[Jpn. J. Parasitol., Vol. 34, No. 4, 301-309, August, 1985]

A Histological Study of the Skin and Nodule during the Course of Diethylcarbamazine Treatment in Onchocerciasis

TATSUYUKI MIMORI

(Received for publication; November 27, 1984)

Key words; onchocerciasis, histology, diethylcarbamazine, mast cell, eosinophil, microfilaria

Introduction

Diethylcarbamazine (DEC) is generally used for the treatment of onchocerciasis. However, DEC may cause serious untoward reactions, known as the Mazzotti reaction, including pruritus, rash and edema of the skin in patients (Mazzotti, 1948). Histopathological changes of the skin after DEC treatment have been reported by several workers in Africa (Hawking, 1952; Connor *et al.*, 1970; Buck, 1974; Connor, 1974; Gibson *et al.*, 1976), America (Martinez, 1949; Tada *et al.*, 1981) and Yemen(Connor *et al.*, 1983). They indicated that when microfilariae (mf) were affected by DEC, inflammatory reactions would be produced and they considered eosinophil as the effector cell. However, there have been no persuasive findings on the mechanism which induce eosinophils to the inflammatory focus.

Therefore, in order to clarify the histological process which occurs during DEC treatment, a histological study was performed chronologically on the skin and nodule of onchocerciasis patients, particularly on the kinetics of mf, mast cells, eosinophils and giant cells. Special attention was paid to the comparison of skin specimens between affected (positive Mazzotti reaction) and unaffected areas of the patients. Furthermore, onchocercal nodules were examined with classification of the nodule into three types on the basis of the nature of included females.

This study was supported by the research grants No. 57041041 and No. 58043041 from the Ministry of Education, Science and Culture of Japan.

Department of Parasitic Diseases, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860, Japan.

Materials and Methods

Patients and material sampling

The patients of onchocerciasis were the laborers of plantation "Corona" in Chicacao located in the south-western part of Guatemala, whose ages were between 17 and 70 years old. During Oct. and Nov., 1982, they were treated with 10 mg/kg pyrantel pamoate (Combantrin®) in order to expel intestinal helminths 3 weeks prior to DEC treatment. All the patients were orally administered with 5 mg/kg/day DEC (Hetrazan®) for 7 consecutive days.

Biopsy specimens of the skin, approximately 1 mm deep by 2-3 mm in diameter, were taken by a corneoscleral punch (Holth type) from the upper body (scapular region, upper arm, chest and abdomen) of the patients 0, 3, 24, 48 and 72 hours after the first dose of DEC. These specimens were taken from the affected skin areas with pruritus, rash and edema and from the unaffected areas without gross changes. Onchocercal nodules were surgically removed from the head, back and waist of the patients at 48, 72 and 120 hours. As controls, 16 nodules were removed for histological comparison from untreated persons living in the same village.

Histological examinations

The specimens of the skin and nodule were fixed in 10 % formalin, embedded in paraffin, and sectioned at 5 microns. Sections were stained with hematoxylin and eosin, May-Grünwald-Giemsa and PAS-hematoxylin.

Table 1 The change of density and location of microfilariae in the skin during the course of DEC treatment

Skin specimens	Hours after first dose	No. of specimens observed	Density of microfilariae*				
			Dermis		Epidermis		
			Reticular layer	Papillary layer	Basal layer	Prickle layer	Granular layer
Pre-treatment	0	26	0.88	0.12	0	0	0
Affected†	3	8	0.63	0.75	0	0	0
	24	9	0	0.78	0.78	0.44	0
	48	6	0.17	0.17	0.50	0.67	0.33
	72	6	0	0	0.17	0.50	0.17
Unaffected	24	3	0.33	0	0	0	0
	72	3	0	0	0	0	0

* Average number of microfilariae found in the serial sections which totaled 200 microns in thickness per specimen.
† Affected skin exhibited pruritus, rash and edema.

