

## Mazzotti Reaction: Clinical and Histological Observation of Onchocerciasis Cases Tested in Guatemala

ISAO TADA\*, TATSUYUKI MIMORI\*, SHIGEO NONAKA†  
AND HORACIO FIGUEROA MARROQUIN‡

(Received for publication; August 10, 1981)

**Key words:** mazzotti test, onchocerciasis, Guatemala, diethylcarbamazine (DEC)

Since Mazzotti (1948) recognized the manifestation of allergic reaction in the onchocerciasis patients given a single dose of diethylcarbamazine (DEC) and suggested its diagnostic utility, this reaction has been called the Mazzotti test. This test has been used especially in Latin America for diagnostic purpose. The histologic changes observed in patients with onchocerciasis given DEC have been described by several workers (Hawking, 1952; Buck, 1974; Gibson *et al.*, 1976). Further, Guerra-Caceres *et al.* (1980) tried to clarify the immune mechanism of this test. All these studies were carried out on the African form of onchocerciasis. The present authors attempted to observe the clinical and histologic changes due to Mazzotti test in patients with American onchocerciasis (Robles' disease). This paper deals with the results obtained.

### Materials and Methods

Four microfilarial positives, laborers of a

---

This study was supported by the Ministry of Public Health, Guatemala, and the Japan International Cooperation Agency (ORCPO Series No. 61).

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

† Division of Dermatology, Nagasaki-Chuo National Hospital, Nagasaki 856, Japan.

‡ Universidad de San Carlos, Seccion de "Enfermedad de Robles", SNEM, Guatemala C.A.

plantation "Milan" located southwestern part of the endemic area of onchocerciasis in Guatemala, were the volunteers for this study. The microfilarial rate among the 245 inhabitants of this plantation was 46.1%. The volunteers showed light to moderate microfilarial density (MFD) ranging from 0.7 to 5.0 microfilariae per mm<sup>2</sup> skin. They were orally administered with 100 mg DEC (Hetrazan) at 11:00 AM. Two skin snips were taken, one for the histologic examination and the other for the assessment of MFD of the monitoring site, from the left shoulder of each volunteer, 0, 2 and 24 hours post-administration. The snips were removed 5 mm apart from the other ones in a shape of a dotted circle. A quantitative skin-snip method (Tada *et al.*, 1974) was used for the assessment of MFD by incubating snips for 12 hours in saline at room temperature (20-24 C). The MFD was expressed as the number of microfilariae per mm<sup>2</sup> skin area. The snips from the monitoring site were fixed in 10% formalin and later used for histological examination with HE and PAS staining. Clinical observation of the patient was also made at each examination time, especially at 24 hours post-administration.

### Results

#### Clinical observation

Approximately one hour after the ad-

Table 1 Results of Mazzotti reaction in 4 microfilarial-positive male volunteers (Plantation "Milan", Guatemala)

Case No.	Age	Signs and symptoms				Microfilarial density*		
		Congestion of bulbar conjunctiva	Pruritus	Chillness and lassitude	Localized skin changes at;	0 hr	2 hrs	24 hrs
50	23	++	++	++	scapular and interscapular	1.2	3.5	3.8
51	20	—	±	—	—	0.7	2.0	0.7
52	28	+++	++	++	left buttock and iliac	5.0	1.7	2.4
53	38	++	++	+++	upper chest	0.9	1.9	7.2

\* Number of microfilariae per mm<sup>2</sup> skin snip.

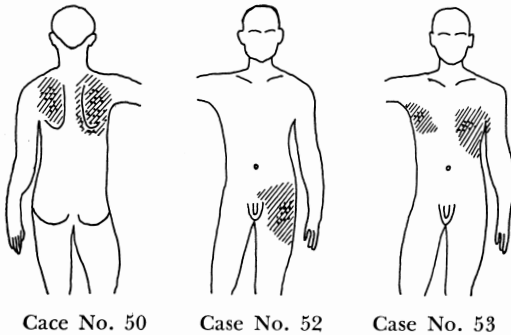


Fig. 1 Schematic illustration of reactions.

ministration, 2 cases (Nos. 50, 52) complained of itching of the whole body. In the case No. 52, strong congestion was seen in the bulbar conjunctiva of both eyes and his skin of the upper trunk showed apparent erythema. At 2-3 hours after the administration, all the cases but No. 51 showed consistent pruritus. Twenty four hours after the DEC administration, all the patients were checked precisely in the naked state. Cutaneous lesions observed were shown in Fig. 1 and summarized in Table 1.

