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PRELIMINARY COMMUNICATION

TOPICAL TREATMENT OF ACUTE HINDLIMB LYMPHEDEMA OF THE RAT USING A TROXERUTIN-PHOSPHATIDYLCHOLINE COMPLEX IN LIPOSOMAL-LIKE MICRODISPERSION

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ABSTRACT

A new reversible complex between troxerutin and phosphatidylcholine (85-90mg/kg per day), in the form of a liposome-like water microdispersion, was topically applied to the rat thigh in an experimental counterpart of acute lymphedema. After four days there was 75% less hindlimb edema (mean decrease 40% of normal compared to control 10% of normal) in the treated compared with the untreated rats. These findings suggest that this drug preparation may be useful to minimize acute peripheral lymphedema in patients.

A reliable and reproducible experimental model for high protein edema is acute lymphedema of the rat thigh (1). This animal model was used to test the efficacy of a new troxerutin-phosphatidylcholine complex, in which the drug is covalently bound to phospholipid molecules in the ratio of 2:1 forming a reversible, liposome-like microdispersion in water (2,3). Previous studies (4,5) have shown that simple water microdispersions of complexes between vasoactive drugs (Bufomedil HCl, Iloprost, some flavons and other polyphenols of vegetable origin) and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine) penetrate rapidly through the epidermal barrier. Furthermore, the duration of the pharmacological effect is much longer than with other topical preparations of these drugs and the minimal active dose is significantly lower (6).

As with other benzopyrones, troxerutin, after injection, reduces high protein edema by increasing tissue proteolysis (7). With reduction in excess tissue protein, edema fluid is resorbed directly into blood capillaries and accordingly, the chief stimulus for chronic, small cell inflammation is removed. Recently it has been shown (8) that troxerutin, carried by phospholipid molecules and applied topically to human skin, increases vasomotion of small peripheral blood vessels thereby improving microcirculatory maldistribution and "stagnant" edema in venous insufficiency of the legs (9). Based on these preliminary studies, we examined the usefulness of the topical preparation of troxerutin in an experimental model of high protein lymphedema.

MATERIALS AND METHODS

Hooded rats (250±25g) were anesthetized with pentobarbital and one hindlimb was made lymphostatic (1). The skin on the medial aspect of the thigh was transversely incised, 1cm below the inguinal ligament. The fascia overlying the femoral vessels was excised and

the vessels undercut with a scalpel for 1.5cm. Care was taken to avoid cutting any major blood vascular tributaries. To obstruct the femoral collecting lymphatics, the tissues around the major blood vessels were compressed several times using fine forceps. Collateral lymphatics were then obstructed by ligatures; these were passed beneath the femoral blood vessels through the musculature of the thigh and around the skin in both medial and lateral directions. Because the resultant acute lymphedema was maximal at 90-100 hours (personal observations), the rats were killed 96 hours after operation. The hindlimb was severed at the ligatures (and at the equivalent position on the opposite control side) and weighed. The tibio-calcaneal joint was disarticulated and the foot was discarded because benzopyrones promote release of histamine in the rat foot making interpretation of the thigh results more reliable with the foot amputated (7). The amount of edema was expressed as the difference between the weight of the operated thigh (L) and the normal, contralateral unoperated one (N) divided by the unoperated one or (L/O-N)/N.

Using a paint brush, 0.7ml of a liposome-like water microdispersion containing 30mg/ml by weight of the complex troxerutin-phosphatidylcholine (T-P) was topically administered daily to each rat in a dose of 85-90mg/kg. There were ten rats in the treated group and ten in the control (no drug) group.

RESULTS AND DISCUSSION

Grossly, the drug treated rats had much less hindlimb edema than the non-drug treated

TABLE 1
Effect of a Complex Troxerutin-Phosphatidylcholine, Topically Administered on Acute Lymphedema of the Rat Hindlimb Thigh (n=10) Compared with Non-Treated (Control) Rats (n=10)

	Normal*	L/O*	Proportn.†	Normal	L/O	Proportn.†
	6.28	8.94	0.42	5.37	6.57	0.22
	6.51	9.18	0.41	7.46	7.61	0.02
	6.75	8.96	0.33	6.49	8.47	0.31
	5.42	7.96	0.47	6.74	6.83	0.01
	7.39	9.73	0.32	7.22	9.03	0.25
	6.62	12.64	0.91	7.23	7.94	0.10
	7.38	9.98	0.35	7.27	5.89	-0.19
	6.15	7.64	0.24	6.02	6.37	0.06
	6.33	7.76	0.23	7.07	9.53	0.35
	7.76	9.76	0.26	7.55	8.01	0.06
Mean	6.66	9.26	0.39	6.84	7.63	0.12
SE	0.22	0.46	0.06	0.22	0.38	0.06

L/O=lymphedema-operated
*weight-grams
†(L/O-N)/N

control rats. The measured volume of edema *(Table 1)* was also significantly less ($0.01<p<0.001$; using a t-test after establishing that there was no significant difference between the variances).

From these preliminary observations, it appears that topical complex troxerutin-phosphatidylcholine is equally effective to other forms of troxerutin (oral or intravenous or intraperitoneal injections) for minimizing high protein edema (7), and in the form of liposome-like microdispersion may prove useful in management of peripheral lymphedema.

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