Table 2 The kinetics of mast cells and eosinophils in the dermis of the skin during the course of DEC treatment

Skin specimens	Hours after first dose	No. of specimens observed	Density of mast cells*		Density of eosinophils*
			Granule-rich	Degranulated	
Pre-treatment	0	26	15.8(8-23)†	2.1(0- 6)(12%)‡	0.1(0- 1)
Affected §	3	8	11.3(6-20)	7.6(2-15)(39%)	0.3(0- 2)
	24	9	9.2(4-17)	6.1(4- 8)(39%)	9.2(0-40)
	48	6	10.7(4-20)	5.5(2-14)(34%)	8.0(0-23)
	72	6	8.8(3-16)	4.2(2- 9)(32%)	4.8(2-12)
Unaffected	24	3	14.7(12-19)	2.7(1- 4)(15%)	0
	72	3	10.7(9-12)	7.3(5- 9)(41%)	0

* The numbers of mast cells and eosinophils were counted in the dermis vertically sectioned (0.25 mm depth from basal layer with 1 mm width).
† Mean (range).
‡ (Number of degranulated mast cells/total number of mast cells) ×100.
§ Affected skin exhibited pruritus, rash and edema.

Histological observations were performed based on the following criteria and methods. In the skin sections, the density and location of mf were recorded by examining 40 serial sections mentioned above. All the numbers of granule-rich mast cells, degranulated mast cells and eosinophils were counted in the dermis vertically sectioned, approximately 0.25 mm below the basal layer by 1 mm wide.

Onchocercal nodules were divided into three types based on the fecundity and activity of female adults contained ; 1) with mf in the uteri (fertile), 2) with empty uteri (nongravid), and 3) degenerating or calcified adult worms (degenerated). Some nodules with mixed categories were excluded. Mf were enumerated by searching all the connective tissue around the worm in the section of the nodule. The count was converted to a density at the number of mf divided by the area of the cut-sur-

Table 3 The kinetics of microfilariae, mast cells, eosinophils and giant cells in nodules during the course of DEC treatment

Condition of adult	Hours after first dose	No. of specimens observed	Density of microfilariae*	Density of mast cells†		Density of eosinophils†	Density of giant cells	
				Granule-rich	Degranulated		Around adult worm‡	In the connective tissue†
Fertile	Untreated	4	3.6(1.1- 9.8)§	33.0(7- 48)	22.8(8-47) (41%) ·‖	55.8(8-187)	0.1(0-0.3)	1.5(0-4)
	48	7	6.3(3.0-42.0)	10.3(1- 22)	15.4(5-28) (60%)	49.7(2-185)	0.1(0-0.6)	1.7(0-9)
	72	3	4.1(2.2- 7.2)	4.3(2- 6)	28.3(24-31) (87%)	260.0(103-362)	0.2(0.1-0.3)	3.7(3-5)
	120	2	32.7(24.9, 40.5)	11.5(8, 15)	29.0(29, 29) (71%)	77.0(48, 106)	0.2(0, 0.4)	36.5(25, 48)
Nongravid	Untreated	8	—	30.3(5- 71)	11.9(1-40) (29%)	30.1(2-112)	0.2(0-0.7)	1.8(0-5)
	48	5	—	32.0(1-104)	32.2(12-39) (42%)	71.0(10-189)	0.9(0-2.9)	1.4(0-3)
	72	2	—	20.0(14, 26)	39.5(17, 62) (66%)	56.0(11, 101)	0.9(0.9, 0.9)	2.0(0,4)
	120	3	—	27.7(15- 44)	18.7(12-26) (40%)	23.0(4- 48)	0.2(0-0.3)	2.7(0-8)
Degenerated	Untreated	4	—	20.5(13- 32)	6.8(2-12) (25%)	63.0(12-161)	1.6(0.5-3.1)	1.5(0-5)
	48	3	—	41.0(13- 47)	7.5(8-26) (42%)	56.0(5- 87)	1.7(0.1-3.0)	0.7(0-2)
	72	3	—	75.7(23-121)	23.7(9-47) (24%)	36.0(0- 62)	1.2(0.1-2.3)	1.3(0-4)

