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INFLUENCE OF SOLVENTS AND SURFACE ACTIVE AGENTS ON THE BARRIER FUNCTION OF THE SKIN TOWARDS SARIN

II. Increase in rate of absorption

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In a previous paper (9) a method was outlined to test the influence of various pretreatments of the skin surface of guinea-pigs on its barrier function in regard to the organophosphorus cholinesterase inhibitor, Sarin, or *iso*propoxy-methylphosphoryl fluoride. It was shown that the time until respiratory arrest gave a satisfactory, indirect measure of the rate of absorption of the test compound provided that the area of absorption was kept constant, that the weight of the animals was uniform, that the absorption was optimal and that evaporation was prevented. In the present paper the results of pretreatment of the skin surface with various organic solvents and surface active agents will be described.

Material and Methods

The main method used was described in detail in an earlier paper (9). A metal ring was glued to the clipped skin of the belly in groups of ten guinea-pigs. Thirty minutes later 0.5 ml of the pretreatment liquid was pipetted on to the skin area within the ring. It was allowed to remain on the area for 1, 5 and 30 minutes (for one compound 1, 5, 10, 15, 20, 30 and 60 minutes). The ring was covered with a cover-glass in order to avoid evaporation. After the pretreatment time the liquid was removed by gentle blotting with dental sorbent rolls. The area was left uncovered in order to allow free evaporation of any remaining liquid. Thirty minutes later, when the skin area always appeared to be completely dry, the animals were challenged, *i.e.* 25 µl of Sarin was applied to the skin surface, the ring covered and the time until respiratory arrest noted. Analytic grade of acetone, ethanol, ether, chloroform and dimethylsulfoxide were used undiluted, like destilled water which served as a control. Furthermore, a 5 per cent water solution of a commersial soap and 0.045 N water solutions of a non-ionic, a cat-ionic and an an-ionic surfactant were used. The non-ionic agent was alkylpolyglycholic ether, the cat-ionic benzethonium chloride, and the non-ionic the sodium salt of alkylethersulfate.

In some experiments the area within the ring was rinsed with 0.5 ml destilled water twice after blotting, and in some additional experiments dimethylsulfoxide, DMSO, was mixed with Sarin in various proportions. In order to get information whether there was a correlation between the increase in rate of absorption and the surface tension reducing capacity of the pretreatment liquids the spread of Sarin on the skin surface was investigated in guinea-pigs. A clipped skin area of 5 by 5 cm was pretreated as earlier (for one minute and without any ring glued to the skin). Thirty minutes later 25 µl of Sarin as a single drop was applied in the center of the skin area. Two diagonal diameters of the visible spot were measured with the aid of a pair of compasses at regular intervals, and the area of the spot was calculated as being an ideal circle. Controls showed that no increase in

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Table 1. *Time in minutes until respiratory arrest in guinea-pigs challenged with 25 µl Sarin applied on a skin area of 3.1 cm² following various forms of pretreatment*

Type of pretreatment	Time in minutes until respiratory arrest pretreatment time in minutes		
	1	5	30
Destilled water	31.4 ± 1.4*	28.9 ± 1.1	29.6 ± 1.0
Acetone	20.2 ± 1.7	17.9 ± 1.4	18.1 ± 1.0
Ethanol	19.4 ± 1.6	17.4 ± 1.6	15.6 ± 1.2
Ether	12.0 ± 1.3	13.6 ± 1.3	11.0 ± 0.9
Chloroform	10.5 ± 1.2	12.7 ± 1.7	11.5 ± 0.8
Dimethyl-sulfoxide	32.0 ± 1.4	30.8 ± 1.2	31.4 ± 1.6
Soap	25.1 ± 1.5	14.2 ± 1.4	12.0 ± 0.9
Non-ionic surfactant	29.8 ± 1.7	24.2 ± 1.5	15.7 ± 1.2
Cat-ionic surfactant	26.5 ± 1.4	18.4 ± 1.7	9.8 ± 0.5
An-ionic surfactant	27.1 ± 1.9	20.2 ± 1.4	7.6 ± 0.4
None	31.2 ± 1.3		

* $\bar{x}$ s.e.m. n=10

Table 2. *The spread of 25 µl Sarin on the guinea-pig skin surface following various forms of pretreatments*

Pretreatment	Area in mm² $\bar{x}$ s.e.m. n=10
None	544 ± 25
Destilled water	551 ± 29
Ethanol	600 ± 43
Ether	613 ± 52
Chloroform	563 ± 54
Dimethylsulfoxide	610 ± 31
Soap	750 ± 28
Non-ionic surfactant	824 ± 63
Cat-ionic surfactant	808 ± 58
An-ionic surfactant	925 ± 76

accuracy could be obtained by addition of a fluorescent material or measuring the skin resistance according to Blank and Finesinger (2) outlining the area and then measure it as above. Controls also showed that the calculation of the spot as being a perfect circle did not give any significant deviations from planimetric measurements.

