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Biochemical lesions in liver and kidney after caecal amoebiasis in rats by *Entamoeba histolytica* and their reversal by antiamoebic drugs

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Abstract. Levels of lipid peroxides in rat caecum, blood, liver and kidney and the capacity of tissue homogenates to form lipid peroxides *in vitro* was enhanced after caecal amoebiasis in rats produced by *Entamoeba histolytica* (IB-1). The activity of hepatic drug-metabolizing enzymes in post-mitochondrial fraction and the cytochrome P_{450} contents in microsomal fraction decreased significantly, while lysosomal enzymes such as acid phosphatase, acid ribonuclease and cathepsin B showed an increase in the liver homogenates of infected animals. These changes were reversed following treatment with the antiamoebic drug, metronidazole.

Keywords. Lipid peroxidation; drug-metabolizing and lysosomal enzymes; cellular injury.

Introduction

It has been reported that intracaecal inoculation of rats with a virulent strain of *Entamoeba histolytica* produces lesion and damage to the wall of caecum (Dutta and Srivastava, 1974). It is not known whether biochemical and histological lesions produced in caecum during amoebiasis, cause secondary damage to other organs like liver and kidney. In view of the reports that prolonged caecal amoebiasis may cause hepatic amoebiasis (Gill *et al.,* 1983a, b), it is imperative that biochemical changes in other tissues are also studied during caecal amoebiasis. Increased lipid peroxidation is considered one of the indices of cellular injury (Sharma *et al.,* 1972; Sato *et al.,* 1979; Suematsu *et al.,* 1977; Nishigaki *et al.,* 1980) and is known to affect structural and functional integrity of biological membranes (Hiramitsu *et al.,* 1976a, b; Tappel *et al.* 1963; Kalish and Di Luzio, 1966; Wills, 1971; Wills and Wilkinson, 1966) and has been implicated in the pathology of ascariasis and filariasis (Gevondyan and Gevondyan, 1974), Hence we have studied changes in lipid peroxidation in caecum, liver, kidney,. brain and blood at different stages of intestinal amoebic infection in rats, the activities of drug-metabolizing and lysosomal enzymes, and the content of cytochrome P_{450} in liver and reversibility of these changes induced by antiamoebic drug metronidazole. The results obtained suggest that although infection is produced in caecum, secondary

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biochemical lesions are produced in liver and kidney, presumably, through increased output of lipid peroxides from infected caecum to blood, and these changes are reversed after administration of the antiamoebic drug, metronidazole.

Materials and methods

Twenty one day old male weanling rats (Druckrey strain) weighing 25-30 g drawn from C. D. R. I. Animal House were fed *ad lib* autoclaved rice diet (15 lbs/sq. inch for 10 min) and had free access to water. Vitamin E (10 mg/kg body weight/day) was also administered orally to all rats till they were sacrificed. The rats were divided into 4 groups after 7 days of this dietary regimen. Three rats were kept in each cage. The rats of group I and II were inoculated intracaecally with 100,000 trophozoites of *E. histolytica* (IB-1) during laprotomy. The methods used for maintaining *E. histolytica* and inoculation procedure in rats were similar to those reported earlier (Krishna Prasad and Bansal, 1982,1983). After two days of inoculation the rats of group II (infected) and group III (uninfected) were orally administered metronidazole (May and Baker). 0·5 ml of metronidazole dissolved in sterile water (100 mg/kg/day) was given for 5 continuous days. The rats of group I and IV received normal saline and served as control. Three rats from group I and II were killed by decapitation on the 3rd and 7th day after inoculation, while the rats of group III and IV were killed on the 5th and 7th day after dietary regimen of one week. All the rats were starved overnight before killing. The liver, brain, kidney and caecum were excised out immediately and washed with chilled normal saline. Blood was collected from the severed blood vessels of the neck.

Monitoring of infection

Infection was ascertained by microscopic examination of caecal contents for the presence of E. histolytica and gross examination of caecal walls for thickening or ulceration, Neal's (1951) method was used for evaluating infection.

Assay of lipid peroxides

Ten per cent (w/v) homogenates of all tissues were prepared in 1·15 % KCI (w/v) using Potter-Elvejhem homogeniser fitted with teflon pestle. Tissue homogenates were incubated in 25 ml Erlenmeyer flasks at 37°C for 2 h in Dubnoff metabolic shaker (120 horizontal strokes per min). Aliquots were withdrawn at 0 h and 2 h for assaying lipid peroxides according to Ohkawa *et al.* (1979). Lipid peroxides in blood was estimated according to Yagi (1976).

