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Entamoeba histolytica: Experimental Chemotherapy of the Liver Abscess by Halogenated Bisphenols

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Abstract

The therapeutic efficacy of halogenated bisphenols against experimental liver abscess in hamster and jird produced by inoculation of axenic strains of *Entamoeba histolytica* (HM-1:IMSS or NOT-7 strain) was investigated. Among the halogenated bisphenols tested, only dichlorophene had a significant therapeutic efficacy, which was evaluated by the liver lesion scores, by orally administrating 50 mg/kg/day for 10 days from the 5th day of inoculation in both models. The highest therapeutic efficacy of dichlorophene was achieved by 100mg/kg/day for 10 days in the same route. Adverse effects by dichlorophene at this dose were scarcely demonstrated. There was not a significant difference in the therapeutic efficacy between this dose of dichlorophene and 35 mg metronidazole/kg/day for 3 or 5 days administered as above. However, administration of the same dose of dichlorophene or metronidazole from the 14th day resulted in a significantly less decrease in the scores. No significant difference was demonstrated in the response to these drugs between the two strains of ameba irrespective of the models and the drug administration protocols employed. Histopathologically, the liver of these experimental models, free from macroscopic lesions after the treatment with either of these two drugs, was still associated with a variety of stages of healing lesions. However macroscopic findings seemed to well reflect the histopathologic healing, as histopathologic healing of the amebic liver abscess by these amebicidal drugs was well correlated with the presence or absence of macroscopic lesion. The healing process was characterized by replacement of a central necrotic mass associated with degenerated amebae by a large number of foam cells, and subsequent formation of granulomas irrespective of the models and drugs employed. Even after trophozoites of *E. histolytica* became no more detectable light microscopically by the drug administration, the amebic antigen was still demonstrated by an immunohistochemical procedure at the sites of original lesions. Electron microscopically, *E. histolytica* was certainly sterilized by dichlorophene, which was characterized by disruption of the plasma and nuclear membrane. These findings suggest that dichlorophene is worth further evaluation against human amebic liver abscess.

Key words: *Entamoeba histolytica:* Protozoa, parasitic; Amebic liver abscess; Dichlorophene; Metronidazole; Chemotherapy

Introduction

Previous investigations in our laboratory disclosed that halogenated bisphenols like bithionol, dichlorophene and hexachlorophene potently inhibited the growth *in vitro* of *Entamoeba histolytica, Giardia lamblia* and *Trichomonas vaginalis* (Takeuchi *et al.*, 1984; 1985; Kawasaki and Takeuchi, 1984). Because of the low toxicity, bithionol and dichlorophene seemed to be worth further evaluation against these protozoan infections. Necessity of novel chemotherapeutic agents against these protozoan infections is quite obvious, since 5-nitroimidazole derivatives including metronidazole, the drug of choice for these infections, have mutagenicity and carcinogenicity (Beard *et al.*, 1988; Chacko and Bhide, 1986). Moreover, an emergence of metronidazole-resistant *T. vaginalis* (Müller *et al.*, 1980; Grossman, III and Galask, 1990) also strengthens the necessity of new drugs. the

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present study was designed, therefore, to evaluate the therapeutic efficacy of these halogenated bisphenols against experimental amebic liver abscess, since the procedure to make experimental amebic liver abscess in animal models has been extensively studied and established (Diamond *et al.*, 1974; Chadee and Meerovitch, 1984). The advantage of liver that it is easier to be examined macroscopically than other target organs of ameba like large intestine also led us to employ amebic liver abscess in this study.

Materials and Methods

Parasite: Pathogenic strains of *E. histolytica* (HM-1:IMSS and NOT-7) were used in this study. These two strains seemed equivalent in their pathogenicity, since both of them belonged to zymodeme II, a typical pathogenic zymodeme proposed by Sargeaunt (1988). Moreover, our preliminary study (Kobayashi *et al.*, unpublished observation) also indicated that they were equally cytopathogenic against NIH:3T3 cell in culture, and produced similar amebic liver abscess in hamster and jird (*Meriones unguiculatus*). After cultivating axenically in BI-S-33 medium (Diamond *et al.*, 1978) for 48 hours at 35.5°C, trophozoites of these strains were harvested and washed as described previously (Takeuchi *et al.*, 1977). Finally, the amebae were washed once and suspended in the medium without serum, and used for further experiments.

Chemicals: Halogenated bisphenols, i.e., bithionol (2,2'-thiobis(4,6-dichlorophenol)), dichlorophene (2,2'-methylenebis(4-chlorophenol)) and hexachlorophene (2,2'-methylenebis (3,4,6-trichlorophenol)) were supplied by Tokyo Kasei Inc. (Tokyo, Japan). Peroxidase-conjugated anti-human IgG goat immunoglobulin (heavy and light chain) was obtained from Miles Laboratories, Inc. (Elkhart, Indiana, USA). Skim milk was purchased from Difco Laboratories, Inc. (Detroit, Michigan, USA). All of the chemicals were of the highest purity commercially available.

Experimental models: Because the susceptibility of aged hamster to *E. histolytica* experimentally inoculated into the liver was significantly lower than that of the young animal (Ghadrian and Meerovitch, 1979), young male golden hamsters of 3 to 4 weeks of age (30 to 40 g body weight), supplied by Sankyo Laboratories Inc. (Tokyo, Japan), were used as an experimental model for amebic liver abscess. In contrast, formation of amebic liver abscess in jird was not affected by age (Chadee and Meerovitch, 1984) and by sex (Kobayashi *et al.*, unpublished observation), adult male and female jirds of 1 to 3 months old (50 to 80 g body weight), which were bred in our laboratory, were also employed in this study. These animals were housed at 22°–25°C, and fed on ordinary mouse pellet *ad libitum.*

These animals were fixed under a light anesthesia with ether, and subjected to laparotomy. Inoculation of trophozoites of *E. histolytica* in amount of 0.1 ml was immediately done into the dorsal portion of the left lobe of liver utilizing a 27 gauge × 3/4 (0.40 × 19 mm) injection needle. Subsequently, the site of inoculation was pressed with a gauze for several minutes to stop breeding, and the peritoneum and skin seemed in a conventional manner. The number of trophozoites experimentally inoculated into hamster and jird was 3×10^6 and 5×10^5 per head, respectively. These manipulations were done aseptically.