Case No. 50: The pruritus with intense itching was seen mostly in the upper half of the body. Induration with marked demarcation was seen in the bilateral scapular and inter-scapular area of the back. The surface of the affected area was flushed and was provided with numerous urticaria of miliary size. The patient complained of burning sensation and pain in this region,

and of general lassitude.

Case No. 51: This case showed no apparent cutaneous and conjunctival changes but a slight itching in some portions of the body surface.

Case No. 52: This case had the sign of acute bulbar conjunctivitis and a marked induration on the skin where the left buttock-iliac-inguinal regions were involved. Flushed and edematous appearance was seen in this area. An intense pain was felt by the patient when the affected skin was touched by fingers. There was no swelling of inguinal and femoral lymphodi. The patient complained of chillness and fatigue, too.

Case No. 53: This case had similar edematous lesions bilaterally in the upper chest area. The surface of these regions was reddish and showed intense pain when touched. The patient was with shaking chills and fever.

Although these volunteers have ever experienced the denodulization several times in the past, they were without any palpable nodules at the time of this study. Further, the provocation with DEC never elicited any nodule-like swelling in this study.

#### *microfilarial density*

The prior MFD of 4 volunteers ranged from 0.7 to 5.0, while the MFD at 2 hours ranged from 1.7 to 3.5 and at 24 hours, from 0.7 to 7.2. As shown in Table 1, there was no significant trend in the change of

Table 2 Histological changes in the skin from the monitoring site of 3 onchocerciasis patients during the course of Mazzotti test

Histological changes	0 hr	2 hrs	24 hrs
	post-administration		
Edema of upper dermis	—	—	—
Capillary dilatation	—	+	—
Congestion of blood vessels	—	+	—~+
Round cell infiltration	+	+	+
Degeneration of collagen	—	±	—
Edema around blood vessels	—	+	+

MFD, so far as measured within 24 hours. The microfilariae emerged from snips at 24 hours post-administration were apparently active and did not show any abnormal movement in saline solution.

#### *histological observation*

Microscopic examination was carried out on the histologic changes in the skin taken from the monitoring site. The summarized histological findings in the sectioned skin from 3 positive reactors (Nos. 50, 52, 53) are as follows (Table 2).

0 hr.: Cell accumulation is not seen around microfilariae located mainly in the corium. The round cell infiltration, composed chiefly of small lymphocytes, is seen to some extent in the corium. A few cell infiltration is present around small blood vessels and capillaries.

2 hrs.: Increase of the cell infiltration and the loosening in the surrounding tissue of small blood vessels are the most marked finding in the sub-papillary layer and reticular layer of the corium. The latter finding may be reflecting the edema of the dermal tissue. A partial degeneration is seen in the collagen. The blood capillaries are dilatated and some erythrocytes are seen congested within the dilatated lumens. Infiltration of round cells is also present in the form of inflammatory-focus in the corium and the subcutaneous tissue.

24 hrs.: The dilatation of the blood capillaries is still present, but it is less intense than that seen in 2 hrs post-administration.

Some microfilariae are aggregated with cells especially the polymorphleucocytes, while others are not surrounded by cells. Apparently the damage of microfilariae is not severe in our specimens, lacking the degeneration of microfilariae. The infiltration of round cells is also seen. However, its intensity is indistinguishable from that seen in the specimens taken at 0 hr. post-administration of DEC. There have been no edema nor remarkable changes in the upper dermis at any period of the observation.

In the tissue taken from the case No. 51, who were considered negative in Mazzotti test, the above mentioned histologic changes are missing.

#### **Discussion**

Mazzotti test is an allergic reaction caused by the oral administration of 50–100 mg DEC to patients with onchocerciasis. It causes pruritus, edema of the skin of face and trunk, erysipela-like lesion on the trunk, congestion of bulbar conjunctiva, pain in the inguinal and femoral lymphnodes. According to Mazzotti (1948), these signs appeared in 59 cases (91%) out of 65 cases tested. No cross reactions were present in the cases infected with other helminths. Thereafter, this test has been used in some endemic areas for the diagnosis of onchocerciasis. However, the diagnostic validity of this test is still considered obscure by some workers. One of our case (No. 51) with microfilariae did not react positively to the DEC provocation. Oomen (1969) reported that 40% of 327 onchocerciasis cases were negative in the test in Ethiopia. However, from the viewpoint of immune mechanism involved and the mechanism of the action of DEC, this reaction had become theme of research by several workers. Hawking (1952) administered DEC on the onchocerciasis patients of Uganda and found numerous foci of inflammatory reaction in the biopsy specimens of skin 18–

