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Effect of Calcium Dobesilate on the Functional Capabilities of Mesenteric Lymphatics in the Guinea Pig

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Summary. The mean time between contractions of lymphangions of mesenteric lymph collectors in anaesthetised guinea pigs was 7.6 ± 6.7 s. The administration of saline had no significant effect on contraction times, nor did 50 mg/kg of calcium dobesilate in saline as a vehicle. However, at dose levels of 100 mg/kg and 200 mg/kg using saline as a vehicle the time between contractions significantly ($P < 0.001$) increased to 11.4 ± 14.8 s and 12.9 ± 23.3 s, respectively. While neither the saline vehicle alone nor calcium dobesilate at any dose level influenced maximal collector diameters significantly, all dose levels of calcium dobesilate significantly ($0.05 > P > 0.01$) reduced minimal lumenal diameters to 30% of their maximum.

The time that the lymphangion spends at its minimal diameter is an indication of the intensity of contraction. Saline alone had no significant effect, but calcium dobesilate at 50 mg/kg and 100 mg/kg significantly ($0.05 > P > 0.01$) increased this time from 0.9 ± 1.2 s to 1.9 ± 1.9 s and 2.0 ± 1.6 s, respectively. At 200 mg/kg of calcium dobesilate, there was a very significant increase ($P < 0.001$) in minimal diameter times to 2.7 ± 1.5 s.

Key words: Calcium dobesilate – Lymphatics – Guinea pig

Introduction

Abnormal capillary resistance and permeability are a characteristic of many diseases including diabetes and chronic venous insufficiency. Calcium dobesilate (calcium dihydroxy-2-5 benzenesulfonate)[1] has been shown both clinically and experimentally to be an angioprotective agent acting on the microcirculation by

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strengthening the walls of the capillaries [1–4]. Also, it protects the endothelial capillary cells by the inhibition of vasoactive substances and desquamation [5, 6], and has been shown to normalise and reduce capillary permeability and excessive transcapillary escape of albumin [7]. Abnormally accumulated interstitial proteins contribute to oedema formation. In view of a report [8] indicating that calcium dobesilate increases thoracic duct lymph flow in dogs, this study was initiated to investigate its action on the functional capabilities of mesenteric lymphatic vessels.

In view of the fact that the gut is an area of fenestrated capillaries whose functions are associated with the need for greater than normal permeability, the use of the intestinal region seemed to be reasonable given the knowledge of the actions of calcium dobesilate, though the properties of the section studied of the vascular circuit represent normal and not pathological functioning.

Materials and Methods

A total of 30 female guinea pigs, body weight 200 ± 20 g, were randomly divided into three groups. (Animals of this weight were necessary since minimal adipose tissue surrounding mesenteric lymphatics was a prerequisite for their clear visualisation).

The animals were fed water (supplemented with 200 mg ascorbic acid per litre) and pelleted food ad libitum. Guinea pigs were given preliminary anaesthesia consisting of 1 ml/100 g of Alphathesin into the muscle of the hindlimbs (dose divided equally between limbs). Front limbs and stomach were shaven, and all hairs removed from the stomach region by the application of sticking plaster (this was necessary to reduce hairs on the operative field).

A vein on the medial aspect of the fore leg was exposed for a distance of 1.5 cm by removal of skin and overlying fascia. Following this, a 25-gauge needle, to which a cannula of known volume filled with physiological saline had been attached, was inserted into the vein. The upper part of the needle and cannula were fixed to the tissue with a drop of tissue glue. The tip of the needle and the cannula were further fixed with narrow (2–3 mm) strips of masking tape. Patency of the line was maintained by the administration of one or two drops of saline per minute during the preparatory stage. Once in place, the animal was administered Alphathesin i.v. by this route until it was in a suitable plane of anaesthesia.

The abdominal region was then opened slightly to the left of the mid-line from 2 cm inferior to the diaphragm and extending for 5–6 cm. At the mid point of this incision, a transverse cut of 3 cm was made. All surgery was performed using a cauterising unit to minimise the risk of bleeding onto the operative site.

