

Comparison of guinea pig sensitization methods

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Up to now, different methods in guinea pig sensitization were used, to assess contact sensitization potential of low molecular weight chemicals. In order to compare the efficiency of the epicutaneous, intradermal and combined sensitization methods, two stronger sensitizers, the water soluble chlormethylimidazoline hydrochloride (CMI) and the lipid soluble 1,3-dinitrochlorobenzene (DNCB) were used for the experiments. Eleven modified sensitization methods were performed. In a first trial each substance was tested on 10 animals. In a further study those techniques which had produced the best results, that is the highest degree of sensitization, were applied to 25 to 30 animals. For comparison of the results of the various methods, a mean sensitization index was calculated. The best results were achieved with the Maximization Test of Magnusson & Kligman (1969) and the Split Adjuvant Technique of Maguire & Chase (1967).

Key words: Challenge tests – chlormethylimidazoline hydrochloride (CMI) – 1,3-dinitrochlorobenzene (DNCB) & sensitization studies in guinea pigs – eleven (11) epicutaneous, intradermal and combined sensitization methods – local skin toxicity – order of sensitization rates.

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This paper reports a comparison of 11 different methods used to assess contact sensitization potential of low molecular weight chemicals.

For the experiments, two strong sensitizers were used:

- (a) water soluble chlormethylimidazoline hydrochloride (CMI)
- (b) lipid soluble 1,3-dinitrochlorobenzene (DNCB) shown in Fig. 1.

CMI is used as an intermediate substance in the processing of some drugs of imidazoline structure. Some years ago three patients who developed a contact dermatitis induced by CMI were examined in the Department of Allergology. Further studies by Schulz &

Schöpf (1967) showed that this substance has a high sensitizing capacity. That is why CMI was used in this study of sensitization methods.

Materials and Methods

The weight of the guinea pigs ranged from 400 to 500 g. The Pirbright white strain was used.

For the epicutaneous application, DNCB was dissolved in:

Ethanol	45 %
Methylcellosolve®	45 %
Tween 80®	10 %

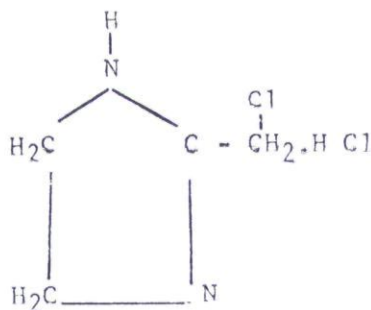
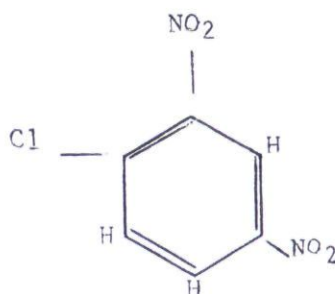


Fig. 1. Chlormethylimidazoline hydrochloride (CMI)



1,3-dinitrochlorbenzene (DNCB)

For dissolving CMI ethanol was replaced by water in the same quantity.

Before starting the sensitization experiments, observations on local toxicity were performed by means of epicutaneous, intracutaneous and subcutaneous applications in animals which had had no previous treatment, and in animals which had been given an injection of 0.1 ml of complete Freund's adjuvant subcutaneously 2 days previously. The adjuvant was mixed with an equal quantity of normal saline. The challenge test was always performed with a modification of a described open epicutaneous method, approximately 10 days after the sensitization procedure. Separate experiments were carried out to determine whether the solvents used were capable of producing an allergic or a toxic reaction, but nothing relevant was found. The various sensitization methods differed in the sensitizing procedure, whereas the challenge tests were always done in the same manner. They consisted of one epicutaneous application 12 to 14 days after the last sensitizing dose. Various concentrations were used to determine the threshold dose.

Method 1

Open epicutaneous method, involved daily applications of 0.05 ml DNCB and of CMI. Skin reactions developed 12 days later. The concentrations were $1/50$ molar DNCB and $1/3$ molar CMI.

Method 2

Closed epicutaneous method, modified version of Buehler (1965). Every second day each test site was covered with a circular bandage. The procedure was continued until the skin showed conspicuous signs of irritation.

Method 3

Intracutaneous sensitization according to Landsteiner (1935) and Draize (1959). Sensitization was carried out with 10 intracutaneous injections three times a week.

Method 4

Subcutaneous sensitization with complete Freund's adjuvant. The allergen was administered only by the subcutaneous route twice, on the first and fourth days together with Freund's adjuvant.

