Introduction

Cardiac glycoside poisoning

Poisoning by *Moraea pallida* Bak. (yellow tulip) (Fig 1) is the most important of all cardiac glycoside induced toxicoses which collectively account for 33% and 10% deaths in large and small stock due to plant poisoning, respectively, in the Republic of South Africa (Kellerman et al., 1996). Yellow tulip contains epoxyscillirosidine (Fig. 2), a bufadienolide type of cardiac glycoside (Enslin et al., 1966).

![Figure 1: Moraea pallida in its natural habitat](image1)

![Figure 2: Structure of epoxyscillirosidine](image2)

Management of poisoning

- **Treatment**
  - Oral administration of activated charcoal is the most effective
- **Prevention**
  - Eradication of yellow tulip is difficult, costly, involve changing the environment (e.g. herbicides, mechanical).
  - Conditioned feed aversion has been found to be a natural mechanism for protecting stock against poisoning.
  - Potential alternative: immunophylaxis with vaccines.

Aim of the study

- Ascertain if antibodies raised against related bufadienolides will cross-react with epoxyscillirosidine.

Objectives

To:

- synthesize epoxyscillirosidine, bufalin and proscillaridin conjugates, to immunize animals.
- evaluate efficacy of the synthesized conjugates in inducing an immunological response.
- determine the degree of cross-reactivity between the different generated antibodies.

Methods and Materials

- Epoxyscillirosidine was extracted, isolated, purified and confirmed using 1H NMR spectroscopy.
- Bufalin and proscillaridin were purchased and together with epoxyscillirosidine were coupled to bovine serum albumin (BSA), ovalbumin (OVA) and keyhole limpet haemocyanin (KLH) to render them immunogenic (Fig. 3).

![Figure 3: Synthesis of immunogens](image3)

- The immunogens (4 mg/ml) were emulsified with an equal volume of Montanide ISA, as an adjuvant (Fig. 4).
- Adult male New Zealand White rabbits (n=15) were randomly assigned to 5 equal groups. Rabbits in groups I, II, III and IV were vaccinated with proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-KLH and epoxyscillirosidine-BSA conjugates, respectively. Group V served as control where animals were administered BSA only (Fig. 5).

![Figure 4: Preparation of vaccine](image4)

- The rabbits were vaccinated on Days 0, 21 and 42 by intradermal injection of 0.1ml of the vaccine at four sites on the dorsum.
- Blood was collected prior to each vaccination and on Day 67.
- An ELISA was performed to determine antibody response.

![Figure 5: Vaccination of rabbits](image5)

Protein Buafadienolide

Results

- Experimental animals developed varying titres of antibodies against proscillaridin, bufalin and epoxyscillirosidine.
- Furthermore, proscillaridin and bufalin antibodies cross-reacted with epoxyscillirosidine (Fig. 6).
- Toxic signs were similarly absent in all the rabbits. However, all rabbits developed reactions at the injection sites, characterized by reddening and slight oedema a day after vaccine administration.
- No rabbit died following the administration of immunogens.

![Figure 6: Optical density values showing degree of reactivity between proscillaridin, bufalin and epoxyscillirosidine antibodies and epoxyscillirosidine in an ELISA](image6)

Discussion

Antibodies could be raised in rabbits against bufadienolide conjugates evaluated herein. Antibodies were synthesized against proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-BSA and epoxyscillirosidine-KLH conjugates. The antibody response was however variable. The KLH conjugate of epoxyscillirosidine induced the highest response. This is likely because KLH is a better carrier protein due to its larger molecular mass, complexity and foreignness which are all properties that determine the degree of immunogenicity of a compound (Kuby, 1994). Antibodies against specific haptons were raised in earlier studies (Landsteiner, 1945). Butler and Chen (1967) first described the production of antibodies against cardiac glycosides. They raised and investigated antibodies specific for digoxin for possible use in immunotherapy (Digibind®). Cross-reactivity among cardiac glycosides has also been reported. Belz et al. (1973) demonstrated cross-reactivity between ouabain, digoxin and digitoxin with proscillaridin. Antibodies against proscillaridin bound ouabain, digoxin and digitoxin, albeit with low binding activity.

Conclusions

- Antibodies were successfully raised against proscillaridin, bufalin and epoxyscillirosidine.
- Furthermore, antibodies against proscillaridin and bufalin cross-reacted with epoxyscillirosidine. However, the degree of cross-reactivity was low.
- The immunogenicity of the bufadienolides may be enhanced by optimizing the vaccines to induce a stronger response.
- The antibodies will be evaluated to determine their neutralization and cross-neutralization efficacy against epoxyscillirosidine in vitro.

References

1. Enslin, P.R., Noon, H., Pienaar, J.J. and Vor Wj, A.J. (1966) Ty. De-Epoxyscillirodenes, the main lae to princi, of Moraea pallida (Blood and Flowers).