Br. J. exp. Path. (1975) **56**, 554

A COMPARISON OF THE EFFECTIVENESS OF SOME ANTI-INFLAMMATORY DRUGS ON THERMAL OEDEMA

N. B. PILLER

From the Department of Zoology, University of Adelaide, South Australia

Received for publication July 22, 1975

Summary.—The efficacy of a group of anti-inflammatory drugs with similar modes of action was tested on thermal oedema. Of these, coumarin and sodium-rutin-sulphate were most effective. A combination of these, marketed as Venolot, although still beneficial, was not as effective as either given singly.

The other drugs tested—levamisole, Reparil and Venoruton—although also of some benefit in treatment of this oedema, did not approach the overall effectiveness of coumarin or sodium-rutin-sulphate. Both drugs are characterized by a very wide safety margin between the therapeutic and the toxic dose. In addition, they are cheap, easy to obtain and can be taken orally. They work by stimulating phagocytosis, enzyme production and thus proteolysis and a subsequent removal of protein and oedema fluid from the injured tissues.

AT PRESENT, there is available a very wide range of anti-inflammatory drugs which are useful in varying degrees for the treatment of high protein oedemas. Recently, a new group of drugs called the benzopyrones have been utilized in the treatment of such oedemas (Földi-Börcsök, 1972; Földi-Börcsök and Földi, 1973; Casley-Smith and Piller, 1975; Casley-Smith, 1975). They have been found to be very effective in reducing the maximal swelling volumes and in improving the rate of resolution of the oedema (Piller, 1975*a*).

Despite the fact that the literature abounds with research articles relating to the anti-inflammatory drugs used and their modes of action, there are very few works on comparisons of such drugs.

This experiment is concerned with a comparison of the efficacy of a number of drugs and their major components. All of the drugs chosen contain either a benzopyrone or an active constituent which behaves similarly. In order for a comparison to be made, the drugs or their active constituents were tested under the same conditions as have been previously used for an estimation of the anti-oedema activity of Venalot and its active constituents (Piller, 1975*a*).

MATERIALS AND METHODS

The drugs to be compared are Venalot (Schaper and Brummer), the active constituents of which are coumarin and sodium-rutin-sulphate; Ketrax (ICI), the active constituent of which is levamisole; Reparil (Dr Madous and Co.), the active constituent of which is aescin; and Venoruton (Zyma) which contains a mixture of mono-, di-, tri- and tetra-0-(β-hydroxyethyl) rutosides. In all cases the optimal recommended dosages are used.

For the experiment, 77 female albino rats weighing 320 $\pm$ 20 g were used. They were randomly divided into groups of 11.

In every group, both rear hind legs of all rats were closely shaven 2 days before the experiment. Two h before the immersion of their hind limbs into hot water the groups were treated as follows:

Group 1. Control group—physiological saline only.

Group 2. Venalot—each ml containing 1·5 mg coumarin and 50 mg sodium-rutin-sulphate, was administered at a dosage of 17·0 ml/kg body weight.

Group 3. Coumarin—each ml containing 2·5 mg in a 2% solution of A.R. ethyl alcohol in physiological saline, was administered at 10 ml/kg body weight.

Group 4. Sodium-rutin-sulphate—each ml containing 42·5 mg in physiological saline, was administered at 10 ml/kg body weight.

Group 5. Levamisole—each ml containing 5 mg in physiological saline, was administered at 10 ml/kg body weight.

Group 6. Reparil—each ml containing 0·03 mg aescin in physiological saline, was administered at 10 ml/kg body weight.

Group 7. Venoruton—diluted so that each ml contained 50 mg of a mixture of hydroxyethyl rutoside in physiological saline, was administered at 10 ml/kg body weight.

All injections were intraperitoneal. After 1½ h, each member of every group was given an intraperitoneal injection of 0·75 ml of a 10% solution of Sagital (May and Baker) in 10% A.R. ethyl alcohol/100 g body weight.

