



PREDICTION OF DRUG-INDUCED IMMEDIATE HYPERSENSITIVITY IN GUINEA PIGS

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Abstract—This study was undertaken to evaluate an assay to assess the risk for drug-induced immediate hypersensitivity reactions. Groups of five to 10 guinea-pigs were given six ip injections of the test compound on days 1, 3, 5, 8, 10 and 12. Aluminium hydroxide was also given in the first injection. At day 33, the animals were given an iv injection of the test compound and the response was recorded by grading the severity of clinical symptoms. Cutaneous passive anaphylaxis was also evaluated in six naive guinea pigs using Evans blue and sera collected from treated animals on day 26. A panel of six positive model compounds (ovalbumin, aprotinin, chymopapain, tetracosactide, cyanocobalamin and procaine), and the negative compound Ribomunyl were tested. Positive systemic and/or cutaneous anaphylactic responses were observed with ovalbumin, aprotinin, chymopapain and tetracosactide whereas no responses were noted with cyanocobalamin, procaine and Ribomunyl. Our results suggest that this protocol can help differentiate positive model compounds (known to induce reactions in man) from negative model compounds, provided that their molecular weight is large enough, but that it is not applicable to substances of low molecular weight.

INTRODUCTION

Drug-induced anaphylactic reactions in humans are relatively common and potentially lethal (Descotes, 1990). However, no standardized and reliable models in either animals or humans are available to predict those reactions that may result in the withdrawal from the market of the drug concerned.

Passive cutaneous anaphylaxis, particularly in the guinea pig, has been used to detect IgE-mediated reactions (Ovary, 1964) whereas systemic anaphylaxis is less commonly used. In order to improve the procedures available, a two-step protocol was designed in Hartley guinea pigs comprising systemic anaphylaxis and passive cutaneous anaphylaxis (PCA). The applicability of this assay was examined using a panel of six positive model compounds that have been reported to induce anaphylaxis in humans and one negative model compound.

MATERIALS AND METHODS

Animals. Hartley male guinea pigs (Charles River, France), weighing 300–400 g at initiation of treatment, were housed in stainless-steel mesh cages, in an air-conditioned building with regulated temperature and humidity, and a 12-hr artificial 'day/night' light cycle. They were given commercial pelleted complete Diet (114C, UAR, France) and softened filtered water *ad lib*.

Test compounds. Ovalbumin, cyanocobalamin and procaine were obtained from Sigma (St Louis, MO,

USA), tetracosactide was from Ciba-Geigy (France), chymopapain from Boots-Dacour (France), aprotinin from Choay (France) and Ribomunyl from INAVA (France).

Systemic anaphylaxis. During the induction phase, the animals were given ip injections (0.5 ml) of one of the test compounds on days 1, 3, 5, 8, 10 and 12. On day 1, 0.2 ml Al(OH)₃ was mixed with the test compound. For each test compound, a group of control animals was treated with injections of saline according to the same administration schedule. The last injection (day 12) was followed by a rest period from day 13 to day 33. On day 26, 3 ml of blood was taken, under light ether anaesthesia, from the retro-orbital sinus of each animal for use in the PCA assay. Sera were separated by centrifugation and deep-frozen. 1 ml of each test compound solution was administered iv on day 33. The dose levels used during induction and elicitation are given in Table 1.

Animals were examined for clinical manifestations of anaphylaxis during the first hour following iv injection and at 4 hr after injection. The clinical manifestations recorded included: nose licking or rubbing of the nose with the anterior limb, piloerection, weakness or loss of muscle tone, gasping respiration, sneezing or coughing (more than three times), retching, râle, cyanosis, convulsions, prostration, and death.

Passive cutaneous anaphylaxis. The presence of antibodies was semi-quantitatively checked in the sera of 'sensitized' animals. Naive animals were given, on the back, intradermal injections (0.1 ml) of serum dilutions from 'sensitized' guinea pigs. 18 hr later, test compounds in 0.5% Evans blue were administered iv

Abbreviation: PCA = passive cutaneous anaphylaxis.

Table 1. Dose levels of test compounds used during induction and elicitation of systemic anaphylaxis

Test compound	Systemic anaphylaxis		
	Induction*	Elicitation†	PCA elicitation
Ovalbumin	0.050	0.200	0.050
Aprotinin	13,000 UIP	26,000 UIP	6500 UIP
Chymopapain	0.125	0.250	0.063
Cyanocobalamin	5	10	2.5
Procaine	3	6	1.5
Ribomunyl	0.5 human dose	1 human dose	0.25 human dose
Tetracosactide	0.250	0.500	0.125

PCA = passive cutaneous anaphylaxis

*mg/animal/injection, unless otherwise indicated.

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Guinea pigs were given six ip induction doses and one iv challenge dose of the test compound mixed in Evans blue. The dose of aprotinin is expressed as protease inhibitor units per animal per injection (UIP).

(1 ml). The cutaneous reaction was observed 30 min after iv injection. Oedema and blue staining at the intradermal injection site indicated a positive reaction. The skin from blue spots was removed to measure the external and internal diameters of the spots. Antibody titres were semiquantitatively evaluated as the reciprocal of the highest dilution that produced a measurable blue spot.