Drug administration and evaluation of the efficacy: Halogenated bisphenols were suspended in distilled water by homogenization in a glass vessel fitted with a Teflon pestle for 1 min at 4°C. Metronidazole was dissolved in distilled water.

Formation of the amebic liver abscess was confirmed macroscopically under laparotomy with a light anesthesia 4 days after inoculation of amebae unless otherwise stated. From the next day, oral administration of halogenated bisphenols or metronidazole in amount of 0.5 ml was initiated utilizing a stomach tube once in morning every day for an appropriate period. The therapeutic efficacy of these compounds was evaluated utilizing the liver lesion scores proposed by Mattern and Keister (1978), after the experimental models were sacrificed 10 days after completion of the drug administration.

Immediately after this macroscopic observation on hamster and jird, portions of the liver,

which became free from macroscopic lesion by the treatment in comparison with the original lesions, were resected, fixed and processed in a conventional manner to make histopathological slides stained with hematoxylin-eosin.

Electron microscopic and immunohistochemical studies: In these studies, 3×10^6 trophozoites of NOT-7 strain were inoculated into the liver of hamster, followed by the treatment with dichlorophene as above. Portions of the abscess lesion, which also became free from macroscopic lesion as judged above 3 days after starting administration of this compounds, were resected and fixed with 10% formalin for 12 hours at 4°C to make histopathological slides.

Another portion of the same original lesion was also resected, fixed with 2% glutaraldehyde in 0.1 M cacodylate-HCl buffer, pH 7.4 for 2 hours at 4°C. The specimen was further processed in a conventional manner for electron microscopic observation and finally embedded in Epok 812. Thin sections were cut with a ultramicrotome (Ultracut-E, Reichart Jung, Austria), and observed with a Hitachi HU-12 AS electron microscope (Hitachi Ltd., Tokyo, Japan) after staining with uranyl acetate and lead citrate.

The same portion was also observed with an immunohistochemical method to investigate localization of amebae or amebic antigens after treatment. The specimen was fixed with 10% formalin, and the tissue sections of 4 to 5 μm in thickness were prepared on slide glass from the paraffin-embedded materials. The whole procedure was essentially based on the conventional method as described by Watanabe and his colleagues (1981) with minor modifications. The endogenous peroxidase activity was suppressed by method of Súarez *et al.* (1987). Non-specific adsorption of anti-amebic antibody to the tissue sections was blocked by incubating the specimens with 3% skim milk dissolved in 15 mM phosphate buffered saline, pH 7.4 for 60 min at the room temperature. As the primary antibody, human serum from a case with confirmed amebic liver abscess and free from other sickness, which showed an indirect fluorescent antibody (IFA) (Takeuchi *et al.*, 1985) titer of $\times$1,024 and diluted 50-fold with 0.05 M phosphate-buffered saline immediately before use, was employed. The peroxidase-conjugated IgG was diluted 200-fold before use. Counterstaining of the nuclei of amebae and liver cells was done utilizing methylgreen (veronal-acetate buffered methylgreen, 1% solution, pH 4.0) according to Barka and Anderson (1962).

Statistics: Statistical analysis of the therapeutic efficacy of the compounds was done by student's *t*-test. Differences with probability (*P*) less than 0.05 was judged significant.

Results

Table 1 summarizes the result of preliminary screening of the therapeutic efficacy of bithionol, dichlorophone and hexachlorophene conducted at 50 mg/kg/day for 10 days. This dose was selected on the basis of our previous experiment on *in vitro* killing of amebae by these bisphenolic derivertives (Takeuchi *et al.*, 1984; Kawasaki and Takeuchi, 1984), and also of the observation by

Table 1 Screening of the efficacy of three halogenated bisphenols against experimental amebic abscess in hamsters

	Liver lesion score					Av. liver lesion score
	0	1	2	3	4	
Control	0	0	0	1	9	3.9
Bithionol 50mg/kg/day, 10 days	0	0	1	2	7	3.6
Dichlorophene 50mg/kg/day, 10 days	2	0	3	2	2	2.2*
Hexachlorophene 50mg/kg/day, 10 days	0	0	0	2	1	3.3

Difference statistically significant as compared to the control. (*P* less than 0.05.)
The compounds administered 5 days after inoculation of amebae.

Yokogawa *et al.* (1963) on the bithionol concentration in blood of human cases with paragonimiasis. At this dose, only dichlorophene was found to significantly reduce the scores.

On the basis of these findings, various doses of dichlorophene were tested and compared with the efficacy of metronidazole at 35 mg/kg/day for 3 days, which was based on one of the prescriptions of metronidazole against human amebic liver abscess, 2.0 to 2.4 g daily for 3 days, as described by Hunter III *et al.* (1976). The data summarized in Table 2 indicated that dichlorophene seemed most effective at 100 mg/kg/day for 10 days in hamster. At this dose, dichlorophene scarcely affected the increase in body weight of the experimental model (Fig. 1). Administration of 200 mg/kg/day dichlorophene for the same period still showed a significant therapeutic efficacy, however, it was lower than that of 100 mg/kg/day for 10 days. Other appreciable adverse effects of dichlorophene were not demonstrated. Moreover, dichlorophene at this dose was as effective as metronidazole judging from the decrease in liver lesion scores. Although prolonged administration of metronidazole, e.g., 5 days, decreased the scores from 3.0 to 0.8, there was not a significant difference in the score decrease between these two doses of metronidazole. Such a significant therapeutic efficacy of dichlorophene and metronidazole was demonstrable irrespective of the two amebic strains employed.