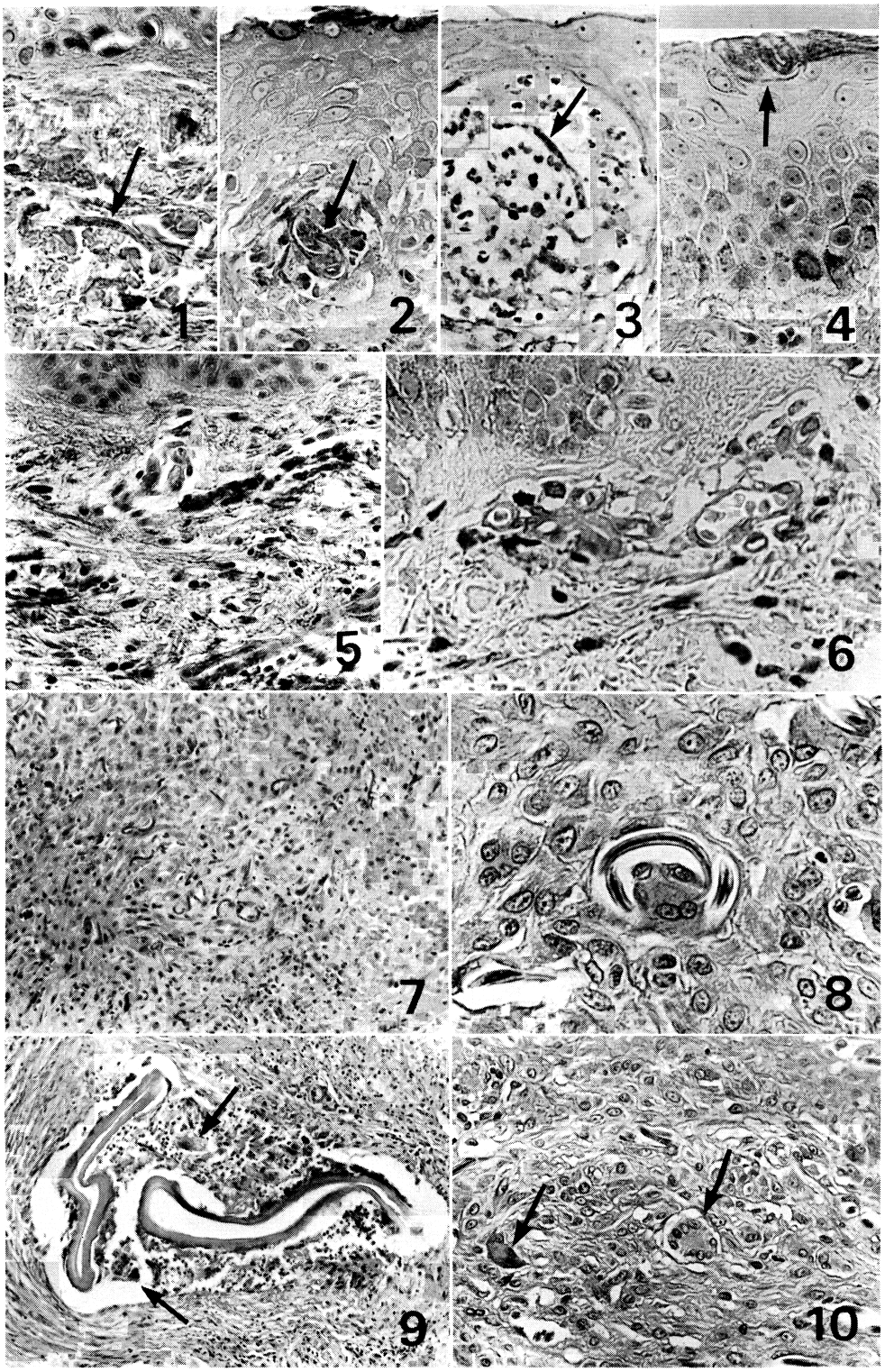
* The number of microfilariae/10 mm² of the connective tissue of nodule.

† The numbers of mast cells, eosinophils and giant cells in the connective tissue of nodule were counted in 20 randomly chosen fields at ×400 magnification.