All experiments were run in groups of ten animals. Both sexes were used in about equal proportions. The weight of the animals was kept fairly constant, 465–480 g, the highest standard error of the mean being ± 12 g. In all 540 guinea-pigs were used.

Results and Discussion

The results are summarized in Tables 1–3 and illustrated by fig. 1. As follows from table 1 pretreatment with destilled water alone has little or no influence on the absorption, indicating also that the blotting does not produce any mechanical injury on the epidermal barrier. With the exception of dimethylsulfoxide, DMSO, all other forms of pretreatment produced an evident decrease in the survival time of the animals when the pretreatment period was 30 minutes. In the case of the two shorter pretreatment periods the organic solvents, still with the exception of DMSO, have about the same effect, while soap and the three surface active agents were considerably less active. The non-ionic surfactant was chosen for a more detailed study of the time factor involved, the results of which are illustrated in Fig. 1. From this it is evident that the barrier destroying activity appears relatively slowly, *i.e.* prolonged contact is necessary in order to reach optimal effect, and the asymptote for optimal effect is reached after 25 to 30 minutes. Two consecutive rinses of the treated area with destilled water (0.5 ml each time) immediately after the blotting had no influence at all

Table 3. *Time in minutes until respiratory arrest in guinea-pigs challenged with Sarin mixed with various proportions of dimethyl-sulfoxide (DMSO) and applied on a skin area of 3.1 cm²*

Amounts of Sarin/DMSO in µl	Time until respiratory arrest in minutes $\bar{x}$ s.e.m. n=10
50/0	32.0 ± 1.1
50/50	10.3 ± 0.6
25/25	10.7 ± 0.4
35/15	18.4 ± 0.9
45/5	25.1 ± 1.2
15/35	17.4 ± 0.8
5/45	32.5 ± 1.5

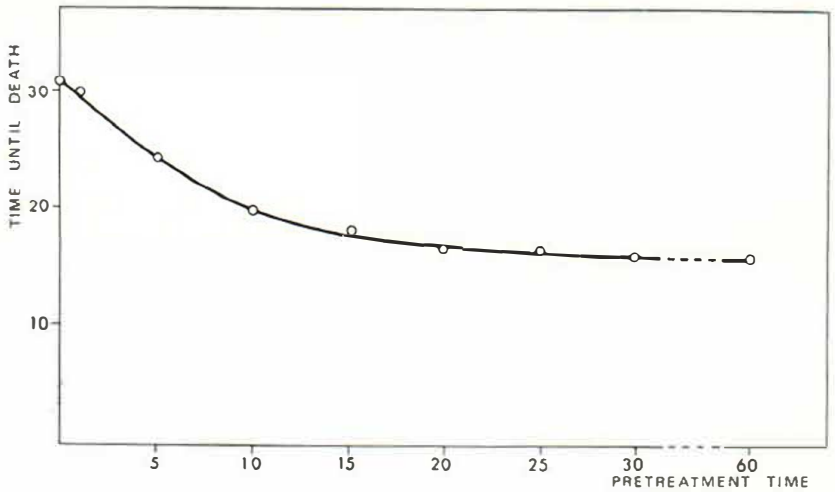

Fig. 1. Relationship between time until respiratory arrest and pretreatment time with 0.045 N alkylpolyglycholic ether in guinea-pigs challenged with 25 μl Sarin applied on 3.1 cm^2. Time is given in minutes. Each points represents the mean of 10 different experiments.

on the survival time. This indicates that the effect on the barrier is not readily reversible, which is in accordance with results obtained by Blohm (6, 7) indicating protein denaturating effects of surfactants.