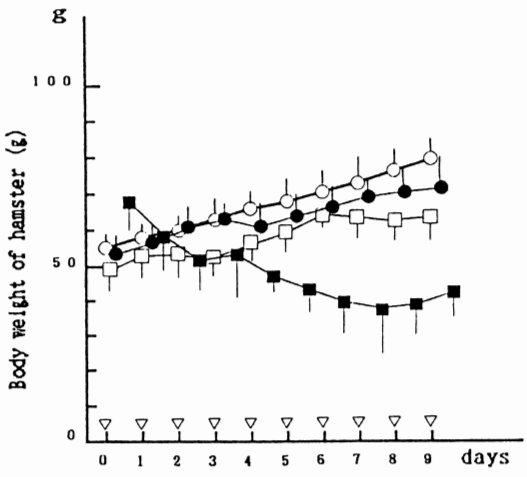


Fig. 1 ○－○ Control, ●－● 100mg/kg/day, □－□ 200mg/kg/day, ■－■ 300mg/kg/day, ▽ Dichlorophene administration.

Changes in the body weight of hamsters after starting administration of dichlorophene. Details as described in the text.

The same experiment was also conducted utilizing jird (Table 3). Oral administration of dichlorophene at 100 mg/kg/day for 10 days also seemed most effective among the various doses tested. Increase in the dose to 200 mg/kg/day seemed to be much less effective. Metronidazole

Table 2 Efficacy of various doses of dichlorophene and metronidazole against experimental liver abscess in hamster

	Liver lesion score					Av. liver lesion score
	0	1	2	3	4	
Control	0	0	2	3	2	3.0
Dichlorophene 75mg/kg/day, 10 days	1	0	2	2	0	2.0
Dichlorophene 100mg/kg/day, 10 days	4	3	1	1	1	1.2*
Dichlorophene 200mg/kg/day, 10 days	2	2	3	1	1	1.7*
Dichlorophene 300mg/kg/day, 10 days	1	2	2	2	1	2.0
Metronidazole 35mg/kg/day, 3 days	3	2	1	1	1	1.4*
Metronidazole 35mg/kg/day, 5 days	6	0	2	1	0	0.8*

*Difference statistically significant as compared to the control. (P less than 0.05.)
The compounds administered as in Table 1.

Table 3 Efficacy of various doses of dichlorophene and metronidazole against experimental liver abscess in jird

	Liver lesion score					Av. liver lesion score
	0	1	2	3	4	
Control	0	0	1	2	2	3.2
Dichlorophene 75mg/kg/day, 10 days	1	0	2	2	0	2.0
Dichlorophene 100mg/kg/day, 10 days	4	0	1	2	0	1.1*
Dichlorophene 200mg/kg/day, 10 days	0	0	0	3	1	3.3
Dichlorophene 300mg/kg/day, 10 days	1	0	2	0	1	2.0
Metronidazole 35mg/kg/day, 3 days	3	1	2	1	0	1.1*
Metronidazole 35mg/kg/day, 5 days	3	0	0	0	0	0.0*

*Difference statistically significant as compared to the control. (P less than 0.05.)
The compounds also given as in Table 1.

at the lower dose appeared as effective as dichlorophene, while prolonged administration of metronidazole for 5 days diminished the scores from 3.2 to 0. However, such a prolonged administration of metronidazole often caused changes in macroscopic appearance of the liver, which were histopathologically characterized by congestion in the hepatic venules and a light necrotic alteration in the liver cells around the central vein (data not shown). Adverse effects of this dose of dichlorophene seemed negligible. For instance, the body weight of experimental jirds was not affected (Fig. 2). In jirds as well, both HM-1 and NOT-7 strains responded equally to these compounds.

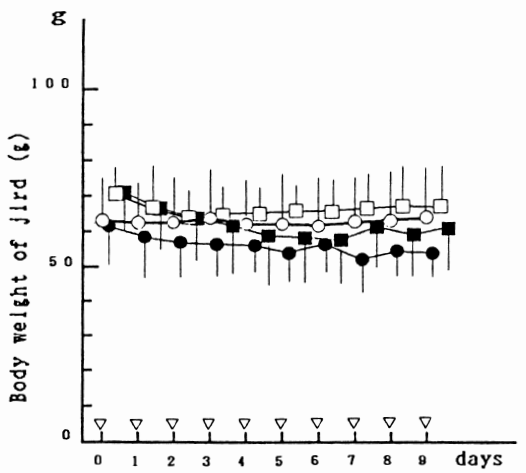


Fig. 2 ○–○ Control, ●–● 100mg/kg/day, □–□ 200mg/kg/day, ■–■ 300mg/kg/day, ▽ Dichlorophene administration.
Changes in the body weight of jirds after starting administration of dichlorophene. Details also as in the text.

Both metronidazole and dichlorophene seemed significantly less effective, when administered from the 14th day of inoculation of ameba (HM-1 strain) into the liver of both models. For instance, oral administration of 100 mg/kg/day dichlorophene for 10 days or 35 mg/kg/day metronidazole for 3 days scarcely affected the liver lesion scores (Table 4). Prolonged administration of metronidazole for 5 days decreased the scores from 3.0 to 2.5; however, this still seemed less effective as compared with the data summarized in Table 2 and 3.

