The Production and Evaluation of Ricin Antitoxins

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SUMMARY

This study was part of a programme of work to develop and evaluate antitoxins that may ultimately be used to protect man from the effects of ricin intoxication. Polyclonal anti-ricin antibodies were raised in sheep following a series of immunisations with ricin toxoid plus Incomplete Freunds Adjuvant. Polyclonal Immunoglobulin G (IgG) was purified from plasma taken after the immunisation series. Despeciated antibody fragments F(ab')$_2$ and Fab', which are considered safer and are candidates for a future human use product, were also made. The protective efficacy of IgG, F(ab')$_2$ and Fab' administered intravenously to mice 2 hours following systemic or inhalational challenge with ricin was investigated. IgG and F(ab')$_2$ protected against ricin intoxication when administered 2 hours following challenge by either route, although IgG was slightly more efficacious. The Fab' fragment was not protective. The efficacy of three dose levels of IgG and F(ab')$_2$ administered intravenously 3, 5, 8, 16 and 24 hours after inhalational challenge with ricin were investigated. Protection achieved (measured by survival and quality of protection) was dose and time related with IgG performing marginally better than F(ab')$_2$. A therapeutic window (100% survival) of 16 hours was established for both antitoxin species. The results of these studies demonstrated that it is feasible to produce an effective ovine polyclonal anti-ricin antitoxin for post-exposure use. Further work towards the manufacture and licensing of an anti-ricin antitoxin suitable for military and civilian use is anticipated over the next few years.

Key words: Ricin, antitoxin, IgG, F(ab')$_2$

INTRODUCTION

Ricin is a 66 kilodalton (KDa) glycoprotein cytotoxin present in the seeds of the castor oil plant (*Ricinus communis*). It consists of two polypeptide chains, called the A-chain and the B-chain which are linked by an easily reduced disulphide bond (1, 2, 3). *Ricinus communis* is grown commercially in many parts of the developing world, including the Middle and Far East, for castor oil production. Although approximately 1,000-fold less toxic than the botulinum toxins, ricin is considered a potential biological warfare agent

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because of the ease and rapidity with which large quantities can be produced and the wide availability of castor oil seeds (4). Historically ricin has been employed in many criminal activities and recently its use as a weapon has been considered by extremist and terrorist groups. At present there is no prophylactic or post-exposure therapy available for the management of poisoned individuals.

The pathological effects and subsequent clinical signs of ricin intoxication depend on the route of exposure, as this dictates the subsequent tissue distribution of the toxin (5). Inhalational exposure produces effects that are confined to the lung (6). Following intravenous or intramuscular administration, lesions eventually develop in the spleen, liver and kidneys (7, 8,) but the lung is not affected (5). There is a lag phase between exposure to ricin and the appearance of toxic effects. This lag phase is related to the dose of ricin and the route via which it is administered (5). However, during this lag phase, although there are no clinical signs of intoxication, irreversible damage has been caused to cells.

Antitoxins are toxin neutralising antibodies, which can provide passive immunity to individuals when administered either before (i.e. prophylactically) or soon after exposure to a toxin. They can be produced following the immunisation of animals using an inactive form of a toxin (e.g. non-toxic subunit or toxoid) or by low levels of toxins themselves. High yields of antitoxins can be generated rapidly in large animals (eg horse/sheep/goats) and using plasmapheresis to collect the plasma can further increase the yield. The plasma produced can then be processed quickly to make a product suitable for use in humans.

The use of antitoxins as therapies for toxin exposure has a number of potential problems. The first is that of immediate adverse effects following a single exposure (anaphylactoid) or anaphylactic reactions on subsequent antitoxin administration. The Fc portion of the IgG molecule in particular may induce this reaction. It is this part of the molecule that tends to be recognised as foreign and elicits host immune responses against the antibody. Removal of the Fc portion (to produce F{ab')$_2$ and Fab') (figure 1), which is referred to as despeciation, reduces the likelihood of these reactions. Other limitations of antitoxins are that timely detection of exposure is required and that the therapeutic window (ie time after exposure antitoxin must be administered to be effective) will be dependent on the toxin and the dose received.