It has been reported that DMSO increases the rate of percutaneous absorption for a number of substances (for references see 13). Since in the present investigation no such effect could be demonstrated when the skin area was pretreated, some additional experiments were run. In these Sarin and DMSO were mixed in various proportions, applied on the skin surface of guinea-pigs as in the other experiments and the time until lethal effect determined. These results are summarized in Table 3. From this it is evident that DMSO increases the rate of absorption of Sarin considerably when they are mixed together. Lethal effect of 5 μl Sarin without any addition of DMSO, applied on the same area of skin in guinea-pigs is seen after 50.2 ± 5 minutes (9). In the present experiments the same effect was obtained in 32.5 minutes when 5 μl Sarin was mixed with 45 μl DMSO. The fact that 50 μl Sarin produces lethal effect at the same time as 25 μl when mixed with equivalent volumes of DMSO is only a confirmation of an earlier investigation, showing that optimal absorption is reached with 25 μl of Sarin applied on an area of 3.1 cm^2 (9). Reduced amounts of both Sarin and DMSO result, as expected, in a delayed respiratory arrest.

Since there may be a correlation between the surface tension reducing activity of the various pretreatment liquids and their absorption promoting activity, *e.g.* by such a mechanism as facilitating penetration into follicles, the spread of Sarin on pretreated skin was determined. Already on non-treated skin Sarin spreads rapidly, and the maximum area is reached already after 1–2 minutes. The spread was always measured 2 minutes after application, since evaporation may disturb the measurements if they are further postponed. The results appear in Table 2, from which it is evident that there is no such correlation as mentioned above. Other possible mechanisms involved in the increase in absorption produced by the various pretreatments are, of course, removal of the lipid surface film and direct barrier destroying effects. Blank and Shapiro (4) have *e.g.* showed that surfactants and soap solution remove some of the amino acid content of the skin surface. To what extent such mechanismus are involved is, however, impossible to judge from the present experiments.

Washing the skin with ether or petroleum ether has little effect on the permeability of skin to salicylates (14), surface ac-

tive agents (3, 5) or alkylphosphate (11). On the other hand, prolonged treatment of skin with acetone, alcohol or hexane will considerably increase the permeability of skin to water (1, 12). Without questioning the accuracy of the various methods used in the above cited papers, it should be pointed out that the present method could be extremely sensitive, at least in the meaning that already a very minute injury to the barrier function of the skin will be readily recorded. This is due to the fact that the skin surface is loaded with a very high dose of toxic material. The LD_{50} of Sarin in the guinea-pig following percutaneous application is thus 5.6 mg per kg bodyweight (8) with an area of absorption restricted to 0.4 cm^2. In the present experiments 27 mg are applied to an area of 3.16 cm^2 in animals weighing about 470 mg. The amount per area unit is about the same (the animals in the LD_{50}-determinations were of approximately the same weight), and thus there are at least 20 LD_{50}-doses applied in the present experiments. With this excess a very minute barrier damage may result in a rapid death of the animal.

Sarin is rapidly hydrolyzed by alkali (10). In at least the case of the soap solution, which had a pH of 10.5, it could be of some importance that the skin surface was more alkaline than in normal animals, *i.e.* some Sarin is inactivated and the effective dose reduced. However, this is not very likely considering the large dose of Sarin and the fact that the absorption of a toxic dose is very rapid. This assumption was supported by the fact that several rinses with saline after pretreatment with soap solution did not change the results.

SUMMARY

The influence of pretreatment of the skin of guinea-pigs with organic solvents and surface active agents on the barrier function towards an organophosphorus cholinesterase inhibitor, Sarin, has been investigated. As a measure of the rate of absorption of the challenging substance the time until respiratory arrest was chosen. Destilled water, used as a control, had no significant effect, while acetone, ethanol, ether and chloroform increased the rate of absorption already following short pretreatment periods. In the case of soap solution and non-ionic, cat-ionic and an-ionic surfactants longer pretreatment was necessary for an optimal effect on the barrier function of the skin. Pretreatment with dimethylsulfoxide had no effect, but when mixed with Sarin in various proportions the rate of absorption of the challenging substance was increased. The spread of Sarin on pretreated skin was also investigated as an indirect measure of the surface tension reducing capacity, and there was no correlation between this and the induced increase in absorption. The results have been discussed with regard to mechanisms which may explain the decrease in barrier function of skin following the various pretreatments.

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