The therapeutic efficacy of dichlorophene and the healing process of experimental amebic liver abscess in the animal models were investigated by some morphological methods. First, histopathological findings on the abscess in hamster inoculated with HM-1 strain and subsequently treated with 100 mg/kg/day dichlorophene for 10 days, were illustrated in Figs. 3 to 5. Fig. 3 shows a view of the control. Conspicuously discrete abscess lesions associated with moderate surrounding fibrosis and infiltration with

Table 4 Efficacy of late administration of dichlorophene and metronidazole against experimental liver abscess in hamsters

	Liver lesion score					Av. liver lesion score
	0	1	2	3	4	
Control	0	0	0	1	2	3.7
Dichlorophene 100mg/kg/day, 10 days	0	0	3	0	3	3.0
Metronidazole 35mg/kg/day, 3 days	0	2	4	4	2	2.5
Metronidazole 35mg/kg/day, 5 days	0	0	4	0	0	2.0*

*Difference statistically significant as compared to the control. (P less than 0.05.)
The compounds were administered 14 days after inoculation of amebae.

lymphocytes and plasma cells were demonstrated, which showed a characteristic chronic inflammation. Furthermore, infiltration with eosinophils and sometimes Langhans type giant cells were also observed. At the center of the lesions was present some necrotic mass. A large number of trophozoites of *E. histolytica* could be detected at the periphery of the necrotic mass or the inner surface of fibrous wall. As demonstrated in Figs. 4 and 5, treatment with dichlorophene reduced the size of the abscess lesion. Inside the lesions, tissue debris as well as a mass of ameba stained darkly with hematoxylin could be detected. As the treatment progressed, the number of amebae in the abscess lesions apparently diminished compared with the control, and the central necrotic mass was started to be replaced with a great many foam cells, which eventually resulted in a granuloma formation. At this stage, the size of granulomas was variable, and they occasionally fused each other. Treatment with 35 mg/kg/day metronidazole for 3 days also indicated essentially the same histopathological findings on the therapeutic effect on amebic liver abscess and its healing process in hamster (Figs. 6 and 7).

Although histopathological properties of amebic liver abscess of jird have been reported to be different from those of hamster (Diamond *et al.*, 1974), the process of abscess healing by these drugs seemed essentially similar. As shown with the control, the abscess in jird by HM-1 strain was characterized by more extensive fibrosis and severe chronic inflammation (Fig. 8). The size of lesion was generally smaller than that of hamster. Administration of dichlorophene at the same dose as above also resulted in a process of healing similar to that observed in hamster (Figs. 9 and 10). However, even after the treatment was initiated, fibrosis was still more extensive in the granuloma of jird, and infiltration with chronic inflammatory cells was more intensive than in hamster. Treatment with the same dose of metronidazole as above also indicated the similar healing process to that in the treated jird with dichlorophene (Figs. 11 and 12).

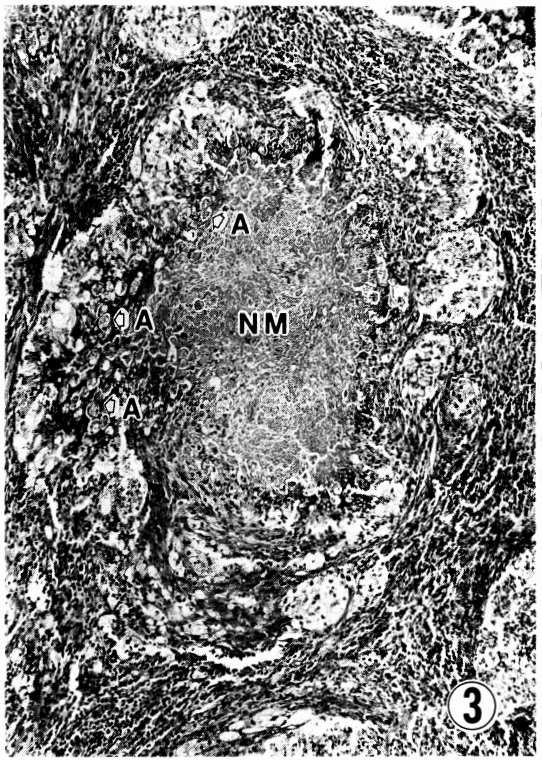
In both experimental models, decrease in the liver lesion scores was generally correlated with histopathological healing except that histopathological lesions were still present after the liver had

Fig. 3 A histopathological view of the control hamster, which was processed as described in the text without drug administration. Amebae inside the abscess wall: A. Necrotic mass at the center of lesion: NM. (× 120)

Fig. 4 Therapeutic efficacy of dichlorophene against the liver abscess in hamster. The necrotic mass (NM) was partially replaced with a large number of foam cells (F). Note the decreased number of ameba (A) compared with Fig. 3. Other details as in the text. (× 120)

Fig. 5 Another view of the same specimen as in Fig. 4. the treatment caused reduction in the size of necrotic mass (NM) and resulted in the granuloma formation. Details also in the text. (× 120)

