The effects of silica on lymph nodes and vessels—a possible mechanism in the pathogenesis of non-filarial endemic elephantiasis

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Abstract

Non-filarial tropical elephantiasis, which occurs in certain volcanic areas of the world, has been postulated to be an obstructive lymphopathy due to the fibrogenic effects of silica absorbed through the plantar skin of bare-footed people. Animal experiments involving the direct intralymphatic injection of fine silica particles have been carried out in order to assess the extent to which this substance can engender lymphatic obstruction and to determine its main site of action. Intralymphatic silica provoked an immediate and intense macrophage reaction with later fibrosis both within lymph vessels and to a lesser extent within lymph nodes. Lymphography indicated that the consequent obstruction resulted more from the effects of silica on vessels than on nodes.

Introduction

The existence of an endemic but non-filarial form of elephantiasis was first reported in Uganda (LOWENTHAL, 1934) and has since been reported in Kenya, Tanzania, Ethiopia, Burundi, Ruanda and Cameroon. The condition is found in the bare-footed sections of agrarian populations engaged in the cultivation of red clays in volcanic regions at altitudes which preclude a filarial aetiology. Inguinal and femoral lymph node biopsies in affected individuals have shown the presence of birefringent particles and associated granulomata (PRICE, 1972). Electron microscopy and micro-analysis show these particles to be within macrophages and to consist principally of silica with varying smaller amounts of aluminium, titanium and iron oxide (PRICE & HENDERSON, 1978). X-ray spectral analysis and other techniques have shown that the proportions of aluminium and silica in the lymph nodes reflect those in the soil (PRICE et al., 1981) and it is therefore probable that these minerals reach the nodes by transit through the afferent lymphatics having been absorbed through the plantar skin. Because of the known fibrogenic potency of silica, the hypothesis has emerged that the disease is an obstructive lymphopathy caused by the fibrotic response to silica of soil origin. The exact site of the obstruction is unlikely to be at the level of the inguinal lymph nodes since the degree of observed fibrosis in the nodes does not appear to be particularly dense (PRICE, 1977; PRICE & HENDERSON, 1979). Obstruction within the afferent lymphatics would seem to be more likely but supportive evidence is currently lacking.

The present work has been carried out to study the histological and lymphographic consequences of direct intralymphatic infusion of a silica suspension in animals.

Materials and Methods

The animals used were 24 New Zealand white rabbits of both sexes weighing 2 to 3 kg. The hind limb lymphatics of rabbits correspond to the usual mammalian pattern having superficial and deep systems (TJERNBERG, 1962; BACH & LEWIS, 1973). In the paw and shank a medial group of two or three subcutaneous (“saphenous”) vessels accompany the long saphenous vein, one of which is selected for cannulation. These vessels, as do the lateral group, drain into the popliteal node from which the efferent vessel joins the deep femoral lymphatics in the thigh. The latter vessels pass to the pelvic nodes (Fig. 1).

The structure of the rabbit popliteal lymph node conforms to the general pattern for mammalian nodes as described by YOFFEY & COURTCHE (1970). The rabbit node, however, shows a more conspicuous division into lobes than in man. The silica used was a sample of pure quartz sand, having a particle size ranging from 0.1 μm to 4.0 μm, most being less than 2.0 μm. Sterile 1% and 3% suspensions of silica were made up in 3% methylcellulose.

The materials and techniques for rabbit lymphatic perfusion have previously been described by GUINEY et al. (1964) and others. It consists of the isolation of an appropriate saphenous lymphatic which has been rendered visible by an injection of a small volume of Patent Blue Violet into the paw and the insertion of a specially designed cannula (ROTT et al., 1964). The material to be infused (silica, methylcellulose or contrast) was delivered via the cannula by a constant rate infusion pump at a rate of 4 ml per hour.

The X-ray contrast medium used was Ultraluid Lipiodol (May and Baker Ltd). The 24 rabbits were divided into two equal sized groups—“A” and “B” numbered one to 12 respectively. Each animal was first sedated using an intramuscular injection of a Fentanyl/Fluanisone mixture. A lymphatic of the right hind limb was then cannulated. The animals of group “A” received 0.3 ml of the 1% silica suspension (3 mg silica) and those of group “B” received 0.3 ml of the 3% silica suspension (9 mg silica). Both groups then received 0.3 ml 3% methylcellulose alone to a lymphatic of the left hind limb (controls). The skin incisions were then closed with catgut.

The first animal in series “A” (A1) was killed after five weeks using an intravenous bolus of pentobarbitone into a marginal ear vein.

