Br. J. Pharmac. (1972), **46**, 254–259.

Effect of external lymph drainage and of coumarin treatment on thermal injury in the rat hind leg

ETHEL FÖLDI-BÖRCSÖK

Lymphological Research Laboratory, Schaper & Brümmer, Salzgitter-Ringelheim, West-Germany

Summary

1. External lymph drainage brings about a significant protective effect in thermal oedema of the rat hind leg. It is suggested that external lymph drainage prevents vasoactive substances drained from the site of injury from passing into the blood stream, which would further increase permeability of the injured blood capillaries.

2. Coumarin (5,6-benzo-alpha-pyron) brings about a significant protective effect against the same injury in sham-operated rats.

3. The strongest protective effect may be attained by combining external lymph drainage with the administration of coumarin.

4. The additional therapeutic effect brought about by coumarin treatment in rats with external lymph drainage is not mediated by an increased flow. The possible mechanisms are discussed.

Introduction

Lymph is known to contain almost all the constituents of plasma (Yoffey & Courtice, 1956) and both the globulin from which kinins are derived and a kinin-inactivating enzyme, kininase (Schachter, 1960). Jacobsen (1966) found two different substrates for kinin-forming enzymes (also present in plasma) in the lymph collected from the hind limbs of anaesthetized animals. Edery & Lewis (1963) have shown that thermal injury results in a considerable increase in lymph flow from the injured extremity with an increase in kinin-forming activity of the lymph as well as histamine. The authors concluded that the increase in kinin-forming activity is the result of a specific activation of the kinin-forming system in the interstitial fluid, brought about by histamine release.

According to Lewis & Wawratschek (1971), there is, after thermal injury, a pronounced increase in the lymph of an inactive form of kallikrein which can be activated by acid treatment (but not by contact with a glass surface). According to these authors, increase in vascular permeability leads to the passage of pre-kallikrein from the plasma into the interstitial space of the injured area. This precursor would be activated by contact with the damaged cells, or possibly by tissue activators released during injury (Lewis, 1959).

Jonsson, Arturson & Änggård (1971) demonstrated the appearance of prostaglandins in lymph from scalded tissue.

Recirculation of kinins and/or other vasoactive substances from the site of thermal injury via lymphatics could lead to an increase in blood capillary permeability at the site of injury. The effect of external lymph drainage was therefore studied on thermal injury.

Since coumarin is a substance known to possess a pronounced anti-inflammatory and lymphokinetic effect (Földi-Börcsök, Bedall & Rahlfs, 1971; Mislin, 1971; Collard, 1971) its influence on thermal injury has also been studied alone and in combination with external lymph drainage.

Methods

Experiments were performed on 80 male Wistar rats (150 ± 20 g body weight) fed R-Altromin and water *ad lib.* Animals were divided into two groups.

Group I (experiments performed under general anaesthesia)

Subgroup 1

After the administration of 3 ml cream (lipid content 7·5 g%) through a stomach tube, 17 ml/kg 0·9% NaCl w/v (saline) was injected i.p. during 90 minutes. The rats were then anaesthetized with nembutal (50 mg/kg i.p.). The volume of the right hind leg, up to the knee joint, was measured with a plethysmograph. Saline was infused (1·5 ml/h) through a cannula inserted in a jugular vein. Intra-arterial pressures were recorded and registered by connecting a catheter inserted into a carotid artery to a Statham pressure transducer which was in turn connected to a Hellige multiscriptor. The thoracic duct was cannulated below the diaphragm. After four 15 min periods when lymph was collected, thermal injury was induced in the right hind leg by immersing it up to the knee joint for 1 min in a water bath at 55° C. Lymph collection was continued for another two hours (eight 15 min periods). Finally, plethysmographic measurements were repeated.

Subgroup 2

The experimental procedure was the same as in subgroup 1, but no saline was injected either intraperitoneally or intravenously.

Subgroup 3

The experimental procedure was the same as in subgroup 1, but, instead of saline, 25 mg/kg coumarin was injected i.p. and a 0·15% solution of coumarin (in 10% propandiol) was infused at a rate of 1·5 ml/h (total dose: 25 mg/kg).