Fig. 6 A histopathological view of amebic liver abscess in hamster treated with metronidazole. A large number of foam cells (F) are present, which is essentially similar to Fig. 4. Other details as in the text (× 120)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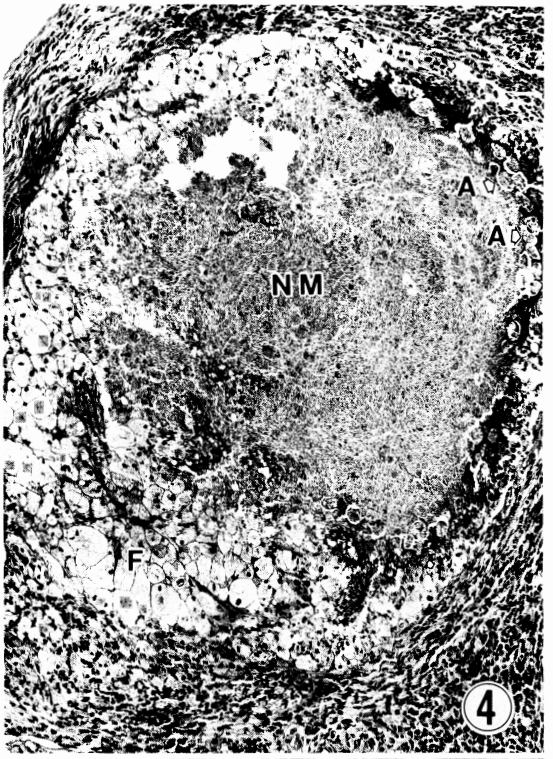
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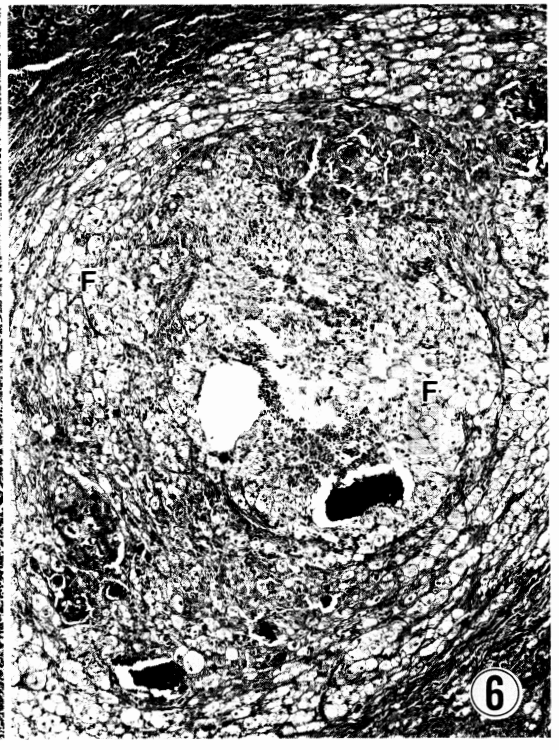
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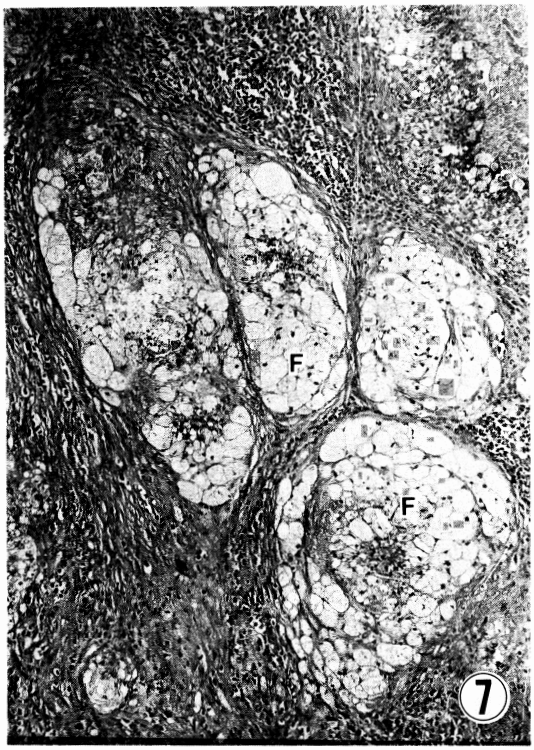
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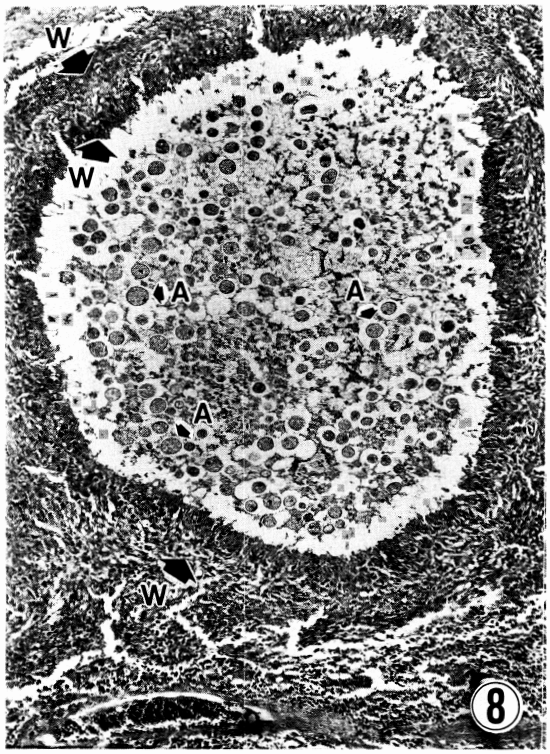
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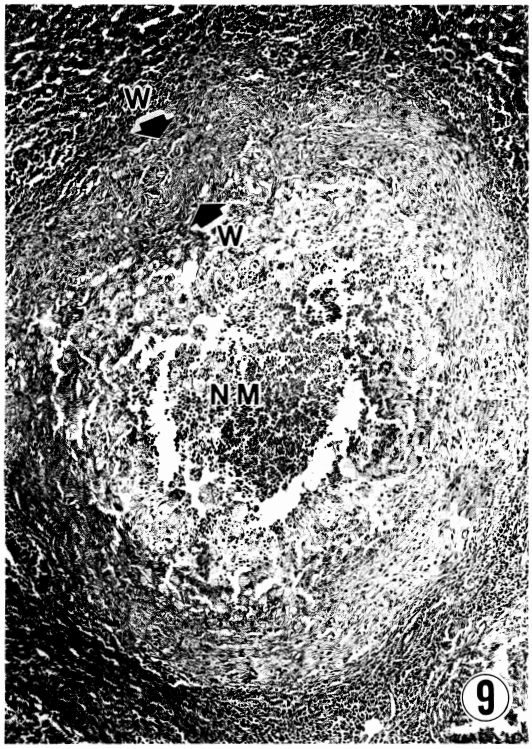
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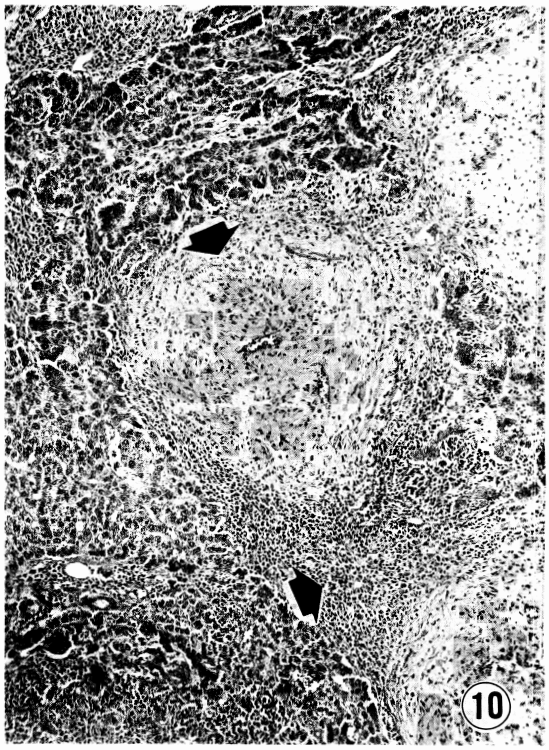
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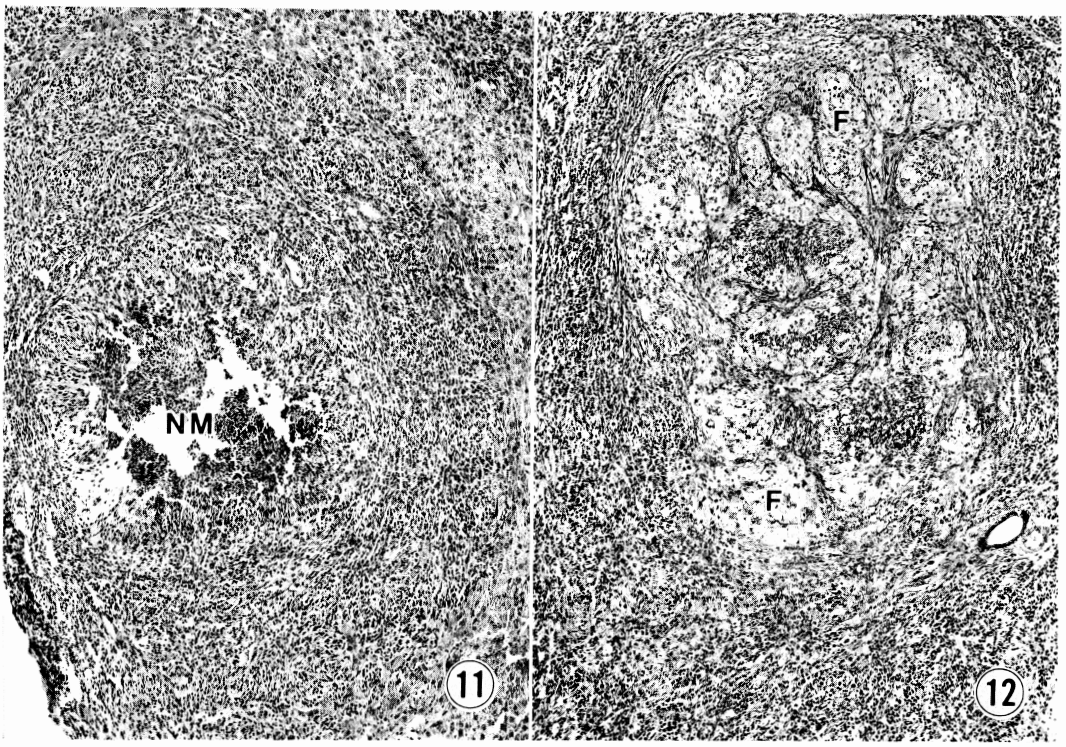


