

Quantitation of Passive Cutaneous Anaphylaxis (PCA) by Using Radiolabelled Antigen

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The major problem of detecting reaginic antibody by passive cutaneous anaphylaxis (PCA) is the quantitation of the dye reaction. Radiolabelled antigen was used in an attempt to quantitate the PCA reaction (Radio-PCA). Antisera containing reaginic antibody against human serum albumin (HSA) were produced in rabbits. These antisera were injected into normal rabbit skin in different dilutions. Twenty-four hours later HSA was injected intravenously either with Evans Blue or as 125-I-HSA. Radioactivity found in antibody-containing skin was significantly higher than in control specimens containing saline or normal rabbit serum, as low as antiserum dilutions of 1:1,000. Compared with the Evans Blue technique Radio-PCA was able to distinguish quantitatively between different antiserum dilutions at a higher level of statistical significance.

Key words: passive cutaneous anaphylaxis.

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The presence of reaginic antibody can be demonstrated either by using specific anti-reagin sera (principle of the radioallergo-sorbent test, RAST) or by passive cutaneous anaphylaxis (PCA). The latter technique was originally described by Prausnitz & Küstner (6) and established as standard technique for the detection of reaginic antibody in animal experiments by Ovary (5).

A major problem with PCA is the quantitation of the test results (10), i.e. the size of the dye reaction (mostly Evans Blue). Various methods calculating the size of extravasated dye by measuring different diameters have been considered to yield satisfactory results (9). Faulk *et al.* (1) improved these methods by using radioactive NaI as indicator substance. It was the aim of

the present study to use the tested antigen itself as an indicator substance and thereby achieve higher specificity and better quantitation.

MATERIAL AND METHODS

Animals. New Zealand white rabbits of both sexes and with an average body weight of 4 kg were used in all experiments.

Antisera. Anti-human serum albumin (HSA) sera were produced in six rabbits by immunization with 0.1 mg HSA (Biotest, Frankfurt)/kg in 0.5 ml of a 1% Al(OH)₃ solution intramuscularly. The animals were bled 4 weeks after immunization by cardiac puncture. Serum was separated by centrifugation at 500 g for 20 min and stored in aliquots frozen at -20°C until used.

Radioactive labelling. According to the method of Franks *et al.* (2) 250 mg HSA were

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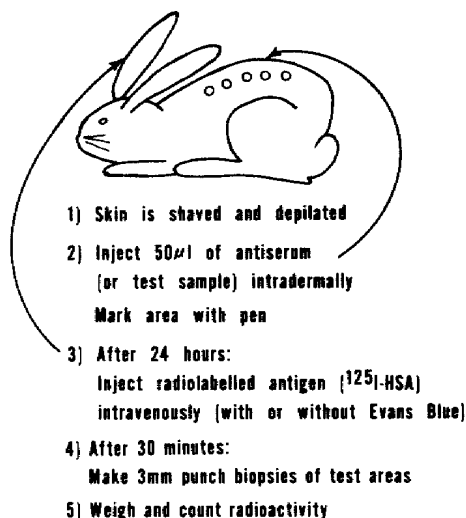


Figure 1: Technique of passive cutaneous anaphylaxis using radiolabelled antigen (Radio-PCA).

incubated with 100 μ Ci 125-Iodine (specific activity 98 μ Ci/ μ g 125 I; Buchler, Amersham) in a NaI/NaIO₃ buffer until a brown ring indicated that the radioactive iodine was bound to the tyrosine rings of the protein. After the labelling procedure, 125-I-HSA was separated from unbound iodine by gel chromatography (Sephadex G-25, Pharmacia, Uppsala).

Passive cutaneous anaphylaxis using Evans Blue. Fifty μ l aliquots of the tested antisera were injected intradermally producing a local wheal of approximately 2 mm diameter. The injection site was marked with a pen. Twenty-four hours later (if not otherwise mentioned) 50 mg HSA were injected in 2 ml 0.9% saline into the ear vein, together with 2 ml of a 5% Evans Blue solution. Thirty minutes after the intravenous antigen injection the animal was killed and the diameter of the Evans Blue extravasation was measured.

Passive cutaneous anaphylaxis using 125-I-HSA (Radio-PCA). In the Radio-PCA experiments the antiserum injection was done as described for the Evans Blue technique.