‡ The number of giant cells/an adult cross section in the nodule.

§ Mean (range).

·‖ (Number of degranulated mast cells/total number of mast cells) ×100.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face of the nodule. The densities of mast cells and eosinophils were expressed as the total number counted in 20 randomly chosen fields at ×400 magnification. Giant cells (foreign body giant cells) were apparently composed of 2 groups by their location in the tissue. In the first population, adjacent to adult worms, the density was expressed by the total number of giant cells per total number of cross-sections of adult worms in a whole nodule section. In the other population, located freely in the connective tissue, the density of giant cells was total number counted in 20 randomly chosen fields at ×400 magnification.

Results

Skin

In the affected areas of the skin, mf migrated from the dermis to the epidermis after DEC treatment as shown in Table 1. Almost all mf were situated in the reticular layer of the dermis before DEC treatment (Fig. 1). In the skin specimens obtained 3 hours after the first dose, about half the mf were in the papillary layer of the dermis. Many mf were seen in the basal layer and prickle layer of the epidermis 24 hours later (Figs. 2, 3). Thereafter, some mf were observed within the granular layer (Fig. 4). Almost all the micro-abscesses, consisting mainly of eosinophils, neutrophils and degenerating mf, were found in the epidermis (Fig. 3).

The kinetics of cells are summarized in Table 2. Eighty-eight percent of the mast cells were granule-rich before DEC treatment, and the proportion of degranulated cells was found to be increased at 3 hours after treatment. At 24 hours, the number of eosinophils was increased from 0.3 at 3 hours to 9.2. Giant cells also appeared in sections obtained at 72 hours. The increase of histiocytes and the loosening and degeneration of the connective tissue were the most marked in the dermis during the course of treatment (Fig. 5). The blood capillaries were dilated and some erythrocytes could be seen in the dilated lumens (Fig. 6). In the skin sections from the unaffected area where mf were rarely found, cellular reactions were much weaker than those seen in the affected area (Table 2).

There were no differences in the severity of histological changes among affected areas regardless of the locality in the body.

Nodule

As shown in Table 3, the density of mf tended to increase with time in the nodules which

Fig. 1 Microfilaria (arrow) in the reticular layer of the dermis before the first dose of DEC. Hematoxylin-eosin stain, ×350.

Fig. 2 Microfilaria (arrow) in the basal layer of the epidermis 24 hours after the first dose of DEC. Hematoxylin-eosin stain, ×350.

Fig. 3 Degenerated microfilaria (arrow) in a micro-abscess, eosinophils and neutrophils are seen, in the prickle layer of the epidermis 24 hours after the first dose of DEC. May-Grünwald-Giemsa stain, ×350.

Fig. 4 Microfilaria (arrow) in the granular layer of the epidermis 48 hours after the first dose of DEC. PAS-hematoxylin stain, ×350.

Fig. 5 Infiltration with histiocytes, and loosening and degeneration of the dermis 48 hours after the first dose of DEC. Hematoxylin-eosin stain, ×250.

Fig. 6 Dilatation of blood capillaries and congestion with erythrocytes in the lumens 3 hours after the first dose of DEC. PAS-hematoxylin stain, ×500.

Fig. 7 Accumulation of degenerated microfilariae in the connective tissue of a nodule including a fertile adult female 120 hours after the first dose of DEC. Hematoxylin-eosin stain, ×100.

Fig. 8 A giant cell attached to a degenerated microfilaria in the connective tissue of a nodule 120 hours after the first dose of DEC. Hematoxylin-eosin stain, ×400.

Fig. 9 Cross section of a degenerated adult worm and giant cells (arrows) in a nodule taken from an untreated patient. PAS-hematoxylin stain, ×100.

