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The action of glucosulphone [N,N^{I}-di (glucose sodium sulphonate) of 4,4^{I}-diaminodiphenyl sulphone] on pancreatic kallikrein

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1. The effects of sulphone have been studied on kinin production in dog plasma and on capillary permeability in the skin of rabbits.

2. Dog plasma was used as a substrate for pancreatic kallikrein *in vitro* and the addition of sulphone caused an inhibition of kinin production, which was dependent on the concentration of the drug.

3. The increased vascular permeability produced *in vivo* by intradermal injections of kallikrein was partially inhibited by sulphone injected intravenously. The same doses of sulphone also inhibited the increase in permeability produced by bradykinin and histamine in the skin of rabbits.

It is well known that glucosulphone exercises a specific action in the chemotherapeutic treatment of leprosy. In treating patients affected with solar prurigo with this drug, we observed that it reduced the pruritus and lesions provoked by the inflammatory processes. To determine the reasons for its beneficial effects, we carried out experiments based on the results of Collier & Shorley (1960), who found that anti-inflammatory drugs such as acetylsalicylic acid, phenylbutazone, amidopyrine and phenazone were very potent in suppressing the bronchoconstrictor action of bradykinin in the guinea-pig.

Collier & Shorley also found that these drugs did not specifically antagonize the action of bradykinin on the capillaries of guinea-pig skin *in vivo*. Hebborn & Shaw (1963) report that sodium salicylate and aspirin are poor inhibitors of kallikrein activity *in vitro*. Despite these results, we investigated the mechanism of the anti-inflammatory action of glucosulphone. Our experiments were of two types: the first was a study of the pharmacological effect of the sulphone on the activation of plasma kinins by pancreatic kallikrein ; in the second type of experiment an *in vivo* study was undertaken to compare the increase in vascular permeability and the vasodilatation produced by such substances as bradykinin and kallikrein when injected intradermally in rabbits before and after administration of the sulphone.

Methods

For the pharmacological assay of the effect of sulphone on kinin formation, the substrate was prepared from dog plasma diluted thirty times with a solution containing 0·15 M sodium chloride and 0·1 M phosphate buffer so that the final solution

was at pH 7·4. The diluted plasma was heated to 62° C for 30 min to inactivate the peptidases. Samples having a final volume of 4 ml., each containing 3·5 ml. of substrate, 0·005 units of pancreatic kallikrein, and the following amounts of glucosulphones, 2, 1, 0·5, 0·4, 0·2 and 0·1 mg, were incubated for 1 hr at 37° C. These samples were compared with control solutions of substrate, kallikrein and 0·15 M NaCl solution. Two blanks were made, one of 3·5 ml. of substrate plus 1 mg of sulphone and the other of 3·5 ml. 0·15 M sodium chloride solution plus 1 mg of sulphone and 1 mg of kallikrein. The final volume of these samples was also 4 ml. The second series of samples contained 4 μg of synthetic bradykinin and either 1 or 2 mg of sulphone made up to a volume of 4 ml. with 0·15 M sodium chloride solution. The samples were incubated at 37° C for 1 hr in a water bath. All samples were tested for their depressor activity on the dog leg preparation, perfused with blood through the femoral artery as described by Binia, Fasciolo & Carretero (1958). The samples were injected into the lumen of the femoral artery and the effect on the perfusion pressure recorded with a Hg manometer in seven experiments. The capillary permeability of the rabbit skin was tested by the method of Miles & Miles (1952), who used guinea-pigs. The rabbits were depilated over a dorsal area lying between the pectoral and pelvic girdles and extending 5 cm on either side of the mid-dorsal line. Pontamine sky blue 6BX (5% in 0·15 M sodium chloride solution) was administered intravenously in the marginal ear vein in doses of 1·2 ml./kg body weight. Half to one hour later, 0·1 ml. of the following solutions were then injected intradermally in the right paravertebral area: 0·15 M sodium chloride, bradykinin 10–100 μg/ml., kallikrein 2·5 u./ml., and histamine 1 mg/ml.