Twenty-four hours later 2 ml of 125-I labelled HSA (25 mg 125-I-HSA/ml) were injected into the ear vein. In some experiments 125-I-HSA was mixed with normal HSA in various concentrations to study the dose-response effect. Thirty minutes after intravenous antigen injection the animals were sacrificed, 3-mm skin biopsy specimens were taken from the marked areas where antiserum had been injected, weighed, and radioactivity counted in a gamma scintillation counter (Packard, Downers Grove, Illinois) (Fig. 1).

Statistics. Unless otherwise indicated results are expressed as mean values ($\bar{x}$) $\pm$ standard error of the mean ($s\bar{x}$). Statistical significance between the means of different groups was calculated using Student's *t* test.

RESULTS

When the radioactivity measured was plotted against the weight of the skin specimen excised (Fig. 2) there was a significant positive correlation in the areas where anti-HSA had been injected. In the observed range between 40 and 230 mg there was no significant change in radioactivity in those areas where normal rabbit serum or saline had been injected. Because of this weight-dependence the results were expressed as radioactivity per gram skin (c.p.m./g) in the following experiments.

In order to study the influence of the total dose of radioactive antigen administered, four rabbits were tested with the same dilutions of anti-HSA, normal rabbit serum (NRS) and saline intradermally, but received different doses of 125-I-HSA intravenously. As Table 1 shows there was an increase in radioactivity over all skin sites with increasing dose of i.v. administered 125-I-HSA. In all samples, however, the anti-HSA-induced counts were significantly higher than those of both controls.

Regarding differences in reactivity between different test animals 40 antiserum samples of different dilutions were investigated in three different rabbits. There was satisfactory

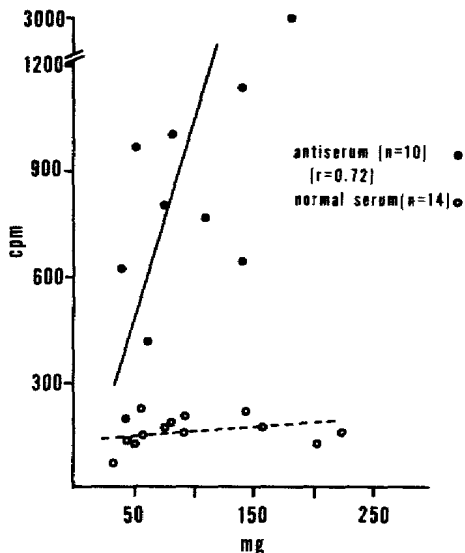


Figure 2: Correlation between radioactivity measured and weight of skin specimen. 10 areas where anti-HSA was injected intradermally are compared with 14 normal rabbit serum test sites (sera undiluted).

reproducibility when the results were expressed on the per weight (c.p.m./g skin) basis (not shown).

The question of major interest was, whether it would be possible by Radio-PCA to quantitate reaginic antibody activity. Therefore six different anti-HSA-sera were serially diluted and tested together with five different normal rabbit sera in the skin of

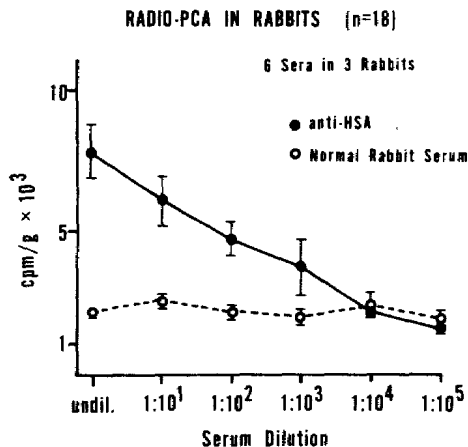


Figure 3: Radio-PCA results of serially diluted anti-HSA and normal rabbit sera (mean values of six sera tested in three different rabbits).

three different rabbits. Fig. 3 shows the results expressed as mean values of 18 anti-HSA injected skin specimens compared with those of 15 NRS controls for each dilution point. There was a direct correlation between antiserum concentration and amount of radioactivity in the biopsied skin area. At a dilution of 1:1,000 anti-HSA-induced counts were significantly higher than NRS counts ($P < 0.02$ for 1:1,000, $P < 0.005$ for the three higher concentrated samples).