Fig. 11 A view of amebic liver abscess in jird treated with metronidazole as in Fig. 6. The central necrotic mass (NM) and surrounding cellular infiltration can be demonstrated. Viable amebae are scarcely seen. (×120)

Fig. 12 Another view of amebic liver abscess in jird treated as in Fig. 6. Note a large number of foam cells with some cellular infiltration. None of viable amebae are identified as in Fig. 11.

become free from macroscopically recognized abscess (data not shown).

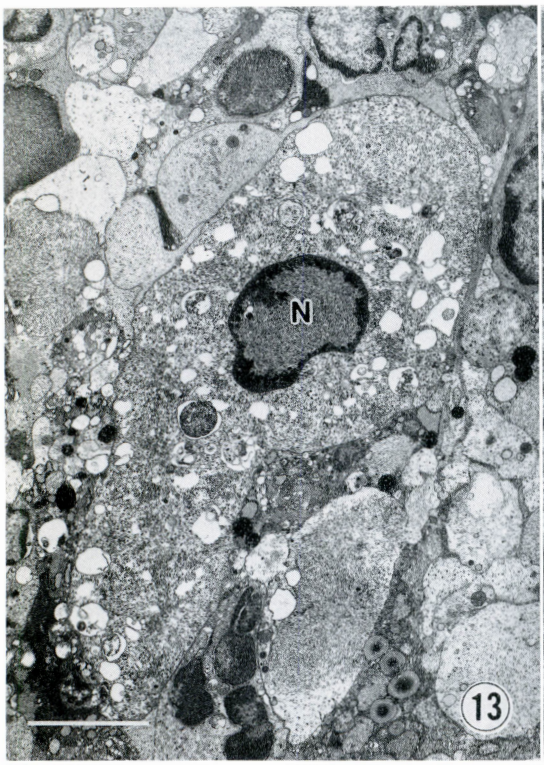
These observations suggested that it took longer to complete original, normal cellular architecture of liver cells than to remove macroscopic lesions, which prompted us to verify amebae were certainly sterilized by dichlorophene despite the decreased number of amebae in the lesions observed by histopathological studies. To solve this, the liver abscess of hamster produced by NOT-7 strain was examined electron microscopically 3 days after starting the drug administration from the 5th day of ameba inoculation. Amebae in the control exhibited no degenerative changes, although they were frequently surrounded by lymphocytes and neutrophils (Fig. 13). In contrast to this, as indicated in Fig. 14, the amebae in the remaining lesions were definitely degenerated by the treatment with dichlorophene. The initial degenerated change was observed in the plasma membrane and some other membranous organelles like the nucleus.