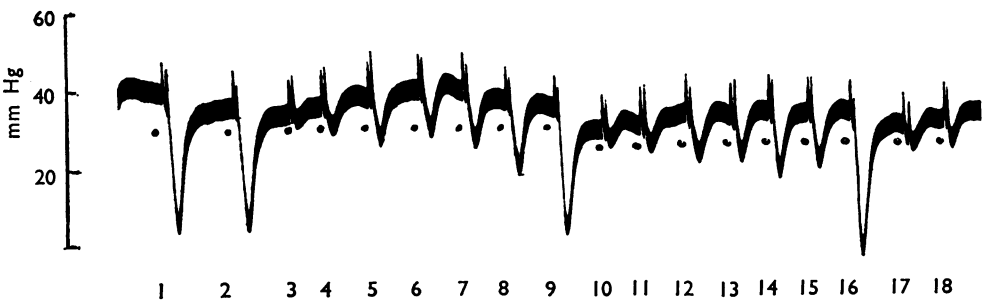

FIG. 1. Experiment showing the inhibitory effect of glucosulphone on plasma kinin formation. Records of arterial pressure in perfused dog's leg. All injections made in a volume of 0·2 ml. K, Kallikrein.

1: Synthetic bradykinin 1 μg/ml.
2: 0·005 u.K + substrate + 0·15 M NaCl solution
3: 0·005 u.K + substrate + 2 mg sulphone
4: 0·005 u.K + substrate + 1 mg sulphone
5: 0·005 u.K + substrate + 0·5 mg sulphone
6: 0·005 u.K + substrate + 0·4 mg sulphone
7: 0·005 u.K + substrate + 0·2 mg sulphone
8: 0·005 u.K + substrate + 0·1 mg sulphone
9: 0·005 u.K + substrate + 0·15 M NaCl solution
10: 0·005 u.K + substrate + 0·2 mg sulphone
11: 0·005 u.K + substrate + 1 mg sulphone
12: 0·005 u.K + substrate + 0·5 mg sulphone
13: 0·005 u.K + substrate + 0·4 mg sulphone
14: 0·005 u.K + substrate + 0·2 mg sulphone
15: 0·005 u.K + substrate + 0·1 mg sulphone
16: 0·005 u.K + substrate + 0·15 M NaCl solution
17: 0·15 M NaCl solution + substrate + 1 mg sulphone
18: 0·005 u.K + 0·15 M NaCl solution + 1 mg sulphone

Twenty-five minutes after these control injections, the rabbits were given 0·1 g/kg or 1 g/kg glucosulphone intravenously (ten rabbits for each dose). An hour later the same volumes and concentrations of bradykinin, kallikrein and histamine solutions as were given to the controls were injected intradermally along the left paravertebral area. Twenty-five minutes later the rabbits were killed by a blow on the head and the skin covering the dorsal area was removed in order to expose and measure the response.

Drugs. We used synthetic bradykinin as supplied by Sandoz of Basilea, Pancreatic Kallikrein (Bayer's Padutine); glucosulphone [N,N'-di (glucose sodium sulphonate) of 4,4'-diaminodiphenyl sulphone] in a 40% aqueous solution (Parke Davis, Promin) and histamine in a 1% aqueous solution.

Results

Effect of sulphones on kinin formation

Figure 1 shows that the substrate plus kallikrein induced a marked reduction in the perfusion pressure of the dog's leg preparation, that the effect of glucosulphone alone was minimal and that when the sulphone was given together with substrate and