Method 5

Combined sensitization by two subcutaneous injections with Freund's adjuvant and five open epicutaneous applications. The first part of the sensitizing procedure was made by the subcutaneous sensitization as described in Method 4. Part 2 of the trial consisted of five epicutaneous applications starting 1 day after the last subcutaneous injection. Care was taken to ensure that the epicutaneous applications were performed to involve the same lymphatic drainage pathway.

Method 6

Combined sensitization by two subcutaneous injections with Freund's adjuvant and one epicutaneous booster application. The subcutaneous injections were made as described in Method 4. The booster application was made by one epicutaneous application in the same region on the eleventh day after the last injection.

Method 7

Combined sensitization by two intracutaneous injections with complete Freund's adjuvant and open epicutaneous application (modified from Maguire & Chase 1967). On the first day every animal was injected with a mixture of Freund's adjuvant and physiological saline five times intracutaneously in an area 25 cm² on the upper flank. Twenty-four hours later the sensitizer was injected at the same sites. From the third day on a series of epicutaneous applications was started for a period of 6 days in the area of the intracutaneous injections.

Method 8

Combined sensitization by two intracutaneous injections with complete Freund's adjuvant and one epicutaneous booster application, that is, the split adjuvant technique of Maguire & Chase (1967). The first part of the experiment is identical with that of

Method 7. In the second part, 10 days later, the allergen was applied epicutaneously.

The challenge tests were performed as already described.

Method 9

Combined sensitization with one intracutaneous injection of Freund's adjuvant and several epicutaneous applications. On the first day every animal received five intradermal injections of the sensitizer mixed with complete Freund's adjuvant. The distribution of the injections was that as described in Method 7. On the second day the epicutaneous applications were started and continued until conspicuous reactions occurred. This was the case for DNCB after 6 days, for CMI after 5 days.

Method 10

Combined sensitization by one intracutaneous injection with complete Freund's adjuvant and one epicutaneous booster application. The intracutaneous injection method was identical with that of Method 9. The booster application was performed on the tenth day after the intracutaneous injection.

Method 11

Guinea pig maximization test according to Magnusson & Kligman (1969). The procedure can be divided into two parts:

A. On the first day every animal received the following injections paired on the left and right sides of the spine somewhat caudally from the shoulder:

1. 0.1 ml complete Freund's adjuvant
2. 0.1 ml test substance without adjuvant
3. 0.1 ml test substance mixed with complete Freund's adjuvant.

These six injections were made within an area of 3 cm² deeply intradermal.

B. On the eighth day after the intracutaneous injections, a closed epicutaneous application followed which remained for 48 h.

After the closed epicutaneous application there was often an enlargement of ulceration and an increase in inflammation. This was regarded as a flare-up phenomenon. For challenge, the open method was used in contrast to the original paper of Magnusson & Kligman (1969).

All the methods described were carried out on 10 animals with each substance. This was regarded as a screening procedure in order to find out the best way to sensitize guinea pigs. In a further study those techniques which had produced the best results, that is, the highest degree of sensitization, were applied to a greater number of animals. Accordingly, groups of 25 to 30 were treated with method numbers 8, 9 and 11. The epicutaneous applications were performed in this trial differently from the original description of Magnusson & Kligman (1969) in the open manner for 2 days, in the vicinity of the intracutaneous injection sites. This can be called Method 11a. The challenge test was as before, open epicutaneous. For comparison of the results of the various methods, a mean sensitization index was calculated.

Results

The following order of sensitization rates was found. The best results (the highest sensitization index) were achieved with

1. Method 11 – Maximization Test of Magnusson & Kligman (1969)
2. Method 8 – split adjuvant technique of Maguire & Chase (1967)
3. Methods 9 and 10 – approximately the same results
4. Methods 5, 6, 7 – about the same results
5. Method 4
6. Methods 1 and 3 – more or less the same results.

It should be noticed that the results of Method 11a, which was the open epicutaneous sensitization, were not as good as the

original technique as proposed by Magnusson & Kligman (1969). Therefore, in the Division of Allergology at Hamburg, the original method of Magnusson & Kligman (1969) is used whenever possible in testing unknown drugs and other materials, sometimes together and in comparison with the epicutaneous technique as described in Method 1.

Conclusions

The sensitization capacity of topical drugs, cosmetics, preservative agents and other substances is of great interest. The question arises as to whether the results of animal experiments are relevant to human beings. As far as we know, one can expect this to be the case, because eczematogenous substances in man have often proved to have a sensitization capacity in guinea pigs.

In this context a new substance, Squaric acid diethylester, was introduced. The experiences with this substance have already been published (Noster et al. 1976).

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