Subgroup 4

Ninety min after the administration of 3 ml cream and the i.p. injection of 17 ml/kg saline the rats were anaesthetized and volume measurements performed. Following this, a lumbar incision was made (sham operation). One hour later, thermal injury was induced. Two hours later, plethysmography was repeated.

Subgroup 5

The experimental procedure was the same as in subgroup 4, but after the administration of cream, 25 mg/kg courmarin was injected i.p. After inducing thermal injury, the same dose was injected again.

Group II (experiments performed in unanaesthetized animals)

Subgroup 6

After plethysmography and i.p. injection of 17 ml/kg saline, rats were anaesthetized with ether and the thoracic duct was cannulated. Lymph collections were started after recovery from anaesthesia. After four 15 min periods when lymph was collected, thermal injury was induced. Lymph collection was continued for another two hours (8 lymph collection periods). Finally, plethysmography was repeated.

Subgroup 7

The experimental procedure was the same as in subgroup 6, but instead of saline, coumarin (25 mg/kg) was injected.

Subgroup 8

Experimental procedure was the same as in subgroup 6, but the thoracic duct was not cannulated, only a sham operation performed.

Subgroup 9

Experimental procedure was the same as in subgroup 8, but instead of saline, coumarin (25 mg/kg) was injected. Lymph protein concentrations were measured by the Kjeldahl method. Data were analysed statistically by Student's *t* test.

Results

Group I (anaesthetized animals)

In sham operated rats oedematous swelling amounted to $1{\cdot}62 \pm 0{\cdot}21$ ml. External thoracic duct drainage reduced thermal oedema significantly, to $0{\cdot}92 \pm 0{\cdot}22$ ml (a 43% protective effect), without saline treatment, and to $1{\cdot}14 \pm 0{\cdot}21$ ml (a 30% protective effect), with saline treatment: there is no significant difference between these two values. Coumarin treatment alone reduced thermal oedema to $0{\cdot}91 \pm 0{\cdot}24$ ml (a 43% protective effect); the difference is statistically significant. The most pronounced protection was brought about by combining external lymph drainage wth coumarin treatment: thermal oedema amounted to $0{\cdot}57 \pm 13$ ml (a 65% protective effect). This value is significantly lower than any other measured in this series (Table 1).

Group II (unanaesthetized animals)

In sham operated animals oedematous swelling amounted to $2{\cdot}12 \pm 0{\cdot}17$ ml. External lymph drainage reduced thermal oedema significantly, to $1{\cdot}47 \pm 0{\cdot}24$ ml (a 31% protective effect). Coumarin treatment alone reduced thermal oedema to $1{\cdot}68 \pm 0{\cdot}26$ ml (a 21% protective effect); the difference is statistically significant. By combining external lymph drainage with the administration of coumarin, thermal oedema amounted to $0{\cdot}90 \pm 0{\cdot}46$ ml; this means, the protective effect was 58%. This value is significantly lower than any other measured in this series (Table 2).

Coumarin treatment did not increase lymph flow, or lymphatic protein output (Table 3).