A section of gut (approximately one third the way along) was then removed from the abdominal cavity an spread carefully out on a heated stage which was covered in Saran wrap (Dow Chemical). All handling was done with large or small cotton buds. The gut was then covered with another layer of Saran wrap. (This is impermeable to O_2, thus helping to maintain PO_2 levels at those which are normally found in the abdominal cavity).

Lymphagions are a section of a lymph collector between two valves. Once an area of the mesentery was exteriorised and prepared, observation of the intensity and frequency of the pulsation of lymphagions in the collecting lymphatics was made using a Zeiss A.C.M. microscope fitted with a Leitz saline immersion lens (× 25). The area was transilluminated with light (30 W QI) which had been filtered through a Balzers B-40546-7 filter. The image was transmitted with a Philips LDM 26 miniature camera fitted with a ½″ separate mesh vidicon tube and observed on a Ikegami televison monitor.

For the assessment of diameter changes a model 407 IPM Image Shearing Monitor was used. Information was recorded on a ¾″ N.V. 9210 National VTR.

Observations were always initiated within 3 min. If this was impossible, the animal was discarded. Total observation timing intervals of 20 min were used.

Table 1. Effect of Doxium on lymphatic function

Group	Contraction frequency (times/min)	Maximum diameter as % of normal maximum	Minimum diameter as % of normal group maximum	Mean and range of time at minimum diameter (20 observations)
Normal	7.57 ± 6.7 (7.9)	100	58.2	0.9 ± 1.15 (0.4–1.15)
Saline	7.31 ± 6.85 n.s (8.2)	98 n.s	54.0 n.s	1.1 ± 0.85 (0.5–2.1) n.s
20 mg/kg Doxium	7.16 ± 7.41 n.s (8.4)	96 n.s	33.4 ↓	1.9 ± 1.85 ↓ (0.3–3.0)
100 mg/kg Doxium	11.4 ± 14.82 ↓↓↓ (5.3)	108 n.s	32.4 ↓	2.0 ± 1.55 ↓ (0.5–3.8)
200 mg/kg Doxium	12.86 ± 23.3 ↓↓↓ (4.7)	90 n.s	28.8 ↓	2.2 ± 1.45 ↓↓↓ (0.5–4.1)

Arrows indicate direction and level of significance:
n.s, not significant; ↓, $0.05 > P > 0.01$; ↓↓, $0.01 > P > 0.001$; ↓↓↓, $P < 0.001$

Animals were randomly allocated to receive either the drug or an equivalent volume of saline following a 10-min period of normal observation. Three dose levels of calcium dobesilate were used; namely 50, 100 and 200 mg/kg corresponding to the 3 randomly assigned groups.

For statistical analysis of contraction frequency, a videotape of each animal in each of the groups was analysed for 10 min of each of either the drug or saline periods. The data from each animal was combined to ascertain the group mean and standard deviation (SD). Student's *t*-test assuming unequal variances was used to determine the significance of the results.

Results

Frequency of Contraction

Normal (Initial). For this, a major lymph collector was found and prepared for examination. These collectors were found by a random search pattern in the viewing area. When a suitable vessel was found, counting and recording was initiated. In each of the ten animals in which all preparatory events were successful, recordings of normal pulsation rates were made for 10 min so that normal fluctuations in rate could be assessed.

The mean time between contractions was 7.578 s ($n = 1{,}580$) with a standard deviation of 6.73, thus in 1 min the lymph vessel under these conditions contracted an average of 7.9 times.

Saline. For this observation period the same lymph vessel was followed. A volume of saline equivalent to that used in drug administration was administered via the leg cannula. Recording was initiated immediately after completion of the injection. Observation was for 10 min.