Fig. 10 Numerous giant cells (arrows) in the connective tissue of a nodule containing fertile adult worm 120 hours after the first dose of DEC. Hematoxylin-eosin stain, ×200.

contained fertile adult females, especially at 120 hours ; counts reached 9 times that of untreated nodules (Fig. 7). Most of the mf present were already degenerating (Fig. 8). In the nodule, the most marked finding was the degranulation of mast cells. The percentage of degranulated mast cells in the nodules containing fertile females (60 %–87 %) was higher than that (24%–66%) of the nodules containing nongravid or degenerated adult worms during DEC treatment. A marked accumulation of eosinophils was seen in the nodules containing fertile adult females at 72 hours. The number of giant cells around the adult worms was high surrounding the degenerated adult worms irrespective of treatment (Fig. 9). However, the density of giant cells locating in the connective tissue portion, where numerous degenerated mf were present, increased significantly in the nodules containing fertile adult female particularly 120 hours after the first dose of DEC (Fig. 10). In these sections, numerous mononuclear cells containing PAS-positive granules in the cytoplasm, which were thought to be macrophages, were also observed. Plasma cells were seen in the nodules containing degenerated adult worms equally in both the untreated and treated groups.

There was no significant change in the morphology of the worm structures, such as the cuticle, muscle, intestine and reproductive organs, in both fertile and nongravid adult worms.

The degree of histological changes in the nodule was essentially the same regardless of the locality in the body.

Discussion

In the present study, it was chronologically shown that mf immediately migrated from the dermis to the epidermis in affected skin following DEC treatment, but not in unaffected skin. Degenerated mf were found in micro-abscesses or intra-epidermal abscesses as previously reported in African patients (Connor *et al.*, 1970 ; Buck, 1974 ; Connor, 1974 ; Gibson *et al.*, 1976). The present finding on the time course of microfilarial destruction in American onchocerciasis was essentially the same with that in Africa.

With regard to the host's killing mechanisms of onchocercal mf after DEC administration, the role of inflammatory cells has been considered important since the reports of Martinez (1949) and Hawking (1952). Recent studies indicate the involvement of eosinophils in the killing process of mf in histological investigations (Buck, 1974 ; Gibson *et al.*, 1976 ; Kephart *et al.*, 1984) and in an *in vitro* study (Greene *et al.*, 1981). As to the trigger for the killing action of eosinophils against onchocercal mf, the type I hypersensitivity mechanism would be plausible in which mast cells with bound IgE degranulate on contact with a cognate antigen from mf. However, there has been no persuasive work on the role of mast cells, so far. The present study of mast cell-eosinophil kinetics clarified quantitatively the relationship between the two : The increase of degranulated mast cells occurred as early as 3 hours after DEC, which was followed by tissue eosinophilia. Through this study, we conclude that mast cell is an important stimulater of eosinophil activation in the potent killing mechanism of onchocercal mf.

Onchocerca volvulus-specific IgE antibody (Weiss *et al.*, 1982) bound to mast cells would react with antigenic substances released from DEC-damaged mf with resultant degranulation as shown in this study. Connor *et al.* (1970) observed an increase of mast cells in post-treatment specimens of the skin ; the highest counts of mast cells were found at 8 days after treatment and many of them were smaller and had compact granules. In this study, the total count of mast cells did not change during the course of DEC treatment, while the degranulation of mast cells occurred earlier than that seen in the above report. Mast cell granules contain eosinophil chemotactic factor of anaphylaxis (ECF-A), histamine and other substances (Wasserman, 1979). Thus the accumulation of eosinophils seen in this study would be triggered by ECF-A released from mast cells, and histamine would promote the dilatation of blood capillaries and edema in the dermis.

The density of degenerated mf increased

in the tissue of the nodules which included fertile adult females during DEC treatment. Boye and Connor (1982) also observed many dying mf in the connective tissue of nodules during DEC therapy. It seems that the mf liberated from gravid females were killed by the combination of DEC and immune mechanisms, and as a result they accumulated in the tissue of the nodule. After the degranulation of mast cell and the accumulation of eosinophils, it was noted that an increase of giant cells was observed in the connective tissue of nodules containing fertile worm in the treated patients (Table 3). This finding is of interest, because the increase of giant cells coincided with the density of mf. It is generally thought that particles or masses of foreign material such as mf, too large to be phagocytosed by ordinary macrophage or monocyte, may initiate the formation of giant cell (Ham and Cormack, 1979). The phagocytic process of mf seen in the specimens was thus quite reasonable.

