Oral Mucosal (Sub-Lingual) Delivery of Vaccines using Zydis® Orally Disintegrating Tablets

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ABSTRACT SUMMARY
The ZYDIS® orally disintegrating tablet has been optimized to produce a stable, freeze dried, dose form for the sub-lingual delivery of vaccines. Using whole inactivated influenza virus as a model antigen, protective immunity following sub-lingual immunization has been demonstrated.

INTRODUCTION
Vaccination is a proven effective means of eradicating or controlling disease, e.g. smallpox, polio, and diphtheria\(^1\). However, vaccination programs in developing countries face significant challenges not least of which are the need for cold chain distribution and storage, potential for needle stick injuries and the need for trained personnel to administer the vaccine\(^2\). Therefore room-temperature stable vaccine formulations, ideally designed for self administration, are an important goal.

The ZYDIS® orally disintegrating tablet has already been proven to provide a room temperature stable and effective delivery vehicle for the sub-lingual immunotherapy of allergens\(^3\). In the current study, the ZYDIS® formulation composition has been optimized using pharmaceutically acceptable excipients to elicit an effective immune rather than tolerogenic response to vaccine antigens.

EXPERIMENTAL METHODS
The study is subject to provisions of the UK Animals (Scientific Procedures) Act 1986 and is therefore subject to Ethical Review Process (ERP). Whole inactivated influenza virus (A/Puerto Rico/8/34 H1N1) was used as a model antigen and incorporated into ZYDIS® formulations and processed using standard conditions for freeze drying. The tablet size was designed to allow sublingual delivery to mice by placing a single tablet under the tongue of the mice. Groups of female Balb/c mice were immunized on days 0, 10 and 20 with various test formulations. On day 27 animals were bled for serum analysis and then received a 50µl intranasal (i.n.) challenge with A/Puerto Rico/8/34 H1N1. Serum analysis included total IgG and subclass analysis. The animals were monitored for signs of infection over 7 days and scored according to a validated scoring system. Animals were scored as 0, 0.5 or 1 (no, mild or moderate clinical signs respectively) for each of the following: pilo-erection, hunched posture, erratic breathing, mobility affected and runny eyes. Changes in body weight and survival rate were also recorded.

RESULTS AND DISCUSSION
The changes in body weight compared to a historical control of an infected animal are presented in Figure 1.

Figure 1: % Body weight change following infection

Animals infected with H1N1 PR8 without immunization lose weight rapidly from day 3 post infection, reaching maximum permissible weight loss of 20% by day 6. All animals immunized with different formulations of a tablet containing influenza antigen showed reduced weight loss. In particular, significant reduction in weight loss was observed in animals receiving formulation 8 (P<0.001), 7 and 4 (P<0.01), compared with the infected control (two-way ANOVA and Bonferroni’s Multiple Comparison Test).

The clinical disease scores following infection of immunized mice are shown in Figure 2.

Figure 2: Clinical disease scores following infection with H1N1 virus
Clinical disease scores also showed significant reduction in severity between groups receiving formulations 8, 7 and 4 compared with the infected only control group (P<0.001 and P<0.05 respectively).

The survival rate data 7 days following infection with H1N1 virus is shown in figure 3.

![Figure 3: % Survival Rate on day 7 following infection with H1N1 virus](image)

The untreated infection control group showed that infection alone with H1N1 resulted in 60% mortality by day 7. A 100% survival rate was achieved for formulation 7 and 90% survival rate for formulation 8.

The H1N1 PR8 specific IgG, IgG1 and IgG2a ELISA endpoint titres are presented in figure 4.

![Figure 4 H1N1 PR8 specific IgG, IgG1 and IgG2a ELISA endpoint titres](image)

Treatment of animals with formulation 8 stimulated significantly higher titres of anti-H1N1 antibody compared with other treatment groups, formulations 7, 10, 4 and 12, resulting in high IgG titres (P<0.01), in particular the IgG2a isotype (P<0.001) when compared by one-way ANOVA and Benferonni’s multiple comparison test).

**CONCLUSION**

In comparison with untreated infected control animals, all treatments resulted in reduced bodyweight loss and clinical disease scores. The most marked reduction in disease was observed following treatment with formulation 8 with less than 4% weight loss and a disease score of less than 0.3 and survival rate of 90%. Protection from disease was associated with high levels of serum IgG dominated by IgG2a indicative of a Th1 response.

The study provides clear evidence that sublingual immunization with ZYDIS® tablets is effective as a means of stimulating potent immunity to influenza antigens. The nature of the formulation of the tablets has a profound effect on the level of immune response stimulated.

The ZYDIS® technology shows great promise for the development of room temperature stable formulations of vaccines for oral delivery thereby eliminating the risk of needle stick injury and enhancing vaccine delivery programs for both developed and developing countries.

**REFERENCES**


**ACKNOWLEDGMENTS**

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