

Evaluation of a new reactive solid phase for radioimmunoassay of serum specific IgE against muscle relaxant drugs

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Until now, immunoassays for detection of anti-muscle relaxant IgE in serum have been performed with the drug coupled to epoxy-activated Sepharose or to RAST papers discs. In the present work we have used a quaternary ammonium-Sepharose in which the quaternary ammonium reactive group (choline chloride) was directly coupled to Sepharose via an ether linkage. 50 μ l of the quaternary ammonium solid phase (QAS) was incubated with 50 μ l of serum for 3 h, washed, incubated 18 h with ^{125} I-anti-IgE and washed again. The results were expressed as the percentage of ^{125} I-anti-IgE adsorbed onto the solid phase. The results were at $1.3 \pm 0.5\%$ for 20 control sera, with an upper normal limit estimated to 2.3%. The within-run reproducibility ranged from 3.2% to 10.0%. The results were significantly correlated with those obtained with either alcuronium-epoxy-Sepharose, choline-epoxy-Sepharose, the RAST-alcuronium or with the RAST-succinyl choline (respectively, $r = 0.66$, $r = 0.80$, $r = 0.81$, $r = 0.40$ and $r = 0.85$). The values obtained with the sera of 83 patients ranged from 0.3 to 38.5%. The sensitivity was estimated at 87.9%, 66.7% and 40.7% with the QAS-RIA, the RAST-succinyl choline and the RAST-alcuronium, respectively. The inhibition of adsorption of specific IgE onto the gel ranged from 13.0 to 90.6% in presence of 130 nmol of soluble muscle relaxants. In 83.3% of 30 cases, the highest inhibition was obtained with the muscle relaxant which was clinically incriminated. In conclusion, the reactive-solid phase which was used in the present work significantly increased the sensitivity of detection of anti-muscle relaxant IgE in serum.

Key words: anaphylaxis; muscle relaxants; quaternary ammonium radical; radio-immunoassay; specific IgE.

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It has been well established, for over a decade, that muscle relaxant drugs can be responsible for anaphylactoid reactions during anaesthesia (1, 7, 18, 23, 25)). Results of skin tests, passive transfer testing and leukocyte histamine release suggested that immunoglobulins were involved in these reactions (8, 9, 19, 24). Baldo & Fisher in 1983 were the first to demonstrate that drug-reactive IgE were involved in these anaphylactoid reactions, using a radio-immunoassay (2). They in-

cubated the sera of patients with the muscle relaxant coupled to epoxy-Sepharose and detected the serum reactive IgE adsorbed to the solid-drug phase using a radio-labeled anti-IgE antibody; they confirmed that substituted quaternary ammonium was the epitope involved in this drug allergy (1, 2). A cross-sensitivity between the different muscle relaxants has been shown, using the "sandwich" RIA with different muscle relaxants coupled to epoxy-Sepharose (2, 3, 5, 4, 10,

11, 13, 20)). Such a cross-reactivity has also been observed by leukocyte histamine release and by skin testing (1, 7, 20). We have compared this epoxy-Sepharose RIA method with skin tests and confirmed the results of Baldo, Harle and Fisher (4, 10, 11, 13, 20). However, the sensitivity of this method has to be improved (6, 11, 20).

In the present work, we evaluate a quaternary ammonium reactive phase to detect the anti-quaternary ammonium IgE in sera of sensitized patients, which can be used routinely with a high sensitivity. The reactive phase was obtained by an ether linkage of choline hydrochloride to galactose units of Sepharose. The test was named QAS-RIA (quaternary ammonium Sepharose-RIA). Comparison with other solid-phases (choline-epoxy-Sepharose, RAST-succinylcholine and RAST-alcuronium) and the clinical evaluation of this test are presented.