Each member of every group was then burnt by immersion of its right hind limb in hot fast-flowing water at 54° for 1 min. The volume of the swelling of both the burnt and the normal paws of each animal of every group was measured by plethysmography as described previously (Piller, 1975*a*). Measurements were made immediately before burning, and then at 3, 6, 9, 12, 15, 18, 24, 30, 36, 48, 72, 96, 150 and 200 h after burning. At every time each paw was measured 3 times and the average recorded. For the analysis of the oedema, a simple representation consisting of 2 regression lines was used. One extended from zero time to the maximal recorded volumes and the other from this volume to the end of the observations. In the calculations, the individual values for each animal of every group was considered.

RESULTS

Effect on rate of formation of thermal oedema (Table I)

The normal rate of swelling for thermally injured paws is approximately 0·07 ml/h. The animals pretreated with Venalot showed no change in this rate of swelling. However, for all of the other active constituents of the drugs there were significant increases. Coumarin significantly increased the rate of formation by 1·75 times ($0·001 < P < 0·01$) while sodium-rutin-sulphate, levamisole and Reparil almost doubled its rate of formation. Venoruton had an even more marked effect on oedema formation by increasing it almost 4 times ($P < 0·001$).

Effect on maximal swelling volume (Table II)

The swelling volumes of the thermally injured paws of the untreated animals rose to approximately 2·63 ml. Those pretreated with Venalot were reduced significantly ($0·01 < P < 0·05$) by 8%. Coumarin and sodium-rutin-sulphate were very much more effective, bringing about reductions of 20% ($P < 0·001$) and 38% ($P < 0·001$) respectively. Pretreatment with Reparil was not as effective, resulting in a 10% ($0·01 < P < 0·05$) reduction. Levamisole and Venoruton had no effect.

Effect on time of maximal swelling volume (Table III)

The paws of untreated thermally

TABLE I.—*Effect on the Rate of Formation of Thermal Oedema*

Treatment	Number	Rate of swelling (ml/h × 10^{-2})	S.e. (× 10^{-2})	P*
Burn	10	6·88	1·27	
Venalot	10	8·55	1·00	—
Coumarin	10	11·10	1·54	↑ ↑
Sodium-rutin-sulphate	10	12·55	2·96	↑
Levamisole	11	12·81	1·99	↑ ↑
Reparil	11	12·21	1·53	↑ ↑ ↑
Venoruton	11	23·05	2·90	↑ ↑ ↑

* Arrows indicate direction and significance of change in the rate of oedema formation:
— NS; ↑ $0·01 < P < 0·05$; ↑ ↑ $0·001 < P < 0·01$; ↑ ↑ ↑ $P < 0·001$.

TABLE II.—*Effect on Maximal Swelling Volume*

Treatment	Number	Mean group paw volume (ml)	S.e.	P*	% Change
Burn	10	2·625	0·0775		
Venalot	10	2·410	0·1294	↓	8
Coumarin	10	2·100	0·097	↓ ↓ ↓	20
Sodium-rutin-sulphate	10	1·63	0·2945	↓ ↓ ↓	38
Levamisole	11	2·522	0·085	—	4
Reparil	11	2·350	0·0939	↓	10
Venoruton	11	2·473	0·0864	—	6

* Arrows indicate significance of reduction in swelling volume:
— NS; ↓ $0{\cdot}01 < P < 0{\cdot}05$; ↓ ↓ $0{\cdot}001 < P < 0{\cdot}01$; ↓ ↓ ↓ $P < 0{\cdot}001$.

TABLE III.—*Effect on the Time of Maximal Swelling Volume*

Treatment	Number	Mean group time (h)	S.e.	P*	% Change
Burn	10	16·5	0·948		
Venalot	10	12·2	0·628	↓ ↓ ↓	26
Coumarin	10	9·6	0·640	↓ ↓ ↓	42
Sodium-rutin-sulphate	10	7·8	0·772	↓ ↓ ↓	52
Levamisole	11	6·82	1·673	↓ ↓ ↓	59
Reparil	11	10·1	1·23	↓ ↓ ↓	39
Venoruton	11	11·4	6·145	—	31

* Arrows indicate significance of reduction in the time of maximal swelling volume:
— NS; ↓ $0{\cdot}01 < P < 0{\cdot}05$; ↓ ↓ $0{\cdot}001 < P < 0{\cdot}01$; ↓ ↓ ↓ $P < 0{\cdot}001$.

injured rats normally reach a maximal swelling volume 16·5 h following injury. With the exception of Venoruton, all of the drugs and their active constituents tested significantly reduced this time. Pretreatment with Venalot brought about a 26% ($0{\cdot}01 < P < 0{\cdot}05$) reduction, while coumarin, sodium-rutin-sulphate, levamisole and Reparil caused very significant ($P < 0{\cdot}001$) reductions of 42%, 52%, 59% and 39% respectively.

