Some observations on the pharmacology of 'deep-heat', a topical rubifacient

A. J. COLLINS,^{1 2} L. J. NOTARIANNI,¹ E. F. J. RING,² AND M. P. SEED¹

From the ¹Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, and the ²Royal National Hospital for Rheumatic Diseases, Bath

SUMMARY A topically applied rubifacient delivered by aerosol (Deep-Heat) was studied. After spray application to the forearms of volunteers, without massage, the erythema produced was measured by thermography and correlated with the concentration of 2 salicylate components of the mixture found in local and systemic venous blood. Maximum erythema occurred at about 30 minutes, while blood salicylate levels were maximal between 20 and 30 minutes after application. Methyl salicylate was absorbed before ethyl salicylate. Over the time period of the erythematous response oxygen levels in local venous blood were raised. Finally, platelets collected from venous blood draining from the sprayed site, when induced to clump by the addition of arachidonic acid in an aggregometer, showed increased resistance to clumping when compared with control cells. The mechanism of these observed phenomena and the mode of action of the constituents of Deep-Heat are discussed.

The *British National Formulary* lists 26 preparations under the heading of rubifacients.¹ These mixtures are normally applied to and rubbed into the skin over an affected part, and are used by the public to treat muscular aches and pains. They are generally acknowledged to act by counterirritancy, but there is no evidence to suggest that they act on any fundamental disease process. Indeed it is probable that the act of rubbing is an important part of their effective mechanism.

In spite of the lack of proved efficacy of these preparations patients with clinically defined rheumatoid arthritis, treated by a rheumatologist with oral anti-inflammatory drugs, superimposed rubs and liniments on to this conventional treatment by choice,² and there is evidence that this practice is normal.³

The majority of rubifacients contain a mixture of substances to achieve a number of effects when rubbed into the skin: an agent to produce a 'medical smell', an agent to produce an erythematous reaction and irritant response from the skin, and an anti-inflammatory agent, normally a salicylate. The combination produces an erythema and irritation which is appreciated as a sensation of warmth.

This study used a preparation delivered by aerosol which avoided the need to rub the skin. Thus we did not mechanically alter the skin by application of the rubifacient. We have attempted to show whether any constituents of the applied mixture were systemically absorbed and, if so, to measure the time course of this effect compared with other pharmacological effects. Because of the recognised effect of salicylate on the prostaglandin system of platelets, an action on these cells was chosen to demonstrate an effect at the cellular level. We did not attempt to demonstrate any beneficial clinical effect of application on inflamed or painful tissue.

Materials and methods

The subjects used in this study were volunteers aged between 20 and 40 years. They had not taken aspirin or other anti-inflammatory medicines during 2 weeks prior to the study.

Rubifacient formulation (Deep-Heat). The rubifacient mixture was produced in an aerosol can. The commercial form of the preparation was used which contained the following ingredients:

Methyl nicotinate	1.6% w/w
2-Hydroxyethyl salicylate	5.0% w/w
Methyl salicylate BP	1% w/w
Ethyl salicylate	5% w/w

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Correspondence to Dr A. J. Collins, University of Bath, School of Pharmacy and Pharmacology, Claverton Down, Bath BA2 7AY.

These substances were in an isopropanol solvent, propelled by trichloro-fluoro-urethane and dichloro-difluoromethane.

In addition a special aerosol was produced which delivered 100 μ l of solvent per burst.

Anatomical site of study. Deep-Heat was sprayed from a metered aerosol on to the anterior aspect of the right forearm of 12 volunteers. An area from 5 cm above the wrist to 5 cm below the antecubital fossa was covered as evenly as possible from a distance of 10–15 cm. When a 'therapeutic' dose was given from the commercial aerosol, the area of the arm was thoroughly wetted with propellant. Even with application from close range, as described, some of the aerosol sprayed beyond the arms and was lost. Thus the amount applied to the forearm was not an exact dose even when metered accurately.

Venepuncture. Venous blood was drawn from a vein in the right antecubital fossa when local estimations were required and from the left antecubital fossa for systemic studies.

For measurement of constituents by high performance liquid chromatography (HPLC) plasma was collected from blood placed in lithium-heparin tubes, then frozen at -20°C until assayed. Six volunteers were studied.

For measurement of platelet aggregation venous blood was collected from the right or left antecubital fossa via a No. 1 hypodermic needle, by gravity, into a plastic tube containing 0.5 ml of 3.8% w/v citrate. Four volunteers were studied.

Venous blood oxygen was measured in blood withdrawn in a plastic 5 ml syringe containing a bead of 1000 U/ml sodium heparin. Blood from either arm was studied.

Skin erythema. Erythema of sprayed skin was measured by thermography. Four volunteers, with their right arm unclothed, remained in an ambient temperature of 20°C for 20 minutes before being sprayed. The thermographic index of an area 10 by 5 cm over the anterior aspect of the forearm was then measured.^{4,5}

Measurement of ethyl and methyl salicylate in venous blood by HPLC. After application of the rubefacient to the anterior aspect of the right forearm blood was collected from the right antecubital fossa as described. The plasma was extracted with ether and the extract evaporated to dryness under nitrogen. The residue was taken up in methanol and applied to a Lichrosorb RP 8 10 μ m column (25×4.5 cm internal diameter), the mobile phase being acetate buffer, pH 2.0 acetonitrile (60:40) with a flow rate of 1.2 ml/min. Detection was by ultraviolet light at 238 nm.