RESULTS

Anaphylactic shock

Animals that were sensitized and challenged with ovalbumin and aprotinin died within a few minutes, whereas only clinical manifestations of anaphylaxis were noted during the first hour after challenge with chymopapain and tetracosactide (Table 2). Some clinical signs were more frequent: nose licking or rubbing of the nose with the anterior limbs, weakness of muscle tone and prostration occurred in virtually all animals, whereas respiratory difficulties, retching, rôle, convulsions and cyanosis were less common. No death or clinical reactions occurred in guinea pigs treated with cyanocobalamin, procaine or Ribomunyl, nor in any unsensitized control animals.

Passive cutaneous anaphylaxis

The diameter and intensity of PCA reactions were similar when measured on the internal and external sides of the skin. The highest dilution that induced a positive response are given in Table 2. Blue spots (often associated with oedema) at the intradermal injection site were observed with ovalbumin, aprotinin and chymopapain. The highest dilution that induced a positive response was 1:512 for both ovalbumin and chymopapain in all six animals. It was 1:512, 1:128 and 1:32 for aprotinin (two animals each). No reactions were observed in unsensitized animals after iv injection of the test compounds (four or five animals per group).

DISCUSSION

Ovalbumin, aprotinin, chymopapain, cyanocobalamin, procaine and tetracosactide have all been reported to induce anaphylaxis in humans (Descotes, 1990). In contrast, the ribosomal and bacterial extract Ribomunyl, an immunostimulating drug which is injected by the deep subcutaneous route, has so far never been associated with anaphylaxis in humans.

With the protocol described here, systemic and/or cutaneous anaphylaxis were induced in guinea pigs that previously had been sensitized actively and/or passively with four positive model compounds (i.e. ovalbumin, aprotinin, chymopapain and tetracosactide). In contrast, no reactions were observed with cyanocobalamin, procaine or Ribomunyl. The severity of the reaction was dependent on the test compound. Injection of ovalbumin and aprotinin induced death within a few minutes, whereas chymopapain and tetracosactide provoked only clinical manifestations of anaphylaxis. Differences in selected elicitation doses may account for these findings. In addition, all four compounds, except tetracosactide, gave a positive cutaneous response.

The mechanism of drug-induced anaphylaxis has been extensively studied (DeSwarte, 1986). High molecular weight substances of foreign origin are complete immunogens, that is to say they can induce the synthesis of specific antibodies (e.g. IgG_{1a} and IgE

Table 2. Systemic anaphylaxis and PCA responses

Compound	Systemic anaphylaxis (no. of animals affected/total no.)		PCA (highest dilution with positive response)
	Clinical signs	Death	
Ovalbumin	5/5	2/5	1: 512
Aprotinin	9/9	4/9	1: 512, 1: 128, 1: 32
Chymopapain	3/5	0/5	1: 512
Cyanocobalamin	0/10	0/10	NR
Procaine	0/10	0/10	NR
Ribomunyl	0/10	0/10	NR
Tetracosactide	5/5	0/5	NR

PCA = passive cutaneous anaphylaxis NR = no response

Guinea pigs were observed for clinical signs of systemic anaphylaxis and death for 1 hr after the challenge injection, and at 4 hr after injection in the 'sensitized' animals. No reactions were observed in 'unsensitized' animals. Serial dilutions of sera from 'sensitized' guinea pigs were injected intradermally into naive guinea pigs and the presence of skin blue spots was recorded.

in the guinea pig) which bind to mast cells with the subsequent release of anaphylaxis mediators upon reintroduction of the substance. Low molecular weight substances can induce anaphylaxis reactions only by acting as haptens. Ovalbumin, aprotinin, chymopapain, Ribomunyl and, to a lesser extent, the peptide tetracosactide have molecular weights that are large enough to be complete immunogens. It is therefore interesting to note that our results correspond to the reactions reported in humans: of the high molecular weight model compounds we tested, only Ribomunyl, which has so far never been reported to induce anaphylaxis in humans, gave negative results in our experimental conditions. In contrast, the low molecular weight model compounds, cyanocobalamin and procaine, gave negative results despite case reports of anaphylaxis in humans.

There was limited discrepancy between systemic anaphylaxis and PCA responses. Tetracosactide was the only model compound that induced systemic anaphylaxis but no PCA response. An explanation may be found in the dose used, as Watanabe and Ovary (1977) suggested that the dose for PCA should be inversely proportional to the molecular weight. In any case, our results suggest that combining systemic anaphylaxis and PCA may not markedly improve the predictability of the proposed model.

Even though both systemic anaphylaxis and PCA models have been used in rats and mice (Arimura *et al.*, 1990; Iwata *et al.*, 1983; Ufkes and Ottenhof, 1984), guinea pigs usually are the preferred species because of their particular sensitivity in the systemic anaphylaxis (Botham *et al.*, 1988) as well as the PCA (Ovary, 1964) models. Our results are in agreement with these previous findings even though no similar study using a panel of model compounds has been published previously.

However, the protocol described here failed to detect the potential of low molecular weight model compounds to induce anaphylaxis. One of the major issues in designing anaphylaxis models therefore remains that is to determine the experimental conditions that can allow low molecular weight substances to act as haptens (Udaka, 1992). Further studies are in progress to address this issue and to improve the predictability of this protocol.

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