Effect on rate of resolution of thermal oedema (Table IV)

The rate of resolution of the oedema is perhaps the most important criterion of the effectiveness of anti-inflammatory drugs. Mild thermal oedema used here to test these drugs normally resolves of its own accord at approximately 0·003 ml/h. Pretreatment with Venalot results in the oedema resolving approximately 3 times as fast ($0{\cdot}01 < P < 0{\cdot}05$) as in the control group. Sodium-rutin-sulphate, Reparil and Venoruton also enhance the resolution by approximately 3 times ($P < 0{\cdot}001$) while coumarin is the most effective, resulting in a 4-times increase ($0{\cdot}001 < P < 0{\cdot}01$) compared with the control. Levamisole had no effect.

TABLE IV.—*Effect on the Rate of Resolution of Thermal Oedema*

Treatment	Number	Rate of resolution (ml/h $\times 10^{-2}$)	S.e. ($\times 10^{-2}$)	P*
Burn	10	0·24	0·33	
Venalot	10	1·03	0·22	↑ ↑
Coumarin	10	1·23	0·15	↑ ↑
Sodium-rutin-sulphate	10	0·94	0·13	↑ ↑ ↑
Levamisole	11	0·58	0·275	—
Reparil	11	1·02	0·36	↑ ↑
Venoruton	11	1·07	0·23	↑ ↑ ↑

* Arrows indicate significance of increase in rate of resolution:
— NS; ↑ $0{\cdot}01 < P < 0{\cdot}05$; ↑ ↑ $0{\cdot}001 < P < 0{\cdot}01$; ↑ ↑ ↑ $P < 0{\cdot}001$.

DISCUSSION

Although the literature abounds with research articles relating to anti-inflammatory drugs and their modes of action, there are very few papers comparing the efficacy of these drugs on a standard reproducible form of inflammation such as a mild burn oedema.

The present experiment is thus concerned with a comparison of the therapeutic usefulness of a number of proprietary drugs and their major components on mild burn oedema.

All the drugs used, with the exception of Reparil (which contains aescin as its active component), are characterized by a very wide safety margin between the LD_{50} and the recommended therapeutic doses. For information relating to Venalot, coumarin and the rutosides see Hazelton *et al.* (1956), Radouco-Thomas *et al.* (1964, 1965), Böhm (1967), Kluken (1971) and Casley-Smith (1975).

Levamisole, previously used as a potent antihelmintic (Lionel *et al.*, 1969; Adam *et al.*, 1973), has recently been shown to be a stimulator of the reticuloendothelial system (RES) and so has been included in this comparison because of the similarity of its mode of action. It also has a wide safety margin between the therapeutic dose and LD_{50} (Thienpont *et al.*, 1966; Raeymakers, Roevens and Janssen, 1967).

Reparil, containing aescin (a saponin), has been used as an anti-inflammatory agent (Girerd *et al.*, 1961; Preziosi and Manca, 1965; Vogel, Marek and Oertner, 1970); again it is included because of a part similarity to the benzopyrones in its mode of action (Girerd *et al.*, 1961; Preziosi and Manca, 1965). However, it has a very narrow safety margin between the therapeutic and toxic doses (Girerd *et al.*, 1961).

Venoruton contains a mixture of hydroxyethyl rutosides, all of which are virtually free of toxicity even in extremely high doses (Radouco-Thomas *et al.*, 1964; Böhm, 1967).

The mode of action of coumarin and related benzopyrones has been elucidated, at least for high protein oedemas. They enhance the normal lysis of the abnormal accumulated proteins of the extracellular compartment (Casley-Smith and Piller, 1974).

