Experimental ocular leishmaniasis

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An experimental study has been made of the ocular lesions produced by *Leishmania donovani* infection. Animals were injected with *Leishmania donovani* subconjunctivally, into the anterior chamber, and into the vitreous. The ocular lesions observed experimentally were conjunctival and corneo-scleral nodules, interstitial keratitis, iridocyclitis, choroiditis, and retinitis together with vitreous haemorrhage.

Subconjunctival and retrobulbar injections of *Leishmania donovani* in hamsters produced visceral leishmaniasis; thus, besides the usual parenteral and intracardiac routes often used for infecting hamsters with *Leishmania donovani*, the ocular route can also be used.

The protozoon *Leishmania*, which is transmitted by the bite of the sandfly (*Phlebotomus*), causes three distinct clinical entities: oriental sore caused by *Leishmania tropica*; kala-azar caused by *Leishmania donovani*; and espundia caused by *Leishmania braziliensis*.

ORIENTAL SORE of the eyelids has been recorded by El Kattan (1935), Kamel (1943, 1945), Wahba (1948), El Said Kahlil Abu Shusha (1949), di Ferdinando (1950), Dobrzanskaya (1964), and Morgan (1965).

The lid is involved only in 2.5 per cent. of cases of cutaneous leishmaniasis (Pestre, 1955), probably because the movements of the lids prevent the fly-vector from biting the skin in this region (Morgan, 1965). Fuchs (1951) described scars of the upper lid as a sequel to leishmaniasis. The conjunctiva is rarely affected in cases of oriental sore (Donatelli, 1950; Gandolfi, 1952), and conjunctivitis when present is due to secondary organisms (Scuderi, 1947). The cornea is occasionally affected by ulcerative keratitis (Chams, 1930) due to direct infection from the fingers (Duke-Elder, 1965).

KALA-AZAR has a specific affinity for the reticuloendothelial system. Various authors, including Lee (1924), Ling (1924), Bhaduri (1927), Recupero (1954), and Tassman, O'Brien, and Hahn (1960), have recorded the presence of retinal haemorrhages in patients suffering from this condition.

ESPUNDIA, naso-pharyngeal leishmaniasis or *Leishmania Americana*, produces ocular lesions in 10 to 20 per cent. of cases (Duke-Elder, 1965). It causes ulcerative granuloma in the nose, which may spread to destroy the lids and conjunctiva (Pessôa and Barreto, 1948; Azulay, 1952). The lid may be affected by way of the naso-lacrimal duct (Machado, Machado, and Moura, 1958). The cornea is often affected by interstitial keratitis (de Andrade, 1942), with aneurysmal formations constituting a leishmanian pannus (Duke-Elder, 1965).

The diagnosis of oriental sore and espundia is made by taking a smear from the edge of the ulcer and staining it with Giesma or Leishman stain, culturing the organism on N.M.N. (Novy-MacNeal-Nicolle) medium, or by an intradermal skin test (Leishmanin test).

The diagnosis of kala-azar is made by detecting the *Leishmania donovani* bodies in smears from splenic, liver, and sternal punctures, cultures in N.M.N. medium, blood examination, and biochemical tests.

The aim of this work was to study experimentally the different ocular lesions that can develop from *Leishmania donovani* infection.

Methods

Seventeen hamsters of the "Golden Syrian type", weighing from 80 to 100 g., and fourteen guinea-pigs of the "South American type", weighing from 320 to 350 g., were used.

The "Kenya strain" of *Leishmania donovani*, which was used in all the experiments, was isolated from the spleen of infected hamsters previously injected with *Leishmania donovani* by the intracardiac route. The anaesthesia used was intraperitoneal Urethane in a dose of 1 to 2 g./kg. body weight.

The specimens removed from the animals, such as enucleated globes and internal organs such as the liver, spleen, and brain, were all fixed in formalin 10 per cent. and embedded in paraffin. Histological sections were cut at a thickness of 6 μ , and stained with both haematoxylin and eosin and Giemsa stain.

The experimental work was carried out according to the following scheme:

- (1) Injection of *Leishmania donovani* subconjunctivally in hamsters and guinea-pigs.
- (2) Injection of *Leishmania donovani* into the anterior chamber of guinea-pigs.
- (3) Injection of *Leishmania donovani* into the vitreous of hamsters and guinea-pigs.
- (4) Examination of the spleen, liver, and brain of animals previously infected with *Leishmania donovani* by subconjunctival or retrobulbar injection.
- (5) Examination of the eyes of hamsters previously infected with *Leishmania donovani* by the intracardiac route.

