ASSESSMENT OF THE ALLERGENIC POTENTIAL OF ALTHESIN AND ITS CONSTITUENTS

P. TACHON, J. DESCOTES, A. LASCHI-LOQUERIE, J. P. GUILLOT AND J. C. EVREUX

SUMMARY

The pharmaceutical formulation of Althesin was shown to have a weak allergenic potential in the guineapig maximization test. Alphadolone and alphaxalone acetate were found to be devoid of such potential, while the status of Cremophor EL remained equivocal.

The aetiology of Althesin-related hypersensitivity reactions is not understood fully as yet. Althesin is a mixture of two steroids, alphadolone and alphaxalone, solubilized by polyethylene glycol glycerol ricinoleate (Cremophor EL). The latter has been incriminated owing to its capacity to induce histamine release, particularly in dogs (Lorenz et al., 1977). However, it seems unlikely that this mechanism can be involved frequently when one compares the lower frequency of hypersensitivity reactions to propanidid, another Cremophor-containing i.v. anaesthetic drug, with that to Althesin (Fisher and More, 1981; Descotes et al., 1982). Complement involvement has also been suggested (Watkins, Appleyard and Ward, 1975; Watkins, Thornton and Clarke, 1979) and its importance confirmed (Radford, Lokyer and Simpson, 1982). An immunemediated response is another possibility (Fisher, 1976; Watkins et al., 1976). In an attempt to investigate this third mechanism, we have studied the sensitizing ability of Althesin and its constituents in guineapigs.

MATERIALS AND METHODS

Standard formulations of Althesin were used. Cremophor EL was purchased from Sigma Chemicals. Alphadolone and alphaxalone were a gift from Glaxo Laboratories.

Animals

Adult albino Dunkin–Hartley guineapigs, weighing from 300 to 400 g at the start of the experiment and originating from standard breedings (Iffa-Credo, l’Arbresle, France) were maintained under recommended conditions. Groups of 10 male and female guineapigs were used for each experiment.

Experimental procedure

All animals were submitted to an intradermal sensitization test, performed with two intradermal injections of Freund’s complete adjuvant as described previously (Magnusson and Kligman, 1969; Brulos et al., 1977). During the induction period, 10 repeated intradermal applications of the substance (0.5 ml per animal) were performed on the skin on the back of the guineapigs. An occlusive bandage was applied for 48 h. After a 12-day rest period, guineapigs were challenged on the abdomen, using the maximum dose which had not caused any orthoergic skin reaction in preliminary studies. Forty-eight hours later, the macroscopic cutaneous response was evaluated, according to a classical scale for erythematous and oedematous lesions (Draize, Woodgard and Calvery, 1944). The macroscopic examination was negative (no allergy) when the score was equal to or less than the score obtained following the first intradermal application. Otherwise, the examination was considered doubtful and a histological examination was undertaken to obtain specific evidence of an allergic reaction, such as skin infiltration with lymphocytes and plasmocytes.

RESULTS

Cremophor EL, alphadolone and alphaxalone acetate were found to be devoid of any sensitizing capacity. In contrast, the pharmaceutical formulation of Althesin behaved as a weak sensitizer when the appropriate area of skin was examined histologically.
The guineapig maximization test is a commonly used procedure for predicting the allergenic potential of chemicals (Maurer et al., 1978; Maurer, Weirich and Hess, 1980) and, despite difficulties of extrapolation from animals to man, these authors consider that even a low level of allergenicity can be detected (Maurer et al., 1979).

In the present experiment, Althesin proved to be allergenic and this is in agreement with the hypothesis of an immune-mediated hypersensitivity reaction in clinical practice (Fisher, 1976; Watkins et al., 1976). That Althesin was allergenic, in contrast to any of its constituents, is apparently paradoxical. However, it should be stressed that, unlike most authors, we assessed the allergenic potential from histological findings and not merely from macroscopic examination. According to the less severe standards, both Althesin and Cremophor EL would have been considered as medium range sensitizers (Maurer, Weirich and Hess, 1980). We believe that the predictive value of the test is enhanced when taking the macroscopic and the histological findings into account simultaneously.

The results indicate that Althesin should be considered as a drug with a weak allergenic potential, while alphadolone and alphaxalone cannot be incriminated as such. In contrast with the experimental work of Glenn and co-workers (1979), who showed the involvement of Cremophor in a mini-pig model, the status of Cremophor remains ill-established in our study. However, the mini-pig model was designed to mimic human "pseudo-allergic" reactions to Althesin, whereas the maximization test was performed to detect an immune-mediated mechanism and this may be the explanation for this discrepancy.

Thus an immune-mediated phenomenon should be considered as one of the mechanisms involved in Althesin-related hypersensitivity reactions.

### ACKNOWLEDGEMENTS

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### REFERENCES


### TABLE I. Skin-sensitizing potential of Althesin and its constituents in guineapigs. Doses per animal used for challenge were 0.5 ml for Althesin and Cremophor EL and 0.1 ml for alphaxalone (9 mg/ml olive oil) and alphadolone (3 mg/ml olive oil). Such doses were the maxima which did not cause any orthoergic response in preliminary studies.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Macroscopic examination (% doubtful)</th>
<th>Histological examination (% positive)</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Althesin</td>
<td>70</td>
<td>10</td>
<td>Sensitizing</td>
</tr>
<tr>
<td>Male</td>
<td>60</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>0</td>
<td>Not sensitizing</td>
</tr>
<tr>
<td>Cremophor EL</td>
<td>60</td>
<td>0</td>
<td>Not sensitizing</td>
</tr>
<tr>
<td>Male</td>
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</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Alphaxalone</td>
<td>0</td>
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</tr>
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<td>—</td>
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</tr>
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<td>—</td>
<td></td>
</tr>
<tr>
<td>Alphadolone</td>
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</tr>
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<tr>
<td>Female</td>
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**EXPERIMENTELLE EINSCHÄTZUNG DES ALLERGENEN POTENTIALS VON ALTHESIN UND SEINER BESTANDTEILE**

**ZUSAMMENFASSUNG**

Beim Maximierungstest am Meerschweinchen konnte gezeigt werden, daß die pharmazeutische Zusammensetzung Althesin ein schwaches allergenes Potential besitzt. Alphadolon und Alphaxalonaacetat erwiesen sich als frei von allergener Wirkung, während die Bedeutung von Cremophor zweifelhaft blieb.

**EVALUATION DU POUVOIR ALLERGENIQUE DE L'ALFATESINE ET DE SES ELEMENTS**

**RESUME**

La présentation pharmaceutique de l'Alfatesine s'est avérée posséder un faible pouvoir allergénique d'après le test de maximisation chez le Coubaye. L'alfadolone et l'acétate d'alfaxalone ne présentent aucune activité de ce type tandis que les résultats concernant le Cremophor EL demeurent équivoques.

**EVALUACION DEL POTENCIAL ALERGENICO DEL ALTESIN Y DE SUS COMPONENTES**

**SUMARIO**

La formulación farmacéutica del Altesin mostró tener un débil potencial alergénico durante las pruebas de maximización efectuadas en cerdos de laboratorio. El acetato de alfadolona y de alfaxalona demostraron carecer de tal potencial, al tiempo que el estatus del Cremofer EL permaneció indefinido.