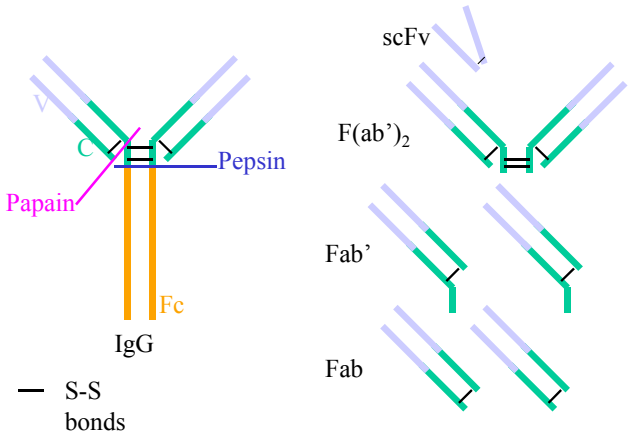


Figure 1 Structure of antitoxins

Whereas it is relatively easy to passively immunise individuals prophylactically with IgG and provide protection against toxin exposure, post-exposure therapy may be more difficult to achieve due to the irreversibility of systemic damage. Since antitoxins are generally not effective at neutralising a toxin once it has entered the target cell it is important to optimise the delivery of antitoxins to the extracellular fluid surrounding the target cells. This could be achieved by utilising the pharmacokinetic properties of despeciated antitoxin fragments (e.g. $F(ab')_2$ and Fab') which are able to distribute more rapidly from the circulation than whole IgG molecule. On a safety perspective the despeciated fragments would have the additional benefit of being more acceptable for use in humans.

Despite the limitations, antitoxins could play an important role in the prophylaxis and therapy of toxins in the event of their use as a Biological Weapon. Compared to vaccines, they are cheaper and more rapid to produce and it is feasible to produce emergency use products in a relatively short time scale.

Results and discussion

Six sheep were administered a series of immunisations with formaldehyde inactivated ricin toxoid in Incomplete Freund's adjuvant to produce hyperimmune plasma. The sheep plasma was fractionated to produce IgG, which was subsequently digested with pepsin to produce the despeciated $F(ab')_2$ fragment. The smaller Fab' fragment was also produced. Following in vitro studies to confirm the neutralising activities of the antitoxins were tested for *in vivo* efficacy to determine the effectiveness of each antitoxin species. When administered intravenously, 2 hours following systemic (ip) ricin challenge, IgG and $F(ab')_2$ were both able to protect from death whereas Fab' did not (figure 2).

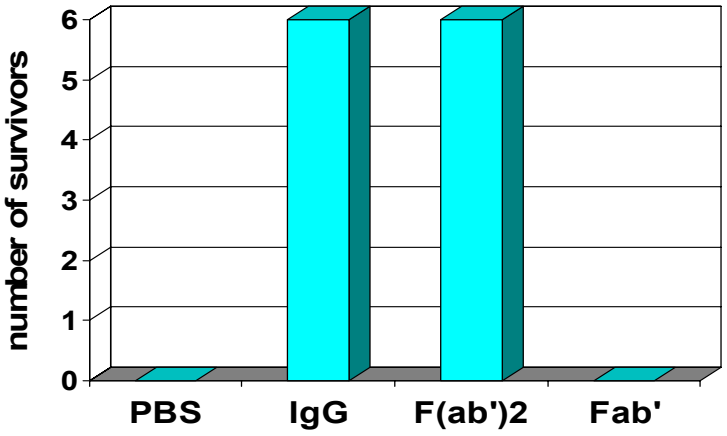


Figure 2. Balb/c mice (6/group) were administered a 3 x LD_{50} dose of ricin. IgG, $F(ab')_2$,Fab' (2.5mg) or PBS were injected intravenously 2 hours after ricin challenge. The number of survivors is shown.

The antitoxins were then tested for efficacy against inhaled ricin in mice by administering them intravenously either 1 hour before or 1 or 2 hours after a 3 LCt_{50} challenge. Both IgG and $F(ab')_2$ were able to protect from death when administered at both the 1 hour and 2 hour time points following ricin challenge. However differences were seen when the antitoxins were administered 1 hour before with the IgG being more effective than the $F(ab')_2$ (figure 3). This was probably due to differences in the pharmacokinetics of the two antitoxins with lower sustained plasma levels of $F(ab')_2$ being seen. Fab' was unable to protect any animals from death at any of the time points and this is consistent with the very low plasma levels seen following intravenous administration.