(1) Injection of *Leishmania donovani* subconjunctivally in hamsters and guinea-pigs

0.2 ml. *Leishmania donovani* (suspended in physiological saline) were injected subconjunctivally into two hamsters, and 0.4 ml. into two guinea-pigs. The left eyes only were injected, leaving the right eyes as controls. Repeated inspections for ocular lesions were made. The animals were killed after 50 days, and the lids together with the globes were removed and fixed and serial histological sections were cut.

(2) Injection of *Leishmania donovani* into the anterior chambers of guinea-pigs

0.1 ml. *Leishmania donovani* suspension was injected into the anterior chamber of the left eyes of three guinea-pigs after an equal amount of aqueous had been aspirated. The animals were examined by the slit lamp for 30, 40, and 50 days respectively; they were then killed, the globes were enucleated and fixed, and serial histological sections were cut.

(3) Injection of *Leishmania donovani* into the vitreous of hamsters and guinea-pigs

0.2 ml. *Leishmania donovani* suspension was injected into the left vitreous chambers of three hamsters and 0.3 ml. into three guinea-pigs. Repeated slit-lamp and fundus examinations were performed for 40, 50, and 60 days; the animals were then killed, the globes were enucleated and fixed, and serial histological sections were cut.

(4) *Examination of spleen, liver, and brain of animals previously infected with Leishmania donovani by the ocular route*

(a) AFTER SUBCONJUNCTIVAL INJECTION

Three hamsters and three guinea-pigs were used. Only two hamsters and two guinea-pigs were injected with *Leishmania donovani* subconjunctivally as described previously. The injection was unilateral in one animal, and bilateral in the other. The third hamster and guinea-pig were kept as controls. The animals were killed after 30 days, the abdomens and heads were opened, and under strict asepsis smears were made from the spleen, liver, and brain. The smears were stained by Giemsa and examined with an oil immersion lens. Histological sections were also cut and stained by haematoxylin and eosin and Giemsa stain.

(b) AFTER RETROBULBAR INJECTION

Three hamsters and three guinea-pigs were used. A retrobulbar injection of 0.3 ml. *Leishmania donovani* suspension was carried out in two hamsters and of 0.4 ml. in two guinea-pigs. The injections were made through the lower conjunctival fornices. The injection was unilateral in one animal and bilateral in the other. The third hamster and guinea-pig were kept as controls. The animals were killed after 30 days. Smears and histological sections were taken from the spleen, liver, and brain as in the previous experiment. The globes were also enucleated and fixed, and histological sections were cut.

(5) *Examination of eyes of hamsters previously infected with Leishmania donovani by the intracardiac route*
Six hamsters were injected with 0.3 ml. *Leishmania donovani* suspension intracardially. They were then repeatedly examined for ocular lesions by split-lamp and fundus examination for a period of three months.

Results

The subconjunctival injection of *Leishmania donovani* caused a severe conjunctival injection, with the development of conjunctival and corneo-scleral nodules (Figs 1 and 2).

FIG. 1 A corneo-scleral nodule in the left eye of guinea-pig after subconjunctival injection of *Leishmania donovani*

FIG. 2 A conjunctival blood vessel crossing the corneo-scleral nodule and encroaching on the left cornea of guinea-pig after subconjunctival injection of *Leishmania donovani*

Interstitial keratitis was also seen by slit-lamp examination. Serial histopathological sections of the nodules showed the presence of round cell infiltration (Fig. 3). The lacrimal gland was also found to be infiltrated with round cells (Fig. 4), and *Leishmania donovani* bodies were evident in sections stained by Giemsa stain.

Injection of *Leishmania donovani* into the anterior chamber of guinea-pigs caused iridocyclitis, keratic precipitates, and hypopyon. Histopathological sections revealed the presence of a round cell infiltration in the cornea, iris, and ciliary body.

FIG. 3 Histopathological section of a conjunctival nodule in a hamster, showing round cell infiltration after subconjunctival injection of *Leishmania donovani*. $\times 350$

FIG. 4 Histopathological section of lacrimal gland of guinea-pig, showing round cell infiltration. $\times 800$

Injection of *Leishmania donovani* into the vitreous of hamsters and guinea-pigs caused retinitis, choroiditis, and vitreous haemorrhage. A greyish yellow-reflex developed in hamsters. Histopathological sections of the globes revealed the presence of round cells and red blood corpuscles in the vitreous (Fig. 5). The retina and choroid were also infiltrated by lymphocytes and plasma cells.