It was shown that DEC had no damaging effect against the adult worm of *O. volvulus* (Ashburn *et al.*, 1949 ; Hawking, 1952 ; Boye and Connor, 1982). In the present study, the density of giant cells around adult worms and the morphology of the adults were not altered by DEC administration. Moreover, the fecundity of the adult females was not damaged by DEC, because mf tended to accumulate during DEC treatment in the nodule with gravid females.

Summary

Histological observations of the skin and nodule of 29 Guatemalan patients with onchocerciasis were performed semi-quantitatively during the administration of diethylcarbamazine (DEC).

In the affected area of the skin, microfilariae appear to migrate immediately from the dermis to the epidermis where they are found encapsulated in micro-abscesses 24 hours later. The increase of the mast cell degranulation was observed at 3 hours after the first dose of DEC, thereafter eosinophils were seen to accumulate in the dermis. Thus the involvement of type I hypersensitivity mechanism in the killing of microfilariae was suggested. An increase of histiocytes, loosening and degeneration of connective tissue, and dilatation of blood capillaries also occurred in the dermis. In contrast, these changes were much weaker in the unaffected skin areas of the same patients. Following DEC treatment the density of degenerated microfilariae increased in nodules containing fertile females. In such nodules, the degranulation of mast cells and accumulation of eosinophils and foreign body giant cells in the connective tissue were more marked than those in the nodules containing nongravid or degenerated adult worms. Adult morphology and the fecundity of females were apparently not affected by DEC treatment.

Acknowledgements

The author is greatly indebted to Prof. I. Tada, Kumamoto University School of Medicine, for his encouragement and valuable advice throughout this study. Thanks are also due to Dr. M. Sakamoto, Institute for Tropical Medicine, Nagasaki University, Dr. M. Korenaga, Kumamoto University School of Medicine, Dr. S. Nonaka, National Nagasaki-Chuo Hospital, and Dr. G. Zea and Dr. J. C. Castro, SNEM, Guatemala, for their excellent collaboration. The author is grateful to Dr. H. A. Godoy B, Director of SNEM, Ministry of Public Health, Guatemala and Dr. T. Suzuki, Chief of JICA Mission for their encouragement and support during this study. I am also highly appreciative of Dr. L. R. Ash of UCLA for reading the manuscript.

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ジエチルカルバマジン投与時におけるオンコセルカ感染者の皮膚および腫瘤の組織学的研究

三森 龍之
（熊本大学医学部寄生虫病学教室）

グアテマラ国において，29名のオンコセルカ感染者にジエチルカルバマジン（DEC）を 5mg/kg/日 7 日間投与し，経時的に採取した皮膚片とオンコセルカ腫瘤を組織学的に検索した．

肉眼的変化の認められた皮膚の部位（マソッティ反応陽性）では，仔虫は DEC 投与開始後の時間経過とともに，真皮層から表皮へ移行し，投与24時間以降の表皮内では変性した仔虫を含む微小膿胞が認められた．肥満細胞の脱顆粒が，DEC 初回投与 3 時間後から観察され，その後，好酸球の集積が認められた．この一連の所見から仔虫殺滅にはⅠ型アレルギー反応が関与していることが示唆された．更に真皮層において，組織球の増加，結合組織の変性，毛細血管の拡張が認められた．これに反して，肉眼的変化の見られなかった部位（マソッティ反応陰性）では，これらの反応は軽度であった．

仔虫を子宮内に含有する成虫（fertile）が存在する腫瘤では，結合組織内に変性した仔虫の増加が認められた．そのような腫瘤の組織における肥満細胞の脱顆粒，好酸球および異物巨細胞の集積は，仔虫を子宮内に含有しない成虫（nongravid）や変性した成虫（degenerated）が存在する腫瘤の場合に比べ著明であった．DEC 投与による成虫の形態学的変化および仔虫産出機能への影響は認められなかった．