24 hours later. The severity of the inflammation was proportional to that of clinical reaction, which was roughly proportional to the MFD. Neutrophil and eosinophil leucocytes appeared in the foci. He speculated that the destruction of microfilariae, with the liberation of foreign protein, would excite an allergic response in the sensitized patient. Buck (1974) describes histologic changes in the biopsy specimen taken 1 hour after the administration of DEC. The observation revealed hyperemia, increased edema and an accumulation of eosinophils around degenerating microfilariae. Later, the microfilariae showed advanced degenerative changes. Similar changes were seen in our Guatemalan cases, though the changes were milder and no degenerative microfilariae were found. The appearance of eosinophils was also scanty in our specimens. Gibson *et al.* (1976) examined the histologic changes elicited in Cameroon patients (MFD; 110–500 per snip), 0 hr–6 days after the initial DEC administration. The skin was taken from the thigh and buttock, where the highest MFD was expected in Africa. Probably due to the unmasking of microfilariae by DEC, histiocytes and eosinophils appeared in close proximity to degenerating microfilariae, acting as the defense mechanism of the host. Further, in their specimens, the increase of fibroblasts in the upper dermis, fibrinoid degeneration of collagen, sclerosis of dermal papillae and phagocytosis of melanin were found. In comparison with the above reports dealing with African cases, it seems that the histological changes are generally more milder in Guatemalan patients. The lesser worm burden due to denodulization campaign and various factors such as the differences in the sampling site, racial factors and pathogenicity of *Onchocerca volvulus* would have played in the formation of less severe histologic figures.

Guerra-Caceres *et al.* (1980) confirmed the

finding that blood eosinophils fell during the course of DEC administration (Mazzotti, 1948) and found an evidence that the eosinophils had an increased capacity to bind to IgG-coated erythrocytes. Thus they speculate that the massive eosinophil degranulation in tissues around DEC-damaged microfilariae could release granule products, which might produce some of the clinical features of the Mazzotti reaction. In our study, skin specimens were taken only from the monitoring site, where the highest MFD is expected, apart from the visible skin lesion. This could be an interpretation to the scarcity of eosinophils in the tissue.

Fazen *et al.* (1976) studied the distribution of microfilariae after a single dose of DEC in Guatemalan patients. They found a consistent decline of microfilariae in the skin for 24 hours and a return to predrug levels by 48 hours. Although the location of snip was not mentioned, the total number of microfilariae of 13 treated cases decreased approximately to one fifth at 8 hours. This phenomenon was attributed to the migration or immobilization of microfilariae in the skin. In our study, the MFD of only 4 cases were assessed, and there was rather an increase at 24 hours post-administration. This discrepancy should be clarified in a future study. Because the difference of skin snip method such as an insufficient incubation time of snips (20 min. in Anderson *et al.*, 1975) would give different result in the MFD, when the microfilariae were partially immobilized.

### Summary

Mazzotti test was performed on 4 onchocerciasis cases in Guatemala. Three cases out of 4 showed intense skin changes within 2 hours and continued at least 24 hours. The manifestations seen were typical: congestion of bulbar conjunctiva, pruritus and marked induration of skin in various portions of the body. The patients complained

of chillness, too. The microfilarial density of the left shoulder did not show any varying tendency within 24 hours of the test. The histological study of the snips from the monitoring site revealed congestion of small vessels, dilatation of capillary vessels and edema of the dermal tissue at 2 and 24 hours after the DEC administration. This evidence shows that the DEC administration elicits not only the drastic cutaneous lesions, but also the diffuse histological changes especially edema even in the cutaneous tissue of unaffected areas of the body.

#### Acknowledgements

We wish to thank Sr. Manuel Reinos C. and Sr. Antonio Solares G., the excellent brigadas of the Ministry of Public Health, Guatemala, who kindly helped us in the field study.