The mean time between contraction was 7.31 s ($n = 644$) with a standard deviation of 6.85. Therefore, in 1 min the lymph vessel under these conditions

contracted an average of 8.2 times. Student's *t*-test showed the difference between the normal and saline contraction times to be non significant [t (2222) = 0.8161].

Calcium dobesilate. Irrespective of whether a dose level of 50, 100 or 200 mg/ml was used the same volume of physiological saline was used as the vehicle.

50 mg/kg: At this dose level the mean time between contractions was 7.16 s (n = 486) with a standard deviation of 7.41 s. Thus, in 1 min the lymph vessel under observation contracted, on average, 8.38 times. Student's *t*-test showed this to be non-significantly different from either the normal (t 2064; 1.00) or saline (t 1122; 0.82).

100 mg/kg: At this dose level, the mean time between contraction was 11.4 s (n = 416) with a standard deviation of 14.82. Thus, in 1 min the lymph vessel under observation contracted 5.26 times. Student's *t*-test showed this time interval to be very significantly slower than normal (t 1994; 5.33, $P < 0.001$) very significantly slower than when saline was administered (t 1058; 5.46, $P < 0.001$) and also very significantly slower than the 50 mg/kg dose (t 894; 5.48, $P < 0.001$).

200 mg/kg: At this dose level there was a further slowing of the interval between contractions to 12.86 with a standard deviation of 23.3 (n = 930), thus in a period of 1 min the lymph vessel under observation would contract 4.66 times. Again Student's *t*-test showed this rate to be very significantly slower (t 2508; 6.77, $P < 0.001$) than normal and also very significantly slower (t 1572; 11.08, $P < 0.001$) than the saline control. There was, however, no significant difference between this dose level and that of 100 mg/kg (t 1344; 1.79, ns).

Maximal/Minimal Diameters

In this section, in view of the fact that a range of vessels with different diameters was examined, percentages have been used instead of absolute measurements. All diameter measurements were made in the central region of the lymphangion. Maximal diameters are expressed as a percentage of the maximal diameter under "normal" conditions. Neither saline nor any dose level of calcium dobesilate had any significant effect on maximal diameters.

Minimal diameters are all expressed as a percentage of this own group maximum. For the normal group, minimal diameter was 58.2% of the maximal diameter. For the saline group it was 54.0%. There was no significant difference between these minimal diameters.

However, for all dose levels of calcium dobesilate there was a substantial reduction in minimal diameters, averaging only 30% of the maximal diameters for their respective groups. These minimal diameters were all significantly ($0.05 > P > 0.01$) smaller than the minimal diameters of the normal group.

Lumenal Closure Times

An investigation of the times that the lymphangion maintained the minimal diameter generally showed wide variations from one lymphangion to the next.

For this reason, all lymphangions were randomly selected from the vessel under observation.

For the normal group the average time at minimal diameter was 0.9 s with a standard deviation of 1.1 s. The range was 0.4–1.6 s.

For the group receiving saline the average minimal diameter time was 1.1 ± 0.8 s with a range of 0.5–2.1 s.

For the group receiving 50 mg/kg calcium dobesilate the average minimal diameter time was 1.9 ± 1.8 s with a range of 0.3–3.0 s, while for the group receiving 100 mg/kg it was 2.0 ± 1.5 s with a range of 0.5–3.8 s. For the group receiving 200 mg/kg it was 2.2 ± 1.4 s with a range of 0.5–4.1 s.

The mean minimal diameter time of the group receiving 50 mg/kg calcium dobesilate was significantly ($0.05 > P > 0.01$) longer than the normal group. The same level of significance also applied for the group receiving 100 mg/kg. For the group receiving 200 mg/kg, the time at minimal diameter was very significantly ($P < 0.001$) longer than the normal group.

Discussion

Our findings show that the 100 and 200 mg/kg dose levels of calcium dobesilate very significantly ($P < 0.001$) slowed the contraction frequency of the mesenteric lymphatic vessels in the first 20 min after administration. The duration of this effect is unknown, but metabolic and pharmokinetic studies [9] indicate maximum blood levels within 3 min of administration and very rapid elimination. Towards the end of our observation periods, a tendency for the contraction rate to speed up was noted, supporting the above evidence.

