any animal.

When two doses of TA1 were administered to male CD-1 mice at different schedules in relationship to vaccination with Fluvirin[®] 2008-2009, the HAI titer was generally greater in animals receiving both TA1 and Fluvirin[®] 2008-2009 vs. those receiving Fluvirin[®] 2008-2009 alone.

Under the different schedules investigated in the current study, the 1.2 mg/kg dose of TA1 appeared to increase the titers more consistently when compared to the other doses utilized.

Furthermore, the best dosing schedule appeared to be TA1 administration seven days prior to and on day of Fluvirin[®] 2008-2009 vaccination on SD 8, as all animals achieved desired anti-influenza antibodies in all tester strains with this regimen.

CONCLUSION

As determined by HAI titer assay, TA1 appears to enhance the formation of anti-influenza antibodies in CD-1 mice vaccinated with two 9 µg doses of Fluvirin[®] 2008-2009; the best dosing regimen appears to be 1.2 mg/kg TA1 given twice: seven days prior to and on the day of vaccination. Overall, the results of this mouse study warranted additional studies in a ferret model; these subsequent studies have achieved similar results validating these findings.

Table 2: Cohort 2 (Control Article/Vaccine Administered on SD 8 and 17)

Group	Treatment	Time of TA1 Administration	TA1 Dose Level (mg/kg/dose)
1	Control Article	Not applicable - Control article (saline) will be administered on SD 8 and 17	0
2	Vaccine only	Not applicable - Vaccine will be administered on SD 8 and 17	0
3	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	0.3
4	Vaccine/TA1	1 hr before and at the same time as vaccine administration on SD 8	
5	Vaccine/TA1	1 hr after and at the same time as vaccine administration on SD 8	
6	Vaccine/TA1	SD 7 – the day prior to and at the same time as vaccine administration on SD 8	
7	Vaccine/TA1	SD 9 – the day after and at the same time as vaccine administration on SD 8	
8	Vaccine/TA1	SD 1 - 7 days prior to and at the same time as vaccine administration on SD 8	
9	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	0.6
10	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	
11	Vaccine/TA1	1 hr before and at the same time as vaccine administration on SD 8	
12	Vaccine/TA1	1 hr after and at the same time as vaccine administration on SD 8	

Group	Treatment	Time of TA1 Administration	TA1 Dose Level (mg/kg/dose)
13	Vaccine/TA1	SD 7 – the day prior to and at the same time as vaccine administration on SD 8	0.6
14	Vaccine/TA1	SD 9 – the day after and at the same time as vaccine administration on SD 8	
15	Vaccine/TA1	SD 1 – 7 days prior to and at the same time as vaccine administration on SD 8	
16	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	
17	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	1.2
18	Vaccine/TA1	1 hr before and at the same time as vaccine administration on SD 8	
19	Vaccine/TA1	1 hr after and at the same time as vaccine administration on SD 8	
20	Vaccine/TA1	SD 7 – the day prior to and at the same time as vaccine administration on SD 8	
21	Vaccine/TA1	SD 9 – the day after and at the same time as vaccine administration on SD 8	
22	Vaccine/TA1	SD 1 – 7 days prior to and at the same time as vaccine administration on SD 8	
23	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	

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3	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	0.3
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6	Vaccine/TA1	SD 7 – the day prior to and at the same time as vaccine administration on SD 8	
7	Vaccine/TA1	SD 9 – the day after and at the same time as vaccine administration on SD 8	
8	Vaccine/TA1	SD 1 - 7 days prior to and at the same time as vaccine administration on SD 8	0.6
9	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	
10	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	
11	Vaccine/TA1	1 hr before and at the same time as vaccine administration on SD 8	
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15	Vaccine/TA1	SD 1 – 7 days prior to and at the same time as vaccine administration on SD 8	
16	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	
17	Vaccine/TA1	TA1 will be administered at the same time as the vaccine on SD 8	1.2
18	Vaccine/TA1	1 hr before and at the same time as vaccine administration on SD 8	
19	Vaccine/TA1	1 hr after and at the same time as vaccine administration on SD 8	
20	Vaccine/TA1	SD 7 – the day prior to and at the same time as vaccine administration on SD 8	
21	Vaccine/TA1	SD 9 – the day after and at the same time as vaccine administration on SD 8	
22	Vaccine/TA1	SD 1 – 7 days prior to and at the same time as vaccine administration on SD 8	
23	Vaccine/TA1	At the same time as vaccine administration on SD 8 and 17	