TABLE 1. *Thermal oedema in anaesthetized rats (Group I)*

Subgroup No.	Procedure	n	$\bar{x}$	$S_{\bar{x}}$	F	t	P
1	Lymph drainage NaCl infusion	12	1·14	0·21	0·92	2·28	<0·05
2	Lymph drainage without treatment	9	0·92	0·22			
1	Lymph drainage NaCl infusion	12	1·14	0·21	2·60	7·03	<0·001
3	Lymph drainage Coumarin infusion	10	0·57	0·13			
1	Lymph drainage NaCl infusion	12	1·14	0·21	0·97	4·93	<0·001
4	Sham operation NaCl infusion	9	1·62	0·21			
1	Lymph drainage NaCl infusion	12	1·14	0·21	0·67	2·04	N.S.
5	Sham operation Coumarin infusion	10	0·91	0·24			
2	Lymph drainage without treatment	9	0·92	0·22	2·69	4·05	<0·001
3	Lymph drainage Coumarin infusion	10	0·57	0·13			
2	Lymph drainage without treatment	9	0·92	0·22	0·90	6·70	<0·001
4	Sham operation NaCl infusion	9	1·62	0·21			
2	Lymph drainage without treatment	9	0·92	0·22	1·19	0·03	N.S.
5	Sham operation Coumarin infusion	10	0·91	0·24			
3	Lymph drainage Coumarin infusion	10	0·57	0·13	1·96	11·79	<0·001
4	Sham operation NaCl infusion	9	1·62	0·21			
3	Lymph drainage Coumarin infusion	10	0·57	0·13	2·9	3·7	<0·005
5	Sham operation Coumarin infusion	10	0·91	0·24			
4	Sham operation NaCl infusion	9	1·62	0·21	0·75	6·55	<0·001
5	Sham operation Coumarin infusion	10	0·91	0·24			

Discussion

External lymph drainage significantly reduces thermal oedema, both in anaesthetized and in unanaesthetized rats. This therapeutic effect cannot be mediated by lymph (=fluid) loss: theoretically, dehydration should *decrease* oedema formation. Copious replacement of water did not abolish the effect of external lymph drainage and, if fluid replacement was combined with the administration of coumarin, the protective effect was even more pronounced.

In the intact animal, vasoactive substances are set free at the site of injury and are transported by the lymph into the blood stream. It is suggested that external lymph drainage prevents recirculation of components of the kinin system (and/or other vasoactive substances) and thus protects blood capillaries of the traumatized area from their additional harmful effects. Coumarin affords a protective effect in the intact animal and increases further the effect of external lymph drainage,

TABLE 2. *Thermal oedema in unanaesthetized rats (Group II)*

Subgroup No.	Procedure	n	$\bar{x}$	$S_{\bar{x}}$	F	t	P
6	Lymph drainage NaCl injection	8	1·47	0·24	0·29	3·06	<0·005
7	Lymph drainage Coumarin injection	8	0·90	0·46			
6	Lymph drainage NaCl injection	8	1·47	0·24	0·48	5·42	<0·001
8	Sham operation NaCl injection	6	2·12	0·17			
6	Lymph drainage NaCl injection	8	1·47	0·24	1·08	1·61	N.S.
9	Sham operation Coumarin injection	8	1·68	0·26			
7	Lymph drainage Coumarin injection	8	0·90	0·46	0·14	6·1	<0·001
8	Sham operation NaCl injection	6	2·12	0·17			
7	Lymph drainage Coumarin injection	8	0·90	0·46	0·31	4·14	=0·001
9	Sham operation Coumarin injection	8	1·68	0·26			
8	Sham operation NaCl injection	6	2·12	0·17	0·44	3·59	<0·005
9	Sham operation Coumarin injection	8	1·68	0·26			

TABLE 3. *Effect of coumarin on lymph flow (A) and lymphatic protein output (B) in rats before (first hour: control values) and after thermal injury (second and third hour)*

	Time	Treatment	n	$\bar{x}$	$S_{\bar{x}}$	F	t	P
(A)	First hour	NaCl	9	0·91	0·28	1·17	0·70	<0·5
		Coumarin	10	0·83	0·26			
	Second hour	NaCl	9	0·80	0·30	1·44	1·76	<0·2
		Coumarin	10	0·58	0·25			
	Third hour	NaCl	9	0·75	0·23	1·17	1·98	<0·1
		Coumarin	10	0·55	0·21			
(B)	First hour	NaCl	9	22·3	8·7	1·60	0·48	<0·7
		Coumarin	10	20·6	6·9			
	Second hour	NaCl	9	22·4	6·04	0·43	0·59	<0·5
		Coumarin	10	20·3	9·1			
	Third hour	NaCl	9	20·5	8·6	1·60	1·43	<0·2
		Coumarin	10	15·6	6·8			