PATIENTS AND METHODS

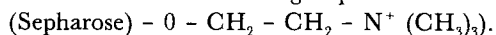
Patients

Eighty-three cases were included in the clinical evaluation of the QAS-RIA. Inclusion criteria were a reported anaphylaxis induced by injection of a muscle relaxant during anaesthesia and negative skin tests to the other drugs which were also administered. The QAS-RIA was compared *a posteriori* with the skin tests, the RIA with muscle relaxant coupled to epoxy-Sepharose, the RAST-alcuronium and the RAST-succinylcholine. Intradermal reaction and prick test were performed as described in (17). The results of the QAS-RIA were also compared to *in vitro* leukocyte histamine release (LHR) in 14 cases. Control sera were from non-allergic subjects and from subjects allergic to common allergens (with normal or high levels of serum total of IgE).

Solid phase

Alcuronium-epoxy-Sepharose and choline-epoxy-Sepharose were prepared as described (11, 20). The QAS-Sepharose was prepared by an ether linkage of choline hydrochloride to galac-

tose units of Sepharose as described (14) with the final formula of the reactive group:



The final specific activity was about 0.20 mmol of quaternary ammonium/ml gel. The paper discs of RAST-alcuronium and of RAST-succinylcholine were obtained from Pharmacia (Bois d'Arcy, France).

RIA procedure

Solid phase drug RIA and RAST to succinylcholine (suxamethonium) and to alcuronium were performed as described (2, 11, 12, 20). The first steps of the QAS-RIA were similar to the previously described for alcuronium-Sepharose and choline-Sepharose (2, 11, 20). 50 μ l of gel suspension was incubated with 50 μ l of serum in duplicate, while shaking, at room temperature

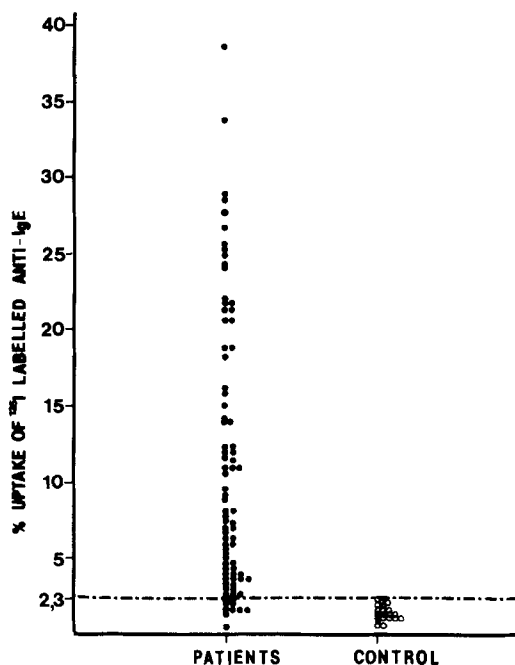


Fig. 1. Percentage of uptake of ^{125}I -labeled anti-IgE onto QAS in the presence of 83 patient sera (●) and of 20 control sera (○). The upper normal limit of uptake percentage (---) was estimated at 2.3%. The results were higher than this limit for 73 of the 83 patient sera, corresponding to a sensitivity of 87.5%.

for 3 h. The gel was then washed 3 times with 3.0 ml of 0.1 mol/l sodium phosphate buffer pH 7.4 containing 0.5 mol/l NaCl and 0.02 % of bovine albumin (w/v). The gel was incubated with 50 μ l of ^{125} I-anti-IgE (Pharmacia) for 18 h and washed again 3 times with the phosphate buffer described above. The results were expressed as the percentage of total γ -radioactivity which was bound onto the gel. The second step was an inhibition step: 50 μ l aliquots of serum were incubated for 1 h with increasing amount of soluble muscle relaxant of 5, 25, 50, 250 and 500 nmol per test tube. This was performed with 10 sera. In a simplified test, the sera were incubated with muscle relaxant at a single dose of 130 nmol. After incubation with the soluble muscle relaxant, the aliquots of serum were treated as described in the first step. Inhibition (I) was estimated from the radioactivity adsorbed to the gel in the second step (B) and from the radioactivity adsorbed to the gel in the first step (A), using the formula:

$$I = \frac{A - B}{A}$$

In vitro leukocyte histamine release

Blood was collected on heparinized tubes, according to (16). Incubation of 50 μ l aliquots was performed for 30 min at 37°C with 5 dilutions from 10^{-1} to 10^{-5} of the commercial preparation of the muscle relaxant