The remaining animals of series “A” were killed as follows: A2,3—15 weeks; A4,5—30 weeks; A7,8—40 weeks, A9,10—50 weeks and A11,12—two years after the silica infusions. Rabbits A7 to A12 (killed at 40 weeks to two
years) also underwent lymphography of both hind limbs—using the same lymphatic as had previously been cannu-
lated—immediately before killing. The animals of group
"B" were killed as follows: B1,2,3—10 weeks; B4,5—25
weeks; B6,7—35 weeks; B8,9,10—40 weeks and B11,12—
46 weeks. Animals B6 to B12 (killed at 35 to 46 weeks)
underwent prior lymphography. Following the death of each
animal, the popliteal nodes were removed. The nodes were
transected at right angles to their long axes at the point of
entry of the silica as indicated by a yellow discolouration on
the surface. The control nodes were transected through the
hilum. In most cases, biopsies of the afferent lymphatics
which had received the infusions were taken. After fixation,
paraffin sections were cut. Staining was by haematoxylin and
eosin or, in some cases, by the silver impregnation method
for reticulin.

Results

Histology

(a) Lymph nodes

None of the control nodes showed any abnorma-
lities except for the presence of deposits of lipiodol
where this had been given.

In the silica treated nodes, there was no noticeable
difference in the degree or rate of development of the
changes between series "A" or "B". Hence the results
in both series will be summarized jointly.

In the earliest node to be examined (A1—36 days)
multiple aggregates of macrophages (granulomata)
were found in the cortex of the node, between which
normal lymphoid tissue was present. In the largest of
these lesions, the central histiocytes were degenerat-
ing as evidenced by foamy vacuolated cytoplasm,
whilst at the periphery, fibroblasts were present (Fig.
2). No collagen was present but stains for reticulin
showed the latter to be present as concentrically
arranged fibres at the periphery of the nodule.

Fig. 1. Normal rabbit lymphangiogram. See text for description.

Polarized light microscopy showed silica particles
both within macrophages and lying free where the
macrophages had degenerated.

By 40 weeks, further coalescence of the granuloma-
tous nodules had taken place and necrosis of the
centrally placed macrophages was prominent.

Animals from this stage onward had also received
lipiodol infusions. Accordingly the characteristic dro-
plets or “vacuoles” were seen in the sections. How-
ever, these were only present between the silicotic
granulomata and within normal lymphoid tissue. The
cellular response to silica had plugged the cortical
lymph sinuses, thus excluding the lipiodol from these
regions.

(b) Lymph Vessels

Afferent lymphatics which had received silica
were easy to identify as they could be seen with the
naked eye as white cords extending up to the node.
No such appearance was present in the control limbs
and, in these, the vessels had first to be rendered
visible by a Patent Blue Violet injection in the foot.

Lymphatics on the control limbs were seen to be
bounded by a mono-layer of epithelial cells sur-
rounded by loose areolar tissue (Fig. 5). On the
silica-treated sides from the earliest onwards, the
vessel lumens were occluded by aggregates of mac-
rophages, giant cells and lymphocytes. Also, chronic
inflammatory cells were present without the original
confines of the vessel—a perilymphangitis. In none of
the vessels examined was collagen present, but silver
impregnation stains showed reticulin fibres within the
lumen. The silica had, therefore, from an early stage
cause an obliteratorive endolymphangitis which, if left
for a sufficient period, would have undergone fibrosis.

Lymphography

All lymphograms showed evidence of varying
degrees of lymphatic obstruction on the silica treated
Fig. 2. Rabbit A1, 36 days after silica infusion. Right popliteal node. Cortical granuloma with surrounding fibroblasts. (H & E × 14)

Fig. 3. Rabbit A4 50 weeks after silica infusion. Right popliteal node. Lymphoid tissue largely replaced by acellular laminated collagen. (H & E × 14)
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Fig. 4. Rabbit All two years after silica infusion. Right popliteal node. Whorled concentric collagenous laminae. (H & E X 35)

side and nodal filling defects were commonly found. Features taken as indicating obstruction were firstly the filling of more than one afferent lymphatic, secondly vessels bypassing the node and thirdly contrast entering the node by a vessel other than the most direct. At least one of these features was seen in every case. An example of the latter two is given in Fig. 4. The over-all findings indicated the presence of obstruction both within the nodes at the site of entry of the silica and also within the vessels which had been used for the silica injection, the latter causing contrast to reach the nodes by alternative vessels. However, in no case was there a total obstruction at the level of the node. The contrast, having bypassed the occluded lymphatic, always reached an unaffected part of the node through which it progressed freely.