All dose levels of calcium dobesilate significantly decreased ($0.01 < P < 0.05$) the minimal diameters as compared to the control situations. This indicates a more intensive contraction of the muscular elements of the lymphangion. concomitant with this was a significant ($P < 0.05$) prolongation of the time the lymphangion spent at its minimal diameter.

The explanation of this may in part derive from a consideration of the known actions of calcium dobesilate. It has been shown to inhibit the effects of vasoactive substances, such as histamine, serotonin and bradykinin [1], it also inhibits endothelial cell desquamation [2]. In addition, it inhibits hyaluronidase [3], which is in part responsible for the degradation of the mucopolysaccharide ground substance of the capillary basement membrane [10], and also slows the excessive formation of hydroxylysine [11]. In diabetic states, calcium dobesilate has been shown to reduce the extravasation of Albumin-I^{131}. The net result of these actions is a protection of the capillary wall with a subsequent reduction of its fragility and hyperpermeability state and a reduced outflow of proteins and reduced interstitial fluid formation. Based on this, the lymph load will be considerably reduced. Since lymph collectors vary their contraction rates according to lymph volumes and pressure [12], it may be that the observations correlate with just this. Alternatively, the more forceful albeit prolonged contractions of the lymphangion recorded in this experiment may indicate a situation which, if it existed in high lymph flow situations, could result in the more efficient and

rapid removal of lymph from tissues. The observation that calcium dobesilate increases thoracic duct lymph in animals in part supports this [8].

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References

1. Kellet DN, Pennington JC, Davies JE (1975) Effect of compound 205-E on capillary permeability in the guinea pig. Experimental report, Huntington Research Centre. Huntington, England
2. Kellet DN, Pennington JC, Davies JE (1975) Effect of compound 205-E on capillary fragility in the guinea pig. Experimental report, Huntington Research Centre. Huntington, England
3. Kappert A (1974) Zur medikamentösen Beeinflussung gestörter Kapillarfunktionen. Z Ther 2:71–89
4. Sevin R, Cuendet JF (1969) Calcium dobesilate in diabetic retinopathy. Ophthalmologica 159:126–135
5. Barry DH, Edwards GP, Kellett DN (1974) The effect of calcium dobesilate on the capillary ultrastructure and histamine induced capillary permeability of guinea pig skin. Huntington Research Centre. Huntington, England
6. Hladovec J (1977) Vasotropic drugs. A survey based on a unifying concept of their mechanism of action. Arzneimittelforsch 27:1073–1976
7. Beyer J, Meissner KO, Happ J et al (1980) Die Wirkung von Calcium Dobesilate auf die Permeation von Plasmaproteinen beim Diabetiker. Dsch Med Wochensch 46:1604–1608
8. Altmann Canestri E, Leguizamon DG, Sanchez CC et al (1977) Etude pharmacologique et clinique dans le traitement de l'suffisance veineuse par le dobesilate de calcium (Doxium). VIe Congrès International de Phlebologie, Buenos Aires, 10–15 Octobre, 1977
9. Benakis A, Glasson B, Bouvier C et al (1974) Metabolism and pharmacokinetics of calcium dobesilate in man. Therapie 29:211–219
10. Thomas J, Dorme N, Sergant et al (1972) Action du dobesilate de calcium sur la resistance et la permeabilitie capillaires et sur le temps de saignement et l'adhesivité plaquettaire modifiés par le dextran. Ann Pharm Fr 30:415–427
11. Hasslacher C (1981) Hemmung der gesteigerten Synthese kapillarer Basalmembran diabetischer Ratten in vivo. Vasa 10:148–150
12. Mislin M (1983) The lymphangion. In: Foldi M, Casley-Smith JR (eds) Lymphangiology. Schattauer, Stuttgart New York, pp 165–173

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