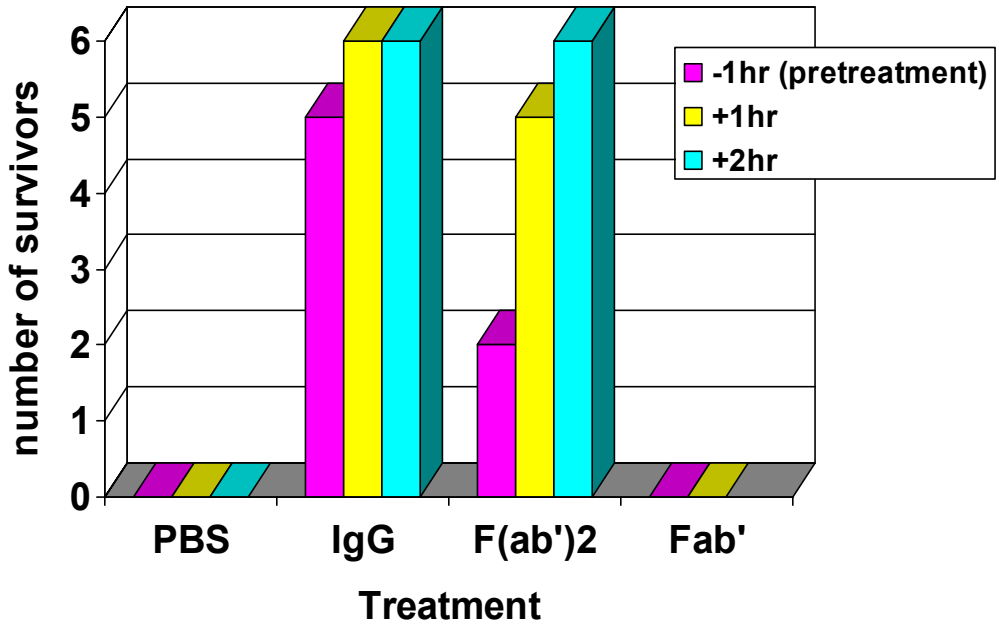


Figure 3. Balb/c mice (6/group) were administered a 3 x LCt_{50} inhalational challenge dose of ricin. IgG, F(ab')$_2$,Fab' (2.5mg) or PBS were injected intravenously either 1 hour before or 1 or 2 hours later. The number of survivors is shown.

The therapeutic window is an important consideration for the concept of use of antitoxins in the event of a suspected BW attack .The therapeutic window for the IgG and F(ab')$_2$ was investigated in a step wise manner, increasing the time after ricin exposure that the antitoxins were administered .. Three dose levels of IgG and F(ab')$_2$ were investigated administered to mice 3 hours after an inhalation ricin challenge. 100% survival was seen for all treatment groups although some transient weight loss was seen in all treatment groups. This was dose dependent and more apparent for the F(ab')$_2$ antitoxin species particularly at the lowest treatment dose (results not shown). This suggests that IgG is the most effective antitoxin species at this time point. However when administered 5 hours after challenge 100% survival was seen with both IgG and F(ab')$_2$ but with less weight loss for the F(ab')$_2$ than the IgG. Thus at a slightly later time point the F(ab')$_2$ was more effective than IgG.

The efficacy of the antitoxins when administered 8, 16 or 24 hours following inhalation challenge with ricin in mice (n=8) was then investigated. Both IgG and F(ab')$_2$ (2.5mg dose) protected from death when administered 8 or 16 hours after an inhalation ricin challenge in mice (figure 4). When administered 24 hours after challenge a break through in protection was seen with approximately 50% survival for both antitoxins. Transient weight loss and visible signs of intoxication were apparent for all time points. However at the 8 hour time point the F(ab')$_2$ gave less weight loss than the IgG (figure 5a) and at the 16 hour time point the IgG gave less weight loss than the F(ab')$_2$ (figure 5b). Therefore at the very early and very late time points IgG performed better than F(ab')$_2$ with F(ab')$_2$ performing better when administered between 5 and 8 hours after ricin challenge .

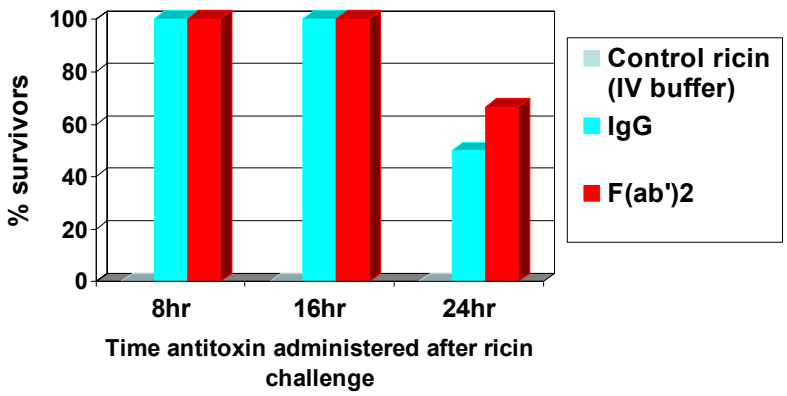
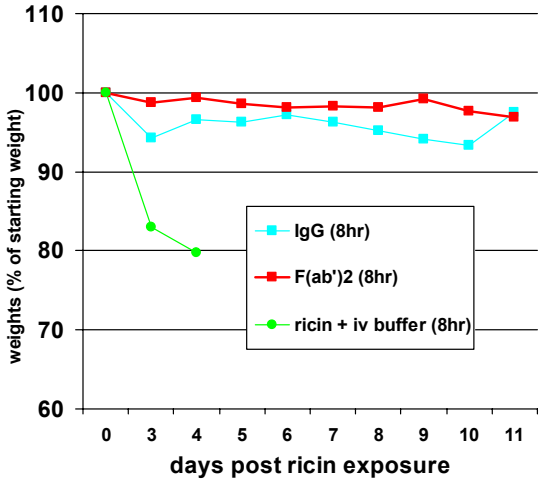