The resulting fragments, because of their size, a concentration gradient directed from the tissues into the blood and a high diffusion coefficient, can rapidly pass down the intercellular junctions to the blood. The excessive amounts of protein are removed and the oedema fluid released (Piller, 1975*a*, *b*).

The macrophages seem to be the cells which are stimulated by coumarin administration since their destruction with silica removes all anti-oedema effects of coumarin (Piller, 1975*b*). *In vitro* work with tissue cultures of macrophages has shown that coumarin stimulates normal proteolysis by approximately 220% (Bolton and Casley-Smith, 1975), thus confirming *in vivo* studies (Piller, 1975*e*, *f*).

Coumarin (and perhaps the other benzopyrones) causes significant increases in liver weights, even when given as a single dose (Piller, 1975*g*). This may indicate an increase in the number of phagocytosing cells (Hoebeke and Franchi, 1973). Thus, the benzopyrones seem to belong to a very large group of RES stimulants (Roos, 1970) encompassing all the active constituents tested in this experiment.

Hoebeke and Franchi (1973) found that levamisole had a dose-related enhancement of carbon clearance from the blood. However, unlike other RES stimulants, levamisole did not cause increases in liver or spleen weights (Hoebeke and Franchi, 1973). Thus, although levamisole stimulates macrophage activity (Schulze and Raettig, 1973) it does not work by increasing cell numbers; rather it has been suggested that it may act by increasing the probability of particle engulfment (Hoebeke and Franchi, 1973).

Reparil is also used as an anti-

inflammatory agent (Vogel and Oertner, 1970). However, since it is only effective against the initial exudative phase of inflammation (Vogel *et al.*, 1970) and because of its toxicity (Girerd *et al.*, 1961), it has only limited use. Reparil, like the benzopyrones, has a weak vasoconstrictor effect due to increased levels of adrenalin which it causes (Girerd *et al.*, 1961).

When considering anti-inflammatory drugs there are 4 very important parameters upon which their effects should be tested. These are represented in Tables I–IV: firstly, considering their effect on the rate of formation of the oedema. All but Venalot significantly increased the rate of swelling. The rate was doubled for all the other drugs, with the exception of Venoruton where it was quadrupled. It would seem then that levamisole and aescin behave similarly to the benzopyrones either by causing injury to the vessel walls directly or indirectly through the release of mediators (Piller, 1975*c*), or by increasing intravascular pressure and flow (Piller, 1975*c*). Any tendency to an increased pressure and flow would enhance leakage of protein and fluid from already existing defects and so increase the rate of oedema formation.

In any high protein oedema the maximal swelling volume of the limb, as well as the time in which it is reached, is very important since this may determine whether the skin of the affected limb will rupture or not. Consequently, any drug which reduces either or both of these parameters would be more useful.

Coumarin and sodium-rutin-sulphate very significantly reduce the maximal swelling volume by 20% and 38% respectively. The combination of these two in Venalot, although causing a significant reduction ($0{\cdot}01 < P < 0{\cdot}05$) was only of the order of 8% for reasons which are discussed elsewhere (Piller, 1975*d*).

A similar situation may also hold for Venoruton which, as mentioned before, also contains a mixture of benzopyrones. Table III shows that all the drugs, with the exception of Venoruton, cause significant reductions ($P < 0{\cdot}001$) in the time at which the maximal swelling volume is reached. Thus, despite the initial increase in the rate of swelling, the drugs generally not only reduce the maximal swelling volumes but also the time at which it is reached. The swelling volume is a good indication of the fluid in the tissues and this is a good indication of the amount of protein. In this respect, coumarin and sodium-rutin-sulphate are the most effective in reducing the abnormal accumulated proteins of the tissue; they do this by causing their proteolysis and removal of the fragments *via* the vascular system (Piller and Casley-Smith, 1975). The slightly different mode of action of levamisole which only results in activation of phagocytosis (Hoebeke and Franchi, 1973) shows that this alone is not enough to reduce the oedema significantly and confirms previous work (Casley-Smith, 1975) that the benzopyrones do not only work in this way.