A comparison of the Radio-PCA technique with the classical Evans Blue

Table 1
Effect of dose of i.v.-administered antigen (125-I-HSA) on skin radioactivity in test areas

Dose 125-I-HSA (c.p.m./mg HSA)	Anti-HSA (c.p.m./g, $n=5$)	Skin radioactivity after injection of normal rabbit serum (c.p.m./g, $n=5$)	0.9% NaCl (c.p.m./g, $n=5$)
0.6×10^4	204 ± 37	25 ± 8	20 ± 5
1.6×10^4	498 ± 23	112 ± 31	97 ± 8
6.0×10^4	3131 ± 400	268 ± 79	238 ± 61
5.5×10^5	7850 ± 1022	2130 ± 117	2205 ± 158

$n=5$ specimens of each tested skin area.

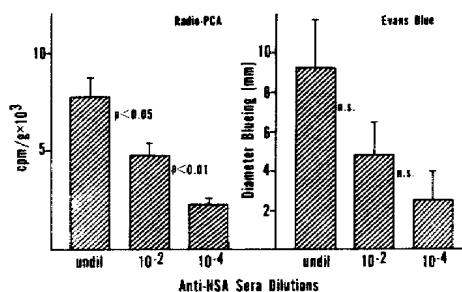


Figure 4: Comparison of six anti-HSA serum dilutions evaluated with Radio-PCA and with Evans Blue in two rabbits (mean values of 12 skin test sites).

method is shown in Fig. 4. By the Evans Blue method it was not possible to distinguish significantly between even hundredfold antiserum dilutions, although the biggest diameters of extravasated dye were observed in the undiluted test sites. Between undiluted anti-HSA and 1:1,000 anti-HSA the difference in mean diameter of blueing was significant at the 1% level. However, in Radio-PCA distinction between undiluted and 1:100 anti-HSA samples, and 1:100 compared with 1:10,000 was easily achieved, with a statistical significance of $P < 0.05$ and $P < 0.01$ respectively.

DISCUSSION

The data presented show clearly that quantitation of passive cutaneous anaphylaxis can be improved by using radio-labelled antigen.

In classical PCA, even with the modification of Faulk et al. (1), the measurable reaction corresponds only to secondary events following the reagin-antigen reaction, such as the release of vasoactive mediators leading to changes in capillary permeability (3) thus allowing the detection of the indicator substance in the extravasal space. By Radio-PCA the antigen-antibody reaction itself is directly quantitated, and proportional to the amount of antigen specifically bound by reaginic antibody. A comparison with the Evans Blue technique

showed clearly that Radio-PCA is the superior method for distinguishing between different anti-HSA-serum dilutions.

A further advantage of this technique is that antibodies of two or more different specificities could be investigated at the same time and in the same sample, when a double – or even multiple – isotope marker is used. Preliminary experiments have shown that this is possible (unpublished observations).

As in every biological assay there are several variables which have to be considered. The size of the skin sample seems to be critical; 3-mm diameter specimens have proven useful. In addition, the total dose of radioactivity administered intravenously influences the results. Therefore all samples must be related to several controls: normal unsensitized skin which gives information about the body distribution of the injected antigen, saline injected skin in order to evaluate the effect of local trauma, and normal rabbit serum for the exclusion of nonspecific antigen binding. The use of a positive standard serum is advisable when absolute values are desired. Comparison of various sera of one experiment should be done in one animal to avoid individual differences in skin reactivity of the test animals.

This technique is not species specific; preliminary experiments have shown similar results in rats (8). It is interesting that orally administered radioactive antigen was measurable by Radio-PCA, thus offering an opportunity to measure enteral absorption of antigens (8).

The major disadvantage of the technique described here is that the animals have to be sacrificed in order to count the radioactivity in the skin test sites. The use of scanning procedures might further improve this technique and allow *in vivo* measurements. Preliminary experiments by Seifert et al. (unpublished observations) have shown that this is possible.

Even if one could avoid the skin excision, Radio-PCA will still remain an experimental technique: the transfer of human serum to

the skin of a healthy volunteer – which is the principle of the Prausnitz-Küstner-test – should be abandoned in clinical use for ethical reasons. The risk of inducing infectious diseases such as hepatitis or syphilis can never be ruled out completely. Here the RAST or in certain cases the passive cutaneous anaphylaxis in the monkey (4) are the methods of choice. In animal experiments, however, where specific anti-IgE is difficult to obtain, Radio-PCA offers a sensitive technique for quantitative demonstration of reaginic antibody.

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