both in anaesthetized and in unanaesthetized rats. Although coumarin is known to increase thoracic duct lymph flow in the dog and in man (Földi, Kovách, Varga & Zoltán, 1962; Bartós & Brzék, 1970) and lymph flow from the lower extremity in the hydrated dog (Calnan & Pflug, personal communication), its protective effect in thermal injury is probably not mediated by increasing lymph drainage from the traumatized extremity since in the present experiments it did not cause an increase in lymph flow. Coumarin, according to our hypothesis, either influences the kinin-forming system, or eventually, protects the target cells directly, or both. It should be stressed, however, that other possibilities do exist. As mentioned above, prostaglandins are released at the site of thermal injury. Possibly coumarin acts somewhere on this system. Lewis (1966, 1967) found increased concentrations of lactic dehydrogenase, glutamic oxalacetic transaminase and glutamic pyruvic transaminase in the lymph of thermally injured limbs; these enzymes are set free as a result of cell damage. Some of these, or other enzymes may prove to be toxic in parts of the body in which they are foreign. Again, coumarin may act by stabilizing cell membranes and/or neutralizing some of the aforementioned substances. Further work is needed to clarify its mode of action.

REFERENCES

Bartós, V. & Brzék, V. (1970). Die Wirkung von Esberiven forte auf die Lymphströmung im Ductus thoracicus des Menschen. *Med. Klin.*, **65**, 1701–1703.

Calnan, J. S. & Pflug, J. J.

Collard, M. (1971). Radiologische Studie über die Wirkung von Pharmaka auf die Lymphgefäße der unteren Extremitäten. *Fortschr. Röntgenstr.*, **115**, 643–649.

Edery, H. & Lewis, G. P. (1963). Kinin-forming activity and histamine in lymph after tissue injury. *J. Physiol., Lond.*, **169**, 568–583.

Földi-Börcsök, E., Bedall, F. K. & Rahlfs, V. W. (1971). Die antiphlogistische und ödemhemmende Wirkung von Cumarin aus Melilotus officinalis. *Arzneimittel. Forsch.* (*Drug Res.*), **21** 2025–2030.

Földi, M., Kovách, A. G. B., Varga, A. & Zoltán, Ö. T. (1962). Die Wirkung des Melilotis-Präparates Esberiven auf die Lymphströmung. *Arztl. Forsch.*, **16**, 99–102.

Jacobsen, S. (1966). Substrates for plasma kinin forming enzymes in human dog and rabbit plasmas. *Br. J. Pharmac. Chemother.*, **26**, 403–411.

Jonsson, E.-E., Arturson, G. & Änggård, E. (1971). Appearance of prostaglandins in lymph from burned tissue. In: *Research in Burns*. Matter, Barclay, and Koničková. Bern: Hans Huber Publishers.

Lewis, G. P. (1959). Plasma-kinin-forming enzymes in body fluids and tissues. *J. Physiol., Lond.*, **147**, 458–468.

Lewis, G. P. (1966). Intracellular enzymes in lymph after thermal injury. *J. Physiol., Lond.*, **186**, 140P–141P.

Lewis, G. P. (1967). Intracellular enzymes in local lymph as a measure of cellular injury. *J. Physiol., Lond.*, **191**, 591–607.

Lewis, G. P. & Wawratschek, W. A. (1971). Effect of thermal injury on the kinin system in rabbit hind limb lymph. *Br. J. Pharmac.*, **43**, 127–139.

Mislin, H. (1971). Die Wirkung von Cumarin aus Melilotus officinalis auf die Funktion des Lymphangions. *Arzneimittel. Forsch.* (*Drug Res.*), **21**, 852–853.

Schachter, M. (1960). Some properties of kallidin, bradykinin and wasp venom kinin. In: *Polypeptides which affect smooth muscles and blood vessels*, p. 241. London: Pergamon Press.

Yoffey, J. M. & Courtice, F. C. (1956). *Lymphatics, Lymph and Lymphoid Tissue*, p. 220. London: Edward Arnold.

(*Received June 5*, 1972)