for a total volume of 150 μ l in Tris-albumin buffer as described (20, 21). The released histamine (Hi) was assayed in the supernatant which was collected after centrifugation at 800 g for 5 min, at 4°C. The total blood histamine (Ht) was measured in a sixth tube in which distilled water was added instead of Tris-albumin buffer and muscle relaxant. The disruption of the blood cells was obtained by freezing and defreezing the sample. Basal histamine (Ho) was determined in a seventh tube in which 50 μ l of blood was incubated with 100 μ l of Tris-albumin buffer. The concentration of histamine in each tube (Hi, Ht and Ho) was determined using the radio-immunoassay described by Morel et al. (22) and commercialized by Immunotech (Luminy, France). The LHR (%) was estimated using the formula:

$$\text{LHR} = \frac{\text{Hi} - \text{Ho}}{\text{Hi} - \text{Ho}}$$

The test was considered positive when more than 15 % of histamine was released. Interpretation of the test was performed as described previously (17).

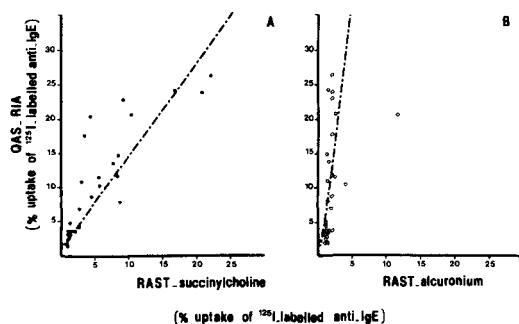


Fig. 2. Comparison between the QAS-RIA and the RAST-succinylcholine (A) in 27 cases of anaphylaxis to muscle relaxant drugs. A significant linear correlation was observed ($r = 0.85$, $P < 0.001$). A lower correlation was observed between the QAS-RIA and the RAST-alcuronium ($r = 0.40$, $P < 0.05$) (B).

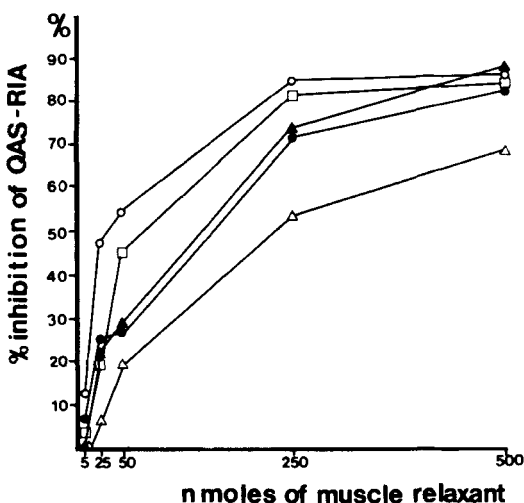


Fig. 3. Typical inhibition curves of the uptake of ^{125} I-labelled anti-IgE onto QAS incubated with patient sera in the presence of increasing amounts of soluble muscle relaxants. The drugs tested were alcuronium (Δ), vecuronium ($\bullet$), suxamethonium ($\blacktriangle$), pancuronium ($\square$) and gallamine ($\circ$).

RESULTS

The within-run reproducibility of QAS-RIA, RAST-succinyl choline and RAST-alcuronium was tested for negative values and for highly positive values. The results are summarized in Table 1. The non-specific adsorption of iodinated anti-IgE IgG onto the QAS gel was estimated to $0.50 \pm 0.05\%$. When the QAS-RIA was performed with increasing dilutions ranging from $5 \cdot 10^{-1}$ to 10^{-2} of a pool of highly positive sera (QAS-RIA = 28.1%), a significant correlation was observed between the obtained values and the calculated values ($= 28.1\% \times \text{dilution factor}$) ($r = 0.95$, $n = 13$, $P < 0.001$).