#### References

- 1) Anderson, R. I., Fazen, L. E. and Buck, A. A. (1975): Onchocerciasis in Guatemala. III. Day-time periodicity of microfilariae in skin. *Am. J. Trop. Med. Hyg.*, 24, 62-65.
- 2) Buck, A. A. (1974): Onchocerciasis. Symptomatology, Pathology, Diagnosis. pp. 20. World Health Organization, Geneva.
- 3) Fazen, L. E., Anderson, R. I., Fazen, L. E. and Figueroa, M. H. (1976): Clinical and laboratory changes consequent to diethylcarbamazine in patients with onchocerciasis. *Am. J. Trop. Med. Hyg.*, 25, 250-256.
- 4) Gibson, D. W., Connor, D. H., Brown, H. L., Fuglsang, H., Anderson, J., Duke, B. O. L. and Buck, A. A. (1976): Onchocercal dermatitis: Ultrastructural studies of microfilariae and host tissues, before and after treatment with diethylcarbamazine (Hetrazan). *Am. J. Trop. Med. Hyg.*, 25, 74-87.
- 5) Guerra-Caceres, J. G., Bryceson, A. D. M., Quakyi, I. and Spry, C. J. F. (1980): Studies on the mechanism of adverse reactions produced by diethylcarbamazine in patients with onchocerciasis—Mazzotti reaction. *Parasite Immunol.*, 2, 121-131.
- 6) Hawking, F. (1952): A histological study of onchocerciasis treated with Hetrazan. *British Med. J.*, 1, 992-994.
- 7) Mazzotti, L. (1948): Posibilidad de utilizar como medio diagnostico auxiliar en la onchocercosis, las reacciones americas consecutivas a la administracion del "Hetrazan". *Rev. Instit. Salubridad Enferm. Trop.*, 9, 235-237.
- 8) Oomen, A. P. (1969): Studies on onchocerciasis and elephantiasis in Ethiopia. pp. 44. De Erven, F. Bohn, N. V.—Haarlem.
- 9) Tada, I. and Figueroa, M. H. (1974): The density of *Onchocerca volvulus* microfilariae in the skin at different times of the day in Guatemala. *Jap. J. Parasit.*, 23, 220-225.

グアテマラのオンコセルカ症におけるマソッティ反応の臨床的ならびに組織学的研究

多田 功 三森龍之

(熊本大学医学部寄生虫病学教室)

野中薫雄

(長崎中央病院皮膚科)

HORACIO FIGUEROA MARROQUIN

(サン・カルロス大学, グアテマラ国厚生省)

4例のオンコセルカ症に対し、ジエチルカルバマジン(DEC) 100 mg を経口投与し、いわゆるマソッティ反応を観察した。うち3例はDEC投与2時間目から皮膚の変化を示し24時間後には明確な形をとった。その所見は眼球結膜の充血、皮膚の掻痒疹、皮膚の局所性硬結でいずれも悪寒戦慄を訴えた。すなわち4例中3例が反応陽性と判定された。DEC投与に伴う皮膚仔虫密度には変化が認められなかった。左肩口のモニター部位における組織学的所見は、小血管の充血、毛細血管の拡張、真皮組織の浮腫であり、これら

は投与2~24時間に著明に認められた。仔虫に対する細胞固集は著明でなく、投与24時間後の検皮法でも仔虫の運動はさほど障害をうけていなかった。これらの成績からDEC単回投与は一部皮膚に激しい丹毒状病変を形成すると共に、全身皮膚に浮腫性変化をひきおこしていることが明らかとなった。更に従来報告されているアフリカ型オンコセルカ症の場合と異なり、中米型ではDEC投与が必ずしも仔虫につよい細胞性反応を起こさないことが注目された。

Explanation of Figures

- Fig. 2 Indurative changes of the back provided with numerous urticaria of millitary size shown 24 hours post-administration of DEC (Case No. 50).
- Fig. 3 Moderate round-cell infiltration around a small blood vessel before the administration (Case No. 53,  $\times 300$ , HE staining).
- Fig. 4 Dilatation of blood capillary 2 hours post-administration (Case No. 52,  $\times 500$ , PAS staining).
- Fig. 5 Erythrocytes are seen in dilatated lumen of blood capillary 2 hours post-administration (Case No. 52,  $\times 500$ , HE staining).
- Fig. 6 Loosening of the dermal tissue around a blood vessel 24 hours post-administration (Case No. 50,  $\times 500$ , HE staining).
- Fig. 7 Microfilaria seen 24 hours post-administration without any aggregating cells (Case No. 53,  $\times 300$ , HE staining). An arrow indicates microfilaria.
- Fig. 8 Microfilaria seen 24 hours post-administration around which an aggregation of polymorph-leucocytes is demonstrated (Case No. 51,  $\times 300$ , HE staining).