Figure 4. Balb/c mice (8/group) were administered a 3 x LCt_{50} inhalational challenge dose of ricin. IgG or $F(ab')_2$ (2.5mg) or antitoxin buffer were injected intravenously either 8hr, 16hr or 24hr later. The number of survivors is shown.

A



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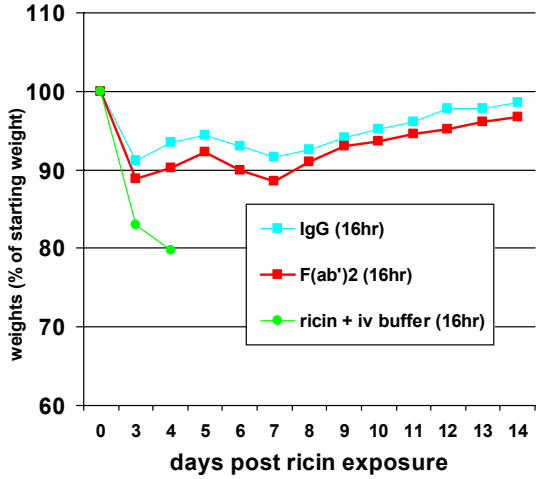

Figure 5. Balb/c mice (8/group) were administered a 3 x LCt_{50} inhalational challenge dose of ricin. IgG or $F(ab')_2$ (2.5mg) were injected intravenously 8hr, 16hr or 24hr later. Weight loss in mice when administered antitoxin a) 8hr or b)16 hr after inhalational ricin challenge is shown.

The difference between the effectiveness of two antitoxin species for the therapy ricin is likely to be due to a combination of pharmacokinetics and the sequali of events that occur following inhalation of ricin. This requires further investigation.

These studies show that it is feasible to produce an antitoxin that can be used for the therapy of ricin intoxication. Whereas IgG demonstrated greater efficacy at some time points, $F(ab')_2$ was more effective than IgG at other time points and may ultimately be more suitable for a human use product. Work to develop this antitoxin further is being undertaken.

REFERENCES

1. BARBIERI L, BALTELLI M, STIRPE F. Ribosomes-inactivating proteins from plants. Biochemica Biophysica Acta. 1993;1154:237–282.
2. LORD, J.M., ROBERTS, L.M. AND ROBERTUS J.D. Ricin: Structure, mode of action and some current application 1994. FASEB L. 8 201-208
3. RUTENBER, E. AND ROBERTUS, J.D. Structure of ricin B-chain at 2.5 A resolution. 1991. Proteins: Structure, Function and Genetics 10 260-269
4. FRANZ, D.R., and JAAX, N.K., 1997. 'Ricin Toxin In Medical Aspects of Chemical and Biological Warfare. Falls Church, VA: *Office of the Surgeon General*. 631-642
5. GODAL, A., FODSTAD, O., INGEBRIGTSEN AND PIHL, A. (1984) Pharmacological studies of ricin in mice and human. Cancer Chemother. Pharmacol 13 157-163
6. GRIFFITHS, G.D., RICE, P., ALLENBY, A.C., BAILEY, S.C. AND UPSHALL, D.G. (1995) Inhalation Toxicology and histopathology of ricin and abrin toxins. Inhalation Toxicology 7 269-288.
7 FODSTAD, O, OLSNES, S, and PIHL, A. (1976) Toxicity, distribution and elimination of the cancerostatic lectins abrin and ricin after parenteral injections into mice. Br J Cancer 32 418-425
8. GRIFFITHS, G.D., LEEK, M.D. and LEE, D.J. (1987) The toxic plant proteins ricin and abrin induce apoptotic changes in mammalian lymphoid tissues and intestines J Pathology 151 221 – 229

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