The results obtained with the QAS-RIA in a group of 20 controls were of $1.3 \pm 0.5\%$. The upper limit of normal results was therefore estimated at 2.3% for the QAS-RIA and at 1.5% for the RAST-succinylcholine and the RAST-alcuronium. The QAS-RIA was higher than the upper normal limit in 73 of the 83 patients, corresponding to a sensitivity of 87.9% (Fig. 1). Anaphylaxis for alcuronium, succinylcholine, gallamine, vecuronium and pancuronium was respectively reported in 7, 33, 13, 21 and 9 cases of the 83 patients. A great majority (80%) of the

patients were females, as previously noted (20). In a subgroup of 27 patients, the QAS-RIA was compared with other tests. The QAS-RIA, the RAST-succinylcholine and the RAST-alcuronium were positive in respectively 88.9%, 66.7% and 40.7% of the cases. In this subgroup, a good correlation was observed between QAS-RIA and the RAST-succinylcholine ($r = 0.85$, $P < 0.001$), but the correlation between QAS-RIA and the RAST-alcuronium was not so significant ($r = 0.40$, $P < 0.05$) (Fig. 2). A significant correlation was also observed between the QAS-RIA and the RIA performed with alcuronium-epoxy-Sepharose ($r = 0.66$, $P < 0.001$) in 22 cases of anaphylaxis to alcuronium, vecuronium and

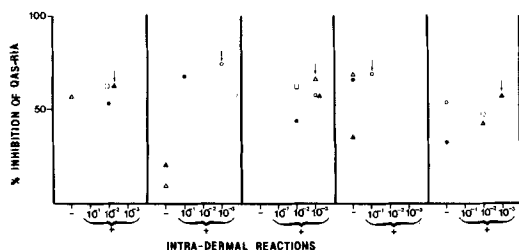


Fig. 4. Comparison among 5 cases between the percentage of inhibition of QAS-RIA by soluble muscle relaxant and the intradermal reaction to the same muscle relaxant. The muscle relaxants tested were alcuronium (Δ), vecuronium ($\bullet$), suxamethonium ($\blacktriangle$), pancuronium ($\square$) and gallamine ($\circ$). The arrows indicate the muscle relaxant incriminated in the patients' anaphylactic reactions. Intradermal reactions were performed with increasing dilutions of the commercial preparation of muscle relaxant. The concentrations of the samples of alcuronium, vecuronium, suxamethonium, pancuronium and gallamine were 5 mg/ml, 4 mg/ml, 10 mg/ml, 2 mg/ml, 20 mg/ml, respectively. The highest inhibition percentage was observed with the incriminated muscle relaxant.

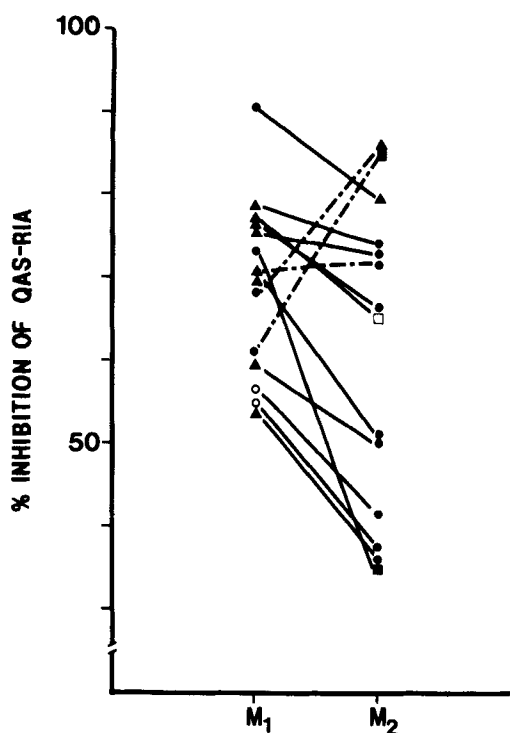


Fig. 5. Comparison of the inhibition of QAS-RIA (%) by the muscle relaxant incriminated in the adverse reaction (M_1) with the inhibition by another muscle relaxant which gave a negative intradermal reaction and a negative leukocyte histamine release (M_2), in 14 cases. Muscle relaxants tested were alcuronium (Δ), vecuronium ($\bullet$), suxamethonium ($\blacktriangle$), pancuronium ($\square$) and gallamine ($\circ$). The highest inhibition percentage was obtained with the incriminated muscle relaxant in 11 of the 14 cases.