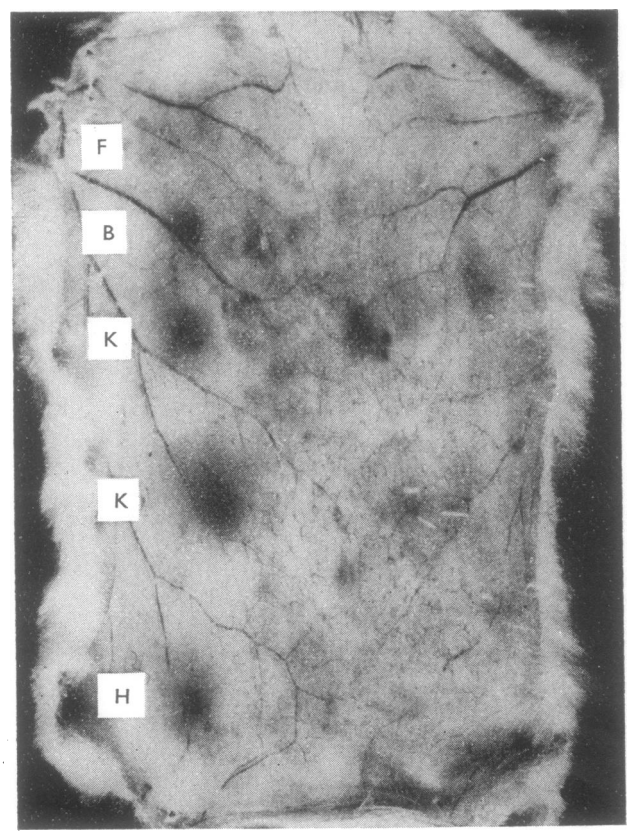

FIG. 2. Skin of rabbit after intravenous pontamine blue (1·2 ml./kg body weight of a 5% solution) showing effects of intradermal injections of 0·1 ml. of solutions of: F, 0·15 M NaCl; B, synthetic bradykinin 10 μg/ml.; K, pancreatic kallikrein 2·5 u./ml.; H, histamine 1 mg/ml. Left, untreated; right, after administration of intravenous glucosulphone (1 g/kg).

kallikrein there was very little reduction in perfusion pressure. The inhibition of the effect of kallikrein plus substrate bore a direct relationship to the amount of sulphone used, thus 100% inhibition was obtained with 1 and 2 mg/4 ml. of sulphone and gradually fell to 75% inhibition with 0·1 mg/ml. The concentration of 0·2 mg/4 ml. is approximately the blood concentration reached during therapy. The assay never showed any inhibition of synthetic bradykinin activity by the sulphone.

Vascular permeability of the rabbit skin

Glucosulphone given intravenously in a dose of 1 g/kg, that is, ten times the therapeutic dose, caused 50% inhibition (reduction of the size and colour intensity) of the inflammatory response to intradermal bradykinin, histamine and kallikrein as shown in Fig. 2. On the other hand, no inhibition was observed when the sulphone was used in a dose of 0·1 g/kg.

Discussion

From the results obtained in the experiments on the perfused dog's leg, it is clear that the quantities of sulphone used have an inhibitory effect on the formation of plasma kinins. The results of the *in vivo* experiments are similar to those of Collier & Shorley (1960), who found that phenylbutazone (100 and 200 mg/kg) and amidopyrine (75 and 150 mg/kg) lessened but did not abolish responses to bradykinin and histamine in guinea-pig skin as shown by blueing in response to intradermal doses of these substances. It should be stressed that our results were obtained with a dose of sulphone which was ten times the therapeutic dose.

REFERENCES

BINIA, A., FASCIOLO, J. C. & CARRETERO, O. A. (1958). A method for the estimation of bradykinin in blood. *Acta Physiol. Lat. Amer.*, **13**, 101–109.

COLLIER, H. O. J. & SHORLEY, P. G. (1960). Analgesic antipyretic drugs as antagonists of bradykinin. *Br. J. Pharmac. Chemother.*, **15**, 601–610.

HEBBORN, P. & SHAW, B. (1963). The action of sodium salicylate and aspirin on some kallikrein systems. *Br. J. Pharmac. Chemother.*, **20**, 254–263.

MILES, A. A. & MILES, E. M. (1952). Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea-pigs. *J. Physiol., Lond.*, **118**, 228–257.

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