Fig. 7 Another view of the same specimen as shown in Fig. 6. Note appearance of a large number of foam cells and cellular infiltration. Viable amebae are not seen. (×120)

Fig. 8 Amebic liver abscess in the control jird. A well defined abscess wall (W) was formed. Amebae (A) were distributed almost evenly inside the abscess lesion with some necrotic mass. (×120).

Fig. 9 A histopathological view of the liver abscess of jird on the healing process by treatment with dichlorophene. The abscess wall (W) became less distinct. NM: necrotic mass. Other findings similar to those in Fig. 4. Details as in the text. (×120)

Fig. 10 Another view of the same specimen as in Fig. 9. Note a granuloma formation and surrounding cellular infiltration like those in Fig. 5. (×120)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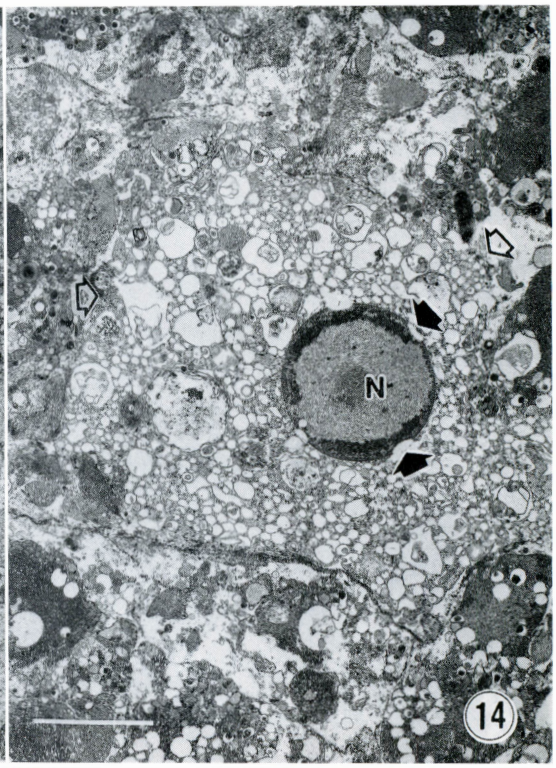
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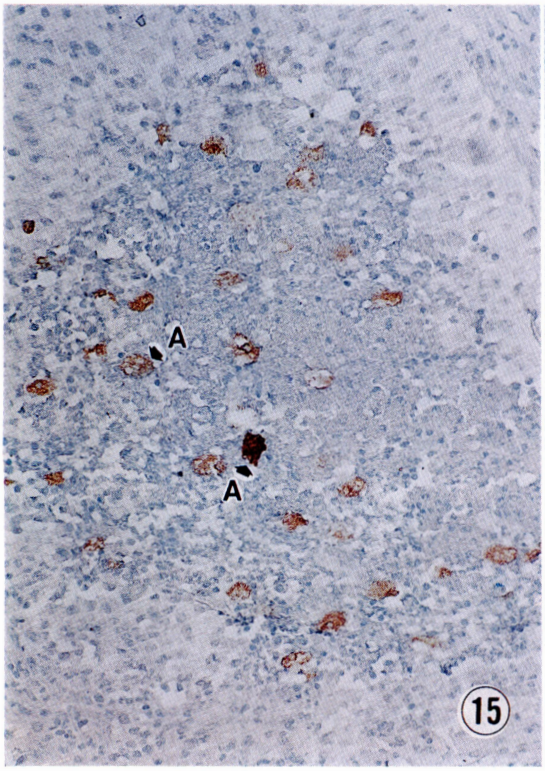
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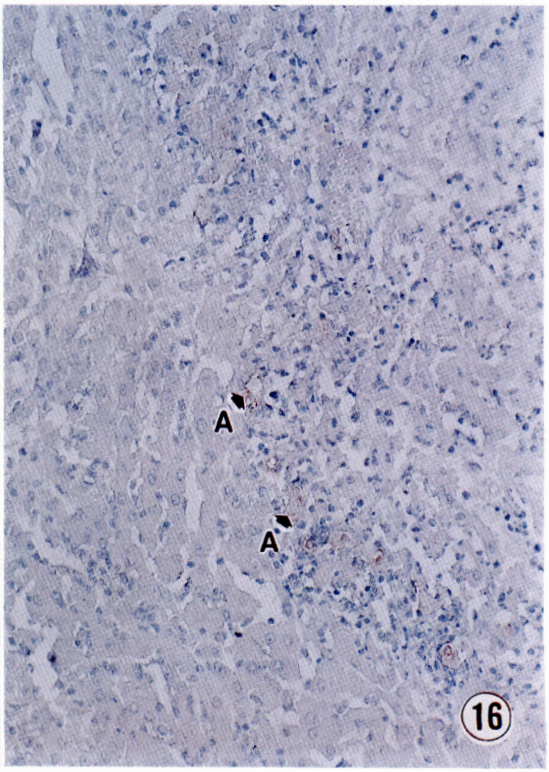
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The healing process of amebic liver abscess was also examined by an immunohistochemical method to further confirm degeneration of amebae. Moreover, the fact that immunological reactions were often involved in the pathogenesis of parasitic infections (Warren, 1982) also prompted the author to conduct this investigation. Fig. 15 demonstrates a distribution of the amebic antigen in the control hamster infected with NOT-7 strain and processed in the same manner as in the electron microscopic study without treatment by dichlorophene. The reaction product by antibody-conjugated peroxidase was deposited around trophozoites of *E.histolytica* which seemed to be viable light microscopically. Treatment by dichlorophene seemed to disrupt amebae as judged from morphological features of the amebae with positive reaction and its intensity. Moreover amebic trophozoites were no more demonstrable or identifiable on the hematoxylin-eosin stained preparations; however, the amebic antigen was still detected at the sites where viable trophozoites had been present (Fig. 16). These findings suggest that the amebic antigen remains in the healing lesions even after amebae were degenerated by the treatment with dichlorophene.