Table 1.

Comparison of the within-run reproducibility of QAS-RIA RAST-succinylcholine and RAST-alcuronium

	Negative test**			Positive test**		
	QAS-RIA	RAST-succinylcholine	RAST-alcuronium	QAS-RIA	RAST-succinylcholine	RAST-alcuronium
Mean* (%)	1.50	1.45	0.80	29.78	16.40	3.85
SD	0.15	0.09	0.08	0.93	1.97	0.46
Coefficient variation (%)	10.00	6.20	9.70	3.20	12.00	11.90

* Results are expressed as the percentage of radiolabeled anti-IgE IgG adsorbed to the solid phase (gel or paper disc).

**Reproducibility for negative and positive tests was estimated from 8 repetitive determinations of the 2 corresponding sera.

pancuronium. A better correlation was observed between the QAS-RIA and the RIA performed with choline-epoxy-Sepharose ($r = 0.80$, $P < 0.001$) in 30 cases of anaphylaxis to either gallamine or suxamethonium (Fig. 2). Skin tests were also performed in the group of patients. The sensitivity of these tests was estimated at 84.2%. The LHR was performed in 14 of the patients who had positive skin tests and positive QAS-RIA. These patients had a reported anaphylaxis for suxamethonium, vecuronium, alcuronium, gallamine and pancuronium in respectively 5, 4, 1, 1 and 3 cases. The LHR was positive in 10 cases.

The QAS-RIA was performed in the presence of increasing amounts of soluble drug for 10 sera (Table 2). Typical inhibition curves are shown in Fig. 3. At a similar dose, the muscle relaxants which were tested gave different levels of inhibition, for doses ranging from 5 to 250 nmol. The inhibition test was performed in the presence of 130 nmol of the incriminated soluble drug for 56 patient sera. The inhibition level varied from 33 to 91% and was significant whichever muscle relaxant was tested. The inhibition test with 130 nmol of soluble drug was performed for each serum with alcuronium, suxamethonium, vecuronium, pancuronium, gallamine and tracrrium in 30 cases. The results were compared with clinical data and with the skin tests. The highest inhibition percentage was observed with the incriminated drug in 25 cases (83.3%). However, a significant percentage was also observed for the other muscle relaxants tested, including the muscle relaxants which gave a negative LHR and a negative skin test. In 14 cases, the percentage of

inhibition for the muscle relaxant incriminated in the anaphylaxis (with positive skin test, positive QAS-RIA and positive LHR) was compared with the percentage of inhibition for another muscle relaxant which gave negative skin tests and a negative LHR. The percentage of inhibition was the highest for the incriminated muscle relaxant in 11 of the 14 cases (78.6%) (Fig. 5). A significant inhibition percentage was also observed for N-methyl morpholinium sulfate (Riom Laboratoire, France) even if lower (7.5–64.0%) than that obtained with muscle relaxants. This drug is a quaternary ammonium compound which has an anti-spasmodic pharmacological effect and only a very weak anti-cholinergic effect.

DISCUSSION

The within-run reproducibility of the QAS-RIA for positive sera was better than that obtained with RAST-alcuronium and RAST-succinylcholine (Table 1) and than that obtained with Sepharose-alcuronium and with Sepharose-choline (11). The non-specific adsorption of ^{125}I -anti-IgE onto the gel was similar to that observed with Sepharose-ethanolamine (11). The binding of IgE to the gel was not due to ionic interactions since the ionic strength of the incubation medium was high (0.5 mol/l of NaCl). The same results were obtained with 1 mol/l NaCl (data not shown). The specificity of the uptake of anti-quaternary ammonium IgE onto the gel was assessed by a 20–90% inhibition of the uptake of specific IgE onto the gel in presence of soluble

Table 2.