Reparil was about as effective as Venalot in reducing the maximal swelling volume. Girerd *et al.* (1961) claim its anti-oedema activity is due to its effects on the vasoconstrictor tone of the capillaries, which is increased due to increased levels of adrenaline. Here it is the vessels of the skin which will be affected, resulting in a subsequent reduction in the number of capillaries from which protein and fluid may escape. However, the vessels of the muscles will undergo vasodilatation, with a subsequent increase in blood flow and leakage of protein and fluid into the tissues. Thus, the final amount of oedema will depend on the ratio of the cutaneous vessels to those in the upper layers of muscle, as well as the degree of injury caused to them by the burn.

The final parameter upon which the effects of the drugs are to be examined is the rate of resolution. Of these coumarin is the most effective and leva-

misole the least. The explanation seems related to their modes of action. Levamisole is limited in its effects because it only stimulates increased phagocytosis and each phagocyte can only ingest a certain amount of material, whereas the enhanced lysis of protein induced by coumarin can continue to occur for as long as the drug is present in adequate levels.

When screening anti-inflammatory drugs to determine their suitability for the treatment of thermally induced oedema, the most important parameters upon which their effect should be examined are their effect on the maximal swelling volume, the time at which it is reached and the rate at which the oedema resolves. In this respect coumarin or sodium-rutin-sulphate alone would be the best therapy. Their combination in Venalot impairs their effectiveness, especially that on the reduction of maximal swelling volume. This does not mean that Venalot, Reparil, Venoruton or even levamisole are not suitable as anti-inflammatory agents, since to some extent they all had a beneficial effect on one or more of the parameters examined here. The results obtained here are not meant to apply to other forms of oedema, they only show that for this type of mild high protein oedema, administration of coumarin or sodium-rutin-sulphate is the most useful therapy. The treatment can be even more effective if the injured area is blown softly with air at 20°C (Piller, 1975*a*).

I am most grateful to the drug companies mentioned in Materials and Methods for supplying the drugs or their active constituents. I also thank the Australian Research Grants Committee for their support given *via* Dr J. R. Casley-Smith.

REFERENCES

ADAM, M., MORGAN, O., PERSAUD, C. & GIBBS, W. (1973) Hyperinfection Syndrome with Strongyloides Stercoralis in Malignant Lymphoma. *Br. med. J.*, i, 264.

BÖHM, K. (1967) *Die Flavonoide.* Aulendorf 1 Wurtt: Cantor.

BOLTON, T. & CASLEY-SMITH, J. R. (1975) An *in vitro* Demonstration of Proteolysis by Macrophages and its Increase with Coumarin. *Experientia*, **31**, 271.

CASLEY-SMITH, J. R. (1975) The Actions of the Benzo-pyrones on the Blood-Tissue-Lymph System. *Folia Angiologica.* Accepted for publication.

CASLEY-SMITH, J. R. & PILLER, N. B. (1974) The Pathogenesis of Oedemas and the Therapeutic Action of Coumarin and Related Compounds. *Folia Angiologica*, Suppl., **3**, 33.

FÖLDI-BÖRCSÖK, E. (1972) Effect of External Lymph Drainage and of Coumarin Treatment on Thermal Injury in the Rat Hind Leg. *Br. J. Pharmac.*, **46**, 254.

FÖLDI-BÖRCSÖK, E. & FÖLDI, M. (1973) Lymphoedema and Vitamins. *Am. J. clin. Nutr.*, **26**, 185.

GIRERD, R. J., DIPASQUALE, G., STEINETZ, B. G., BEACH, V. L. & PEARL, W. (1961) Anti-Edema Properties of Aescin. *Archs int. Pharmacodyn.*, **133**, 127.

HAZELTON, L. W., TUSING, B. R., ZEITLIN, R., THIESSEN, J. & MURER, H. K. (1956) Toxicity of Coumarin. *J. Pharmacol. exp. Ther.*, **118**, 348.