FIG. 5 Histopathological section of vitreous chamber of hamster, showing round cell infiltration and red blood cells after injection of *Leishmania donovani*. $\times 600$

The examination of the internal organs of animals previously infected with *Leishmania donovani* by the ocular route, revealed the following results:

After subconjunctival injection

The hamsters showed a marked enlargement of the spleen 30 days after the injection, as compared with the spleen of the control hamsters (Figs 6 and 7). Smears from the spleen of the infected hamsters revealed numerous *Leishmania donovani* bodies, some in the macrophages but mostly extracellular (Fig. 8). Smears from the liver and brain also revealed *Leishmania donovani* bodies, but these were very scanty. Histopathological sections stained by Giemsa also revealed the bodies which were more numerous in the splenic sections.

FIG. 6 Hamster showing marked enlargement of the spleen after subconjunctival injection of *Leishmania donovani*

FIG. 7 Control hamster, showing normal spleen

FIG. 8 Smear from spleen of hamster, showing *Leishmania donovani* bodies in the macrophages, but mostly extracellularly after subconjunctival injection of *Leishmania donovani*. Oil immersion $\times 4,850$

The guinea-pigs injected subconjunctivally with *Leishmania donovani* did not show any evidence of such bodies in smears from the spleen, liver, or brain. A culture from the spleen on N.M.N. medium was performed, but also proved negative.

After retrobulbar injection

The hamsters also showed a marked enlargement of the spleen 30 days after the injection, as compared with the control animal. *Leishmania donovani* bodies were also evident in smears and histopathological sections of the liver and brain, as in the previous experiment. The organs from the guinea-pigs were also unaffected. The retrobulbar injection did not affect the animal's eyes, except for a mild conjunctival injection with mucous discharge.

It was noticed that the hamsters which were bilaterally injected, whether by the subconjunctival or retrobulbar route, showed a severer infection than those injected in one eye only.

The examination of the eyes of hamsters previously infected with *Leishmania donovani* through the *intracardiac route* showed that only two of six hamsters infected with visceral leishmaniasis or kala-azar developed ocular lesions. Ciliary injection, aqueous flare, and interstitial keratitis developed in the right eye of one of the hamsters 50 days after the injection. It was difficult to examine the lens and fundus because of the corneal lesion. The animal died 8 days later from kala-azar. The second hamster developed bilateral lesions. A corneo-scleral nodule was noticed in the right eye 2 months after the injection. An interstitial keratitis developed later and the whole cornea was opaque by the third month. The left cornea also showed an interstitial keratitis 9 days after the appearance of the right nodule. The cornea became gradually opaque, and the animal died 3 months after the injection from kala-azar.

Discussion

Reviewing the literature on the subject of ocular leishmaniasis, Metelkin (1928) found that corneal lesions could be produced experimentally in dogs; an interstitial keratitis was produced with the presence of the parasites in the cornea.

Bollinger and Macindoe (1950) injected the "Australian opossum" parentally with the blood of Indian patients suffering from kala-azar. Ocular lesions were produced in the form of interstitial keratitis, iritis, cyclitis, complicated cataract, choroiditis, retinopathy, and papillitis.

Manson-Bahr (1954), discussing the ocular lesions in kala-azar, stressed the findings of Wright who showed that retinal haemorrhages occurred in the posterior segment similar to those seen in malaria. Such haemorrhages were attributed by Recupero (1954) to vascular fragility, a low platelet count, and a prolonged prothrombin time.

Sorsby (1963) stated that retinal haemorrhages in kala-azar were probably due to anaemia. He discussed the condition of "post kala-azar dermal leishmaniasis", where small vascularized nodules occasionally appear in the episclera close to the limbus. The nodules may extend to the cornea which becomes opaque and infiltrated, showing superficial and deep vascularization.

In our experimental study on ocular leishmaniasis, we observed that subconjunctival injection of *Leishmania donovani* in hamsters and guinea-pigs produced conjunctival and corneo-scleral nodules together with interstitial keratitis. The lacrimal gland was also affected.

Visceral leishmaniasis developed after subconjunctival and retrobulbar injections of *Leishmania donovani* only in hamsters, as guinea-pigs have been found to be poor hosts for *Leishmania*.

Injection of *Leishmania donovani* into the anterior chamber and vitreous of animals caused signs of iridocyclitis, choroiditis, and retinitis, together with vitreous haemorrhage.

Intracardiac injections with *Leishmania donovani* produced corneo-scleral nodules and interstitial keratitis in only some of the animals injected.

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