When the histological findings were compared with the lymphogram in each animal, it was apparent that the degree of obstruction—i.e., number of obstructive features on the lymphogram—was determined by the total duration of exposure to silica rather than the amount of collagen seen on the histological sections. In general, the greater the elapsed time after the silica infusion, the greater the degree of lymphographic obstruction. Since at any one time point, the amount of collagen present varied between animals, it appeared that the progressive cellular response to silica was the chief factor engendering the disturbance of flow, rather than the intensity of the fibrotic response.

Discussion

The response of living tissues to challenge by crystalline silica is by rapid phagocytosis of the particles by macrophages. Once within a macrophage, silica exerts a cytotoxic effect by the disruption of lysosomal membranes and the consequent autolysis of the cell (ALLISON et al., 1966). A factor, as yet undefined, is subsequently liberated which stimulates collagen synthesis by fibroblasts (ALLISON et al., 1977). The fibrotic response to silica is a slow process, and in the present animal model even at two years we did not observe the dense fibrous reaction such as is seen after very prolonged industrial exposure to silica dust. However, there is no reason to doubt that such an end result would have obtained given a sufficient duration of exposure.

The experiments of DRINKER et al. (1934) in which oedema of the hind limbs of dogs appeared following repeated intralymphatic injections of silica and quinine hydrochloride are well known. In those animals, the clinical picture was somewhat ambiguous due to the occurrence of repeated infections in many of the limbs and, lacking the benefit of lymphographic techniques, Drinker and his colleagues were unable to confirm the site or even the very existence of lymphatic blockade. Our experiments, using X-ray lymphography have shown that the part cellular, part fibrous reaction produced by silica did cause unequivocal lymphatic obstruction. Furthermore, the work of Drinker et al. does not permit any conclusion regarding the specific
Fig. 5. Rabbit B11 40 weeks after infusion. Upper—afferent lymphatic, control limb. Normal appearance (H & E $\times$ 100). Lower—afferent lymphatic after silica infusion. The lumen is occluded by a mass of chronic inflammatory cells. There is also a marked peri-lymphatic inflammatory response. (H & E $\times$ 35)
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Fig. 6. Rabbit A10. Lymphangiogram 50 weeks after infusion. Right side—the site of entry to the node of the afferent lymphatic used for the silica infusion is represented by a large nodal filling defect; the vessel itself is occluded and therefore not opacified. The node fills by an alternative vessel entering it medially and another, collateral, vessel bypasses the node completely. Left side—normal.

actions of silica alone, since those workers also infused quinine hydrochloride which is itself a potent sclerosant, noted for its effectiveness in the injection treatment of varicose veins.

The design of our experiments meant that observation could only be made of the changes in one (injected) lymphatic and a corresponding small area of the node into which that vessel drained. It has been shown that such a vessel becomes consistently occluded following silica infusion and that the same pathological process extends for a short distance into the lymph nodes at the point where the afferent lymphatic enters it. In no case was there widespread involvement of the node. It was apparent that such silica as does enter the node becomes arrested at an early stage and the granuloma thus produced is localized to the cortex close to the site of entry of the afferent lymphatic.

Functionally, it appeared that the reaction in the afferent lymphatics was of greater significance than that in the nodes. Whereas the silica produced total occlusion of the injected lymphatic, the reaction in the node was so localized that contrast could flow freely through the node by way of unaffected lymph sinuses. It is probable that the involvement of the node would have been even less proportionately, had smaller quantities of silica been used, most of which would have been fixed by macrophages in the lumen of the afferent lymphatic. The quantities used in these experiments were large relative to the load which could be absorbed through the feet of elephantiasic patients. Silica-laden macrophages have been shown to be “sticky”, i.e., their motility is diminished (GAAFAF & TURK, 1970), so that they tend to accumulate within lymph vessels, their transit to the draining lymph nodes thus being retarded. Hence, the cytopathic effect of silica is inflicted on lymph vessels at an earlier stage than on the nodes.

Can lymphoedema result from occlusion of lymph trunks without co-existent obstruction at the level of the draining lymph nodes? Lymphographic and histological evidence in patients with one type of primary lymphoedema indicates that limb swelling can be associated with under developed or completely absent distal lymphatics in the presence of perfectly normal inguinal and iliac lymph nodes (KINMONTH, 1982).

Conclusion

The predominant obstructive effect of silica within the lymphatic system is on the lymphatics themselves and not on the draining lymph nodes. These findings support the hypothesis that if silica is absorbed through the plantar skin and enters lymphatics then lymphostasis can result. Further experiments involving the injection of silica particles into the foot pads of animals are required, to confirm that such particles do enter lymphatics from the interstitial space and that such a route of entry is associated with the same changes as have been demonstrated here following direct intralymphatic introduction.

References


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