Discussion

Metronidazole, which was first developed for treatment of *T. vaginalis* infection, has been applied to amebic infection, and is now the drug of choice for treating invasive amebiasis. Recent investigations, however, indicated that new drugs in place of metronidazole are urgently needed, as judged from the serious adverse effects and the occurrence of resistant *T. vaginalis*. (Alvarez *et al.*, 1983; Sloan *et al.*, 1983; Müller *et al.*, 1980; Grossman III and Galask, 1990). However, in view of enormous economic expenditure for development of new drugs against parasitic diseases, reevaluation of currently available drugs has been recommended to broaden their applicability.

The present investigation indicated that oral administration of dichlorophene, which has been employed for treating human cestode infections (Reynolds, 1989), showed a significant therapeutic efficacy against experimental amebic liver abscess in hamster and jird. Because the dose of metronidazole employed in the present experiment appeared to be equivalent to a single one shot dose of this drug against human amebic liver abscess (Hunter, III *et al.*, 1976), the therapeutic efficacy of dichlorophene may be similar to that of metronidazole at least in regard to amebic liver abscess.

The reason of difference in the therapeutic efficacy between hamster and jird at 200 mg/kg/day is not known. The experimental model for amebic liver abscess in hamster and jird has been characterized in some details as noted above. In particular, differences in the histopathological features from those of human amebic liver abscess have been stressed (Pérez-Tamayo and Brandt, 1975). In spite of such qualitative differences, the amebic liver abscess in hamster and jird is of apparent importance. For instance, changes in the pathogenicity of ameba cultivated under various conditions has been assessed utilizing these experimental models

Fig. 13 An electron microscopic view of amebic liver abscess in the control hamster. An ameba is surrounded by neutrophil and lymphocyte; however, none of degenerative changes of the trophozoite can be observed. The nucleus (N) seems intact. Bar = 5 μm.

Fig. 14 Amebic liver abscess in hamster treated with dichlorophene as in Fig. 4. The membrane of nucleus (N) was partially disrupted (black arrows). In addition, the plasma membrane was extensively damaged (white arrows). Bar = 5 μm.

Fig. 15 Distribution of the amebic antigen in the liver of control hamster without drug administration as detected by immunohistochemical procedure. The reaction product of peroxidase is found on amebic trophozoites (A) in the lesion, which appeared viable judging from their morphological properties. (×240)

Fig. 16 Distribution of the amebic antigen in the liver of hamster treated with dichlorophene as in Fig. 4. The reaction product was still detectable; however, the intensity was much less than in Fig. 15, suggesting the amebae were destroyed. (×240)

(Mirelman *et al.*, 1986). However, to our knowledge, these experimental models have not been tested for evaluation of amebicidal compounds. Although the present study demonstrated that a successful removal of amebic liver abscess was achieved macroscopically by the drugs when administration was initiated soon after formation of the abscess lesion, the late administration resulted in a failure in the treatment. Since previous histopathological study (Diamond *et al.*, 1974) on experimental amebic liver abscess in hamster and jird showed that formation of the fibrous wall in the liver lesion required approximately 2 weeks, administration of these drugs from the 14th day of inoculation of amebae may result in lower penetration of these drugs into the lesion. Because the amebae are always located inside the abscess wall (Pérez-Tamayo and Brandt, 1975), if the rate of drug penetration into the lesion decreases, the efficacy of treatment is also expected to diminish. Recent studies by Ohtomo and his colleagues (Ohtomo *et al.*, 1991) suggested that metronidazole can penetrate enough through the abscess wall in humans. Less extensive fibrosis in human amebic liver abscess (Pérez-Tamayo and Brandt, 1975) may also account for this difference at least partially.

The present finding that dichlorophene seemed more effective in the treatment of experimental amebic liver abscess than bithionol and hexachlorophene appeared to be in contrast to the previous data on *in vitro* killing of *E. histolytica* trophozoites (Takeuchi *et al.*, 1984; Kawasaki and Takeuchi, 1985). It is possible that absorption and distribution of dichlorophene may be responsible for this difference in the anti-amebic action. In addition, although we tried to evaluate the bisphenolic derivatives dissolved in NaOH and subsequently diluted with 0.1 M HEPES buffer, pH 7.4 as described previously (Takeuchi *et al.*, 1984), the compounds prepared in this manner were significantly less effective in treating the experimental amebic liver abscess than those suspended in distilled water (data not shown).

Studies for evaluating the efficacy of these drugs by histopathological, electron microscopic and immunohistochemical methods indicated some interesting features of the action of these drugs against amebic liver abscess. It is not known if human amebic liver abscess also responds to these compounds in the same manner as the present experimental models; however, the fact that histopathological lesions on the healing process still remained even after the liver became free from macroscopic lesions is stressed. These findings may suggest that the liver lesion scores based on the macroscopic observation of amebic liver abscess may not always be applicable to evaluation of the chemotherapeutic agents against experimental amebic liver abscess, although we could observe the parallel correlation of macroscopic and microscopic healing in the present models. Disruption of the amebae confirmed electron microscopically and immunohistochemically also supports the significant efficacy of dichlorophene. Pathophysiological functions of the amebic antigen localized in the liver which had been free from viable amebae are still not known.

In conclusion, the present findings suggest that at this dose of dichlorophene, appreciable adverse effects were scarcely demonstrated. Moreover, the lack of serious side effects of dichlorophene like carcinogenicity and mutagenicity, led us to suggest that it is worth further testing against human amebic infection. Although it sometimes causes nausea, vomiting and other gastrointestinal symptoms and precautions might be exercised to its extensive use for those with impaired liver functions, the fact that liver enzymes are usually within normal limit in amebic liver abscess (Wolfe, 1991) appears to support our view.

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