Measurement of platelet aggregation. Platelet aggregation was measured by a modification of the

method of Silver *et al.*⁶ Blood was collected as previously described (9 ml blood added to 1 ml citrate) and centrifuged at 200g for 10 minutes. The platelet rich plasma (PRP) was aspirated and stored at room temperature. Platelet aggregability was then measured by taking a 200 μ l aliquot of PRP, which was stirred at 1100 rpm at 37°C in a plastic tube in a Malin dual channel aggregometer. Arachidonic acid (about 0.4 mM) was added to achieve a threshold concentration which caused platelets to clump. Platelets collected after spraying a forearm with Deep-Heat were aggregated by this method, and the amount of arachidonic acid required to achieve aggregation was compared with the threshold value.

Venous blood oxygen. Oxygen in venous blood was measured using an Instrumentation Laboratories 113 blood gas analyser with an oxygen electrode. Blood from both arms of 2 volunteers was taken for both local and systemic measurements.

Results

When the anterior aspect of the right forearm was sprayed with a small volume of Deep-Heat (500 μ l), a visible erythematous skin reaction developed within approximately 10 minutes. The reaction was measured by thermography, and a typical time profile of the erythema is shown in Fig. 1. The initial fall in the index was the result of cooling owing to evaporation of aerosol vehicle from the skin.

Concurrently, blood taken from the right antecubital fossa was assayed for ethyl and methyl salicylate to show the extent of local absorption of these compounds. The absorption profile of both these constituents in local venous blood drained from the sprayed site was similar to the time course of the erythematous response.

To assess the systemic blood levels achieved by the salicylate components of the spray the right forearm of one subject was completely wetted with the aerosol, then blood was taken from both the right and left antecubital fossae for the next 90 minutes. The blood was assayed for ethyl and methyl salicylate. The result for one volunteer is shown in Fig. 2 and the results for the group given in Table 1. In this and other experiments the absorption of methyl salicylate preceded that of ethyl salicylate, but the latter appeared in venous blood at a higher concentration. A comparatively high concentration of the salicylates was found in local venous blood for an hour after application, but in blood taken from the opposite arm they were detected up to only 20 minutes after application and at the lower limit of the sensitivity of the assay.

Serial venous blood samples taken from a vein draining a forearm sprayed with Deep-Heat became

Fig. 1 The thermographic index, measured over 70 minutes, of the anterior aspect of a right forearm after application of 500 μ l of Deep-Heat spray. Samples of venous blood taken from the right antecubital fossa over the same period showed the rapid absorption of both ethyl and methyl salicylate as measured by high performance liquid chromatography. The subject was a white female, aged 22 years. ■=Methyl salicylate. □=Ethyl salicylate.

visibly bright red over the following 30 minutes. When the oxygen concentration in this blood was measured, an increase in venous blood oxygen was found which showed a profile similar to the thermographic index curve measuring erythema. No significant increase in oxygen concentration took place in blood from the opposite arm (Fig. 3).

Deep-Heat was tested for its capacity to alter the clumping of platelets. The right forearm of volunteers was sprayed with Deep-Heat and the anterior surface completely wetted. Venous blood samples were then withdrawn from the right antecubital fossa and the harvested platelets tested for their capacity to clump in the presence of arachidonic acid (AA) in an in-vitro test. The results obtained (Fig. 4) suggest that the platelets collected from such blood were made more resistant to AA induced clumping, but only for a short time, the maximum effect being reached at 15 minutes after application of the spray. The effect was not seen in platelets from venous blood from the opposite, untreated arm.

Discussion

Rubifacient preparations are traditional mixtures.

Fig. 2 Local and systemic venous blood levels of ethyl and methyl salicylate over 90 minutes after spraying the right anterior forearm with a 'therapeutic' dose of Deep-Heat. After spraying the right forearm sufficiently to wet the whole anterior surface, venous blood was taken from the right and left antecubital fossae. The salicylates were measured in plasma by HPLC. The subject (volunteer 3) was male, aged 40 years. ■=Methyl salicylate. □=Ethyl salicylate. A: Local blood from right antecubital fossa. B: Systemic blood from left antecubital fossa.

Table 1 The mean local venous blood level of ethyl and methyl salicylate over 90 minutes after spraying the right anterior forearm with a 'therapeutic' dose of Deep-Heat in 6 volunteers. Values represent the mean plasma level $\pm$ SD of 6 samples taken over 90 minutes from each volunteer. Conditions for the collection of blood are stated in the text. These mean values are to be compared with the plasma profile over 90 minutes shown in Fig. 2.