Figure 1: Number of Mice Achieving Protective Tiers After TA1 in Cohort 1

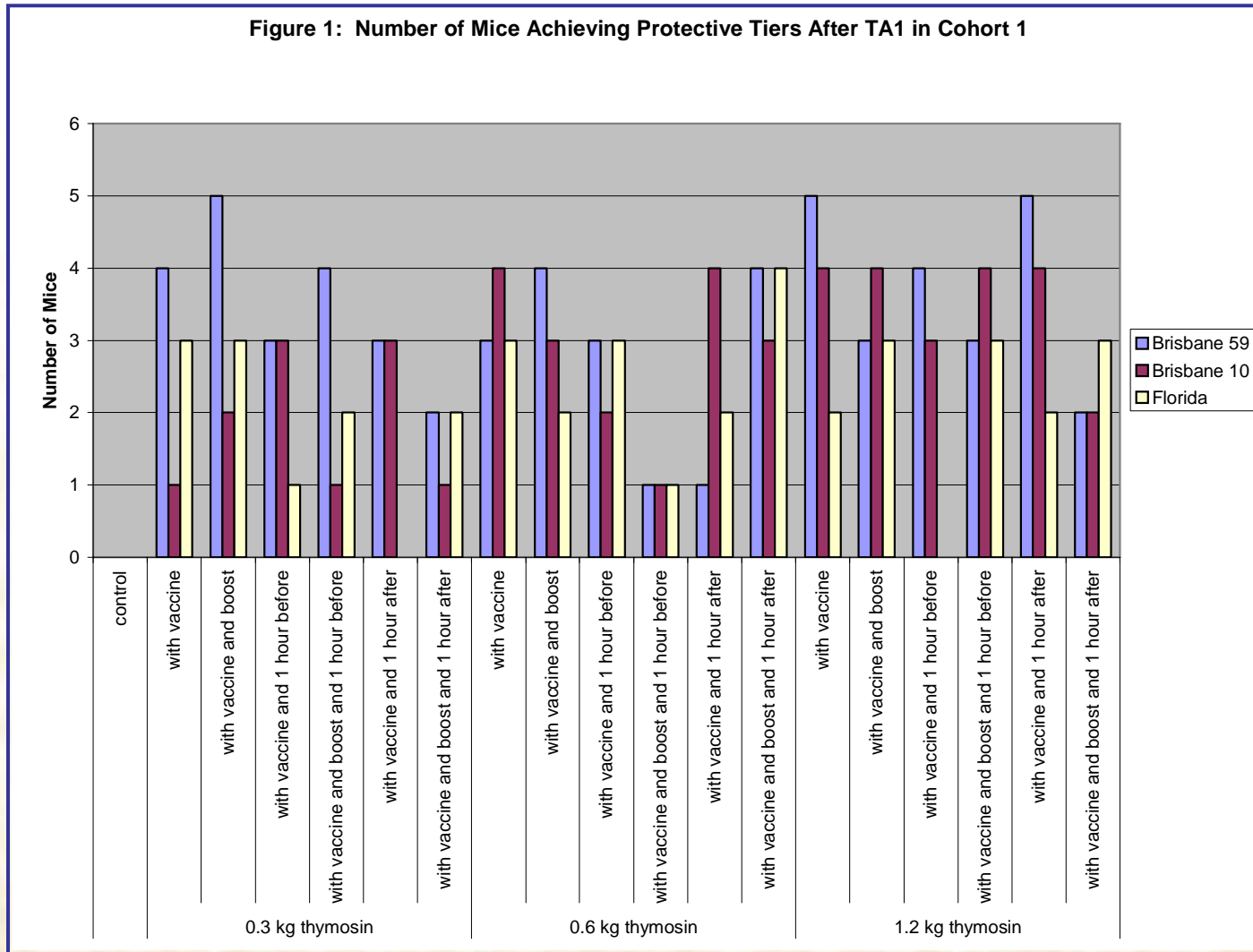


Figure 2: Number of Mice Achieving Protective Tiers After TA1 in Cohort 2