HOEBEKE, J. & FRANCHI, G. (1973) Influence of Tetramisole and its Optical Isomers on the Mononuclear Phagocytic System. Effect on Carbon Clearance in Mice. *J. Reticuloendothel. Soc.*, **14**, 317.

KLUKEN, N. (1971) A Clinical Double Placebo Experiment for Trying to Objectivate the Therapy with Remedies Against Venous Diseases. *Therap. Woche.*, **21**, 72.

LIONEL, N. D., MIRANDO, E. H., NANAYAKKARA, J. C. & SOYSA, P. E. (1969) Levamisole in the Treatment of Ascariasis in Children. *Br. med. J.*, ii, 340.

PILLER, N. B. (1975*a*). The Resolution of Thermal Oedema at Various Temperatures under Coumarin Treatment. *Br. J. exp. Path.*, **56**, 83.

PILLER, N. B. (1975*b*) Ineffectiveness of Coumarin on the Treatment of Thermal Oedema in Macrophage-free Rats. *ArzneimForsch.* Submitted for publication.

PILLER, N. B. (1975*c*) Benzo-pyrones: Their Selective Injury to Rabbit Vascular Endothelium. *J. Clin. exp. Pharmacol. Physiol.* In the press.

PILLER, N. B. (1975*d*) Benzo-pyrones and their Effect on the Resolution of Thermal Oedema. *Folia Angiologica.* Submitted for publication.

PILLER, N. B. (1975*e*) Influence of Benzo-pyrones on the Acid and Neutral Protease Levels in the Skin, Muscle, Serum and Lymph of Rats with Various High Protein Oedemas. *J. exp. Med.* Submitted for publication.

PILLER, N. B. (1975*f*) Further Evidence for the Induction of Proteolysis by Coumarin. *Br. J. exp. Path.* In the press

PILLER, N. B. (1975*g*) An Explanation for the Effect of Coumarin on Rat Liver Weights. In preparation.

PILLER, N. B. & CASLEY-SMITH, J. R. (1975) The Effect of Coumarin on Protein and PVP Clearance

from Rat Legs with Various High Protein Oedemas. *Br. J. exp. Path.* Accepted for publication.

PREZIOSI, P. & MANCA, P. (1965) The Anti-oedematous and Antiphlogistic Action of Aescin and its Relation to the Hypophyseal–Adrenal System. *ArzneimForsch.*, **15**, 404.

RAEYMAEKERS, A. M., ROEVENS, L. F. & JANSSEN, P. A. (1967) The Absolute Configurations of the Optical Isomers of the Broad Spectrum Antihelmintic Tetramisole. *Tetrahedron Letters*, **16**, 1467.

RADOUCO-THOMAS, S., GRUMBACH, P., NOSAL, G. L. & RADOUCO-THOMAS, C. (1964) Anti-inflammatory Effect of Trihydroxyethyl rutoside Assessed in the Granuloma Pouch. *Life Sci.*, **3**, 459.

RADOUCO-THOMAS, S., GRUNBACH, P., NOSAL, G. L. & GARCIN, F. (1965) Etude Toxicologique de quelques factors P. *Therapie*, **20**, 879.

Roos, B. (1970) Macrophagen: Herkunft, Entwicklung und Funktion. In *Immune Reactions*. Eds. A. Studer and H. Cottier. Berlin: Springer.

SCHULZE, H. J. & RAETTIG, M. J. (1973) Increase of Peritoneal Macrophage Activity of the Mouse by Levamisole. *Verh. Dt. Ges. inn. Med.*, **79**, 622.

THIENPONT, D., VANPARIJS, O. F., RAEMAEKERS, A. M., VANDENBERK, J., DEMOEN, P. J., ALLEWIJN, F. J., MARSBOOM, R. P., NIEMEGEERS, C. J., SCHELLEKENS, K. H. & JANSSEN, P. A. (1966) Tetramisole (R8299) a New Potent Broad Spectrum Antihelmintic. *Nature, Lond.*, **209**, 1084.

VOGEL, V. G., MAREK, M. L. & OERTNER, R. (1970) Studies on the Mechanisms of Therapeutic and Toxic Actions of the Horse Chestnut Saponin Escin. *ArzneimForsch.*, **20**, 703.