Inhibition of adsorption of IgE to QAS-gel in the presence of increasing amounts of soluble muscle relaxant drugs

	Drug added (nmol)				
	5	25	50	250	500
Suxamethonium	6.0±4.9	20.7±10.8	27.6±10.8	59.6±14.5	71.8±10.6
Vecuronium	15.1±9.5	29.9±11.5	35.2±13.2	63.1±10.9	72.5±9.7
Alcuronium	17.3±4.5	25.9±14.2	33.5±21.5	54.7±20.7	59.5±18.7
Pancuronium	18.4±13.7	35.3±13.9	49.3±13.1	71.9±11.6	77.3±9.1
Gallamine	21.8±14.5	35.6±19.0	42.1±17.9	61.7±21.0	68.6±20.9

* Results are expressed as mean±SD ($n = 10$ sera for each drug tested)

muscle relaxant. The results of the QAS-RIA were well correlated with those obtained with choline-epoxy-Sepharose and with the paper disc of RAST-succinylcholine (Fig. 2) but not with those obtained with alcuronium-epoxy-Sepharose and with RAST-alcuronium. Moreover, the percentage of radioactivity which was bound was respectively 2.3-fold and 1.6-fold higher than that obtained with choline-epoxy-Sepharose and with RAST-succinylcholine (Fig. 2). In addition, the sensitivity of detection of drug-reactive IgE antibodies was higher with the QAS-RIA than with muscle relaxants coupled either to epoxy-Sepharose or to RAST paper disc (6, 11, 20). According to Baldo et al. the differences observed in frequency of detection of specific IgE antibodies may be due to the nature of the drug solid phase (6); they hypothesized that the substituted ammonium ion of the drugs was less accessible for binding to the IgE antibodies, after chemical coupling (6). Quaternary ammonium compounds other than muscle relaxant drugs can be used in the preparation of solid phase to overcome this problem (6) as was the case in the present study. Another way is to avoid the use of a spacer molecule for coupling the drug to Sepharose. The QAS was prepared by ether linkage of choline chloride to Sepharose, in the absence of any spacer. In the alcuronium-epoxy-Sepharose or choline-epoxy-Sepharose, a spacer (epoxirane) is used to link the drug to the solid phase. This spacer can be responsible for non-specific interactions of the solid phase with serum IgE.

A significant inhibition was observed for all the muscle relaxants tested, whichever muscle relaxant was incriminated in the anaphylactoid reaction. This confirmed the cross-sensitivity of the

anti-drug IgE antibody with all the muscle relaxants (4, 20). Other quaternary ammonium compounds such as N-methyl morpholinium sulfate can also inhibit the binding of IgE to QAS. However, the percentage of inhibition which is obtained with this molecule is about 2-fold lower than that observed with muscle relaxant drugs. This could mean that the affinity of anti-quaternary ammonium IgE for the drugs depends not only on the presence of quaternary ammonium but also on the region of the molecule which includes the quaternary ammonium substitute.

The comparison between inhibition tests and clinical data and skin tests showed that the highest inhibition was obtained with the incriminated drug, in about 83 % of the cases.

One may suppose that the muscle relaxant which gives the lowest inhibition is a possible candidate for further anaesthesia, on the condition that this elicited drug also gives negative skin tests and negative histamine release. In the present study, the drug which gave a negative skin test and a negative LHR induced a lower inhibition than the muscle relaxant incriminated in the anaphylaxis, in 11 of 14 cases. In three cases, the drug which was elicited from the skin test, from the LHR and from the inhibition test of QAS-RIA was used in further anaesthesia without any reaction (21). However, a more extensive study is needed to evaluate whether the inhibition test of QAS-RIA can be used as one of the tests predicting the risk of cross anaphylaxis and the harmlessness of a muscle relaxant that could be used later (21).

In conclusion, we have improved the RIA method for detecting anti-muscle relaxant IgE in serum using a quaternary ammonium compound

coupled to Sepharose. Compared with muscle relaxants coupled either to epoxy Sepharose or to RAST paper discs, this new reactive solid phase increases significantly the sensitivity of detection of specific IgE.

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