Volunteer	Sex	Mean level of methyl salicylate in plasma (ng/ml)	Mean level of ethyl salicylate in plasma (ng/ml)
1	F	0.233 $\pm$ 0.165	0.164 $\pm$ 0.069
2	M	0.021 $\pm$ 0.029	0.007 $\pm$ 0.014
3	M	2.296 $\pm$ 1.490	0.874 $\pm$ 0.520
4	F	0.390 $\pm$ 0.212	0.618 $\pm$ 0.454
5	F	0.169 $\pm$ 0.224	0.230 $\pm$ 0.310
6	F	0.0283 $\pm$ 0.037	0.042 $\pm$ 0.049

They usually contain salicylates and other substances that cause a feeling of warmth and wellbeing when they are applied to the skin. Their effect on muscle or joint pain is difficult to quantitate, but some evidence exists that they do relieve rheumatic pain.⁷ If their popularity with the public is used as a gauge, they are successful medicines, with a market in the UK estimated to be worth £7m. However, they are not formally used to treat joint or muscle pain.

Fig. 3 Changes in oxygen concentration of venous blood taken from the right antecubital fossae of 2 subjects (A and B) over 90 minutes. The right anterior forearms ● had been sprayed with Deep-Heat at zero time. For comparison, oxygen concentration in blood taken from the left antecubital fossae ■◇ over the same period is also shown.

Fig. 4 Resistance to clumping demonstrated by platelets harvested from venous blood taken from the right antecubital fossa after the right forearm was sprayed with Deep-Heat at zero time. Platelets were induced to clump in an aggregometer by the addition of arachidonic acid. The Figure shows the amount of arachidonic acid required to induce clumping after the arm was sprayed at zero time. Numbers are the number of subjects tested at each point. Bars = SEM. The value at 15 minutes was significantly elevated ($p < 0.05$).

It is difficult to separate the beneficial effect of rubbing from the pharmacological effects of the ingredients of rubefacient preparations, both may have a counterirritant effect. For the study of the pharmacology of the ingredients we therefore chose an aerosol form of rubefacient whose manufacturers

state that massage is not necessary, and we relied purely on the ingredients of the mixture to achieve an effect.

It is difficult to know which component of the mixture caused the changes that have been measured; probably nicotine was responsible for the majority of the recorded vasodilatation. Crockford *et al.*⁸ demonstrated the effect of topically applied nicotine (Trafuril, tetrahydrofurfuryl nicotinic acid ester) and observed that the skin of a denervated limb did not produce an erythematous response. Their findings and the observation of Peterson *et al.*,⁹ who found that lepromatous skin with incompetent cutaneous innervation failed to react to nicotine, suggest that the vasodilatation is mediated by a nervous reflex rather than by the direct action of nicotine on the smooth muscle of blood vessels. The entry of topically applied nicotine is usually via hair follicles and sweat glands and is enhanced by hydrated skin,^{10 11} and its effect is thus modified by skin temperature and hydration. We observed that the erythema produced was less when the skin was cold and that the initial erythema was perifollicular.

Increase in the oxygen concentration in venous blood after application of Deep-Heat may have been due to the creation of shunts between capillaries and venules by the vasodilatory action of nicotine, causing the mixing of arteriolar and venous blood. Alternatively, nicotine does have the direct capacity to reoxygenate reduced haemoglobin, an action involving nicotinamide adenine dinucleotide (NAD),¹² which has been used to rejuvenate butcher's meat. The increase in oxygen concentration shown in Fig. 3 appears to involve only venous blood draining from the sprayed forearm; venous blood taken from the opposite arm was unaffected.

Similarly, a significant concentration of the salicylate component of the spray was found only in venous blood draining from the sprayed arm. Methyl salicylate was consistently absorbed before ethyl salicylate, but the latter, probably because a greater amount was applied, achieved a higher blood level. Systemically the levels detected were at the limit of the assay.

The premise that these salicylates, once absorbed, may affect the prostaglandin system was apparently demonstrated. Fig. 4 shows the apparent increased resistance to clumping of platelets taken from venous blood from the sprayed forearm of several volunteers and induced to clump by AA. Platelet clumping is a cellular event influenced by thromboxane A_2 .¹³ The system is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs via inhibition of the enzyme cyclo-oxygenase.¹⁴ Inhibition of platelet cyclo-oxygenase by the applied salicylate mixture, and thus a decrease in cellular thromboxane A_2 , may be the

mechanism by which this happened. This phenomenon was shown only in platelets harvested from local blood. However, salicylate is a poor inhibitor of cyclo-oxygenase, and it may be that resistance to clumping was caused by a different mechanism, for instance by the release of prostacyclin, a potent inhibitor of platelet aggregation, from blood vessel walls,¹⁵ perhaps by the vasodilatory effect of the applied nicotinate.

None of the pharmacological effects described here may contribute to the action of this or any other rubifacient in the treatment of soft tissue or joint pain, but the combination of counterirritancy, increased local blood flow owing to blood vessel dilatation, enhanced oxygen concentration in local venous blood, and an antiaggregatory effect on platelets would suggest an anti-inflammatory action. All these effects seem to be local phenomena restricted to the treated site and we have no evidence that structures deeper than the skin are affected or that there was any systemic effect. However, the changes observed were real pharmacological effects, illustrating that this type of medicine is not therapeutically inactive and may demonstrate useful pharmacological principles.

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