Fractionation of liver homogenate

The homogenate was centrifuged at 1000 g for 10 min to remove cell debris and the resultant supernatant at 29,000 g for 15 min to separate the mitochondrial pellet. The mitochondrial pellet was washed twice by resuspending it in 1·15% KCl (w/v) and centrifuging at 29,000 *g* for 15 min. All the supernatants were combined and centrifuged at 105,000 *g* for 60 min. The microsomal fraction thus obtained was washed

once by resuspending in 1·15 % (v/v) KCl and recentrifuging at 148,000 *g* for 60 min. All these centifugations were performed at 0–4°C.

Enzyme assay in liver

Drug-metabolizing enzymes aniline hydroxylase (Cochin and Axelrod, 1959) and aminopyrine N-demethylase (Kato and Gillette, 1965) were assayed in post-mitochondrial supernatant. Lysosomal acid phosphatase (Wright *et al*., 1972), acid ribonuclease (De Duve et al., 1955) and Cathepsin B (Mycek, 1970) were assayed in the unfractionated homogenate without freezing and thawing. Cytochrome P_{450} content was assayed in microsomal fraction according to the method of Omura and Sato(1964).

Results

All the rats following inoculation with *E*. histolytica developed caecal amoebiasis as evidenced by thickening of the caecal wall with ulceration and appearance of mucous in the caecal lumen. The results summarized in table 1 show that the level of lipid peroxides in rat caecum, blood, liver and kidney increased progressively after amoebic infection in the caecum but there was no significant change in the level of lipid peroxides in the brain. The tissue injury in caecal walls during amoebic infection causes increased lipid peroxidation in caecum and there is an increased out put of lipid peroxides from the site of infection to blood. The capacity of the homogenates of caecum, liver and kidney of infected rats to form lipid peroxides during *in vitro* incubation at 37°C was also more than that in the tissues from uninfected rats. It is possible that certain pro-oxidant factor(s) are elaborated during amoebic infection. The results of table 2 show that the activities of drug-metabolizing enzymes aminopyrine N-demethylase and aniline hydroxylase in the post-mitochondrial supernatant and cytochrome P_{450} content of the liver microsomal fraction decreased significantly in the infected group as compared to that of the uninfected one. On the other hand activities of lysosomal enzymes *viz*. acid phosphatase, acid ribonuclease and cathepsin B increased through infection. It is possible that these changes in liver are consequences of increased lipid peroxidation.

After 5 days of treatment of infected rats with metronidazole, thickening and ulceration of caecal walls and mucous contents were reduced to normal levels. The results of table 3 demonstrate that the level of lipid peroxides in blood, caecum, liver and kidney were also restored to normal values. Production of lipid peroxides *in vitro* by liver and caecum homogenates decreased considerably after 5 days of drug-treatment. The results presented in table 3 also show that the activities of aminopyrine N-demethylase, aniline hydroxylase, acid phosphatase acid ribonuclease, cathepsin B and contents of cytochrome P_{450} were similarly restored after treatment with metronidazole. However, when metronidazole was administered to uninfected rats, the activities of these enzymes remained unchanged as compared to those of untreated rats.

Discussion

It is clear from the above results that establishment of amoebic infection causes increase in the level of lipid peroxide *per se* in caecum, liver and kidney and the capacity of

Table 1. Lipid peroxidation in caecum, liver and kidney.

Days after infection		Uninfected			Infected		
		0 h	2 h	Lipid[a] peroxides *in vitro*	0 h	2 h	Lipid peroxides *in vitro*
Caecum	0	77 ± 6·8	136 ± 11·7	59	76 ± 8·8	143 ± 13·2	67
	3	76 ± 6·8	128 ± 12·4	52	106 ± 10·8[b]	182 ± 20·4[b]	76
	7	88 ± 7·2	146 ± 13·7	58	134 ± 11·7[b]	252 ± 24·6[b]	118
Blood	0	0·64 ± 0·054	—	—	0·66 ± 0·042	—	—
	3	0·65 ± 0·051	—	—	0·84 ± 0·062[b]	—	—
	7	0·64 ± 0·038	—	—	1·12 ± 0·078[b]	—	—
Liver	0	186 ± 17·6	406 ± 28·7	220	184 ± 18·5	472 ± 30·6[b]	228
	3	196 ± 16·6	416 ± 28·7	220	268 ± 24·6[b]	612 ± 50·6[b]	394
	7	208 ± 18·9	428 ± 22·4	220	294 ± 16·8[b]	756 ± 42·8[b]	462
Kidney	0	141 ± 12·6	277 ± 22·4	136	156 ± 14·3	296 ± 16·8	140
	3	151 ± 13·7	292 ± 17·6	141	176 ± 9·8	332 ± 21·6	156
	7	162 ± 16·6	286 ± 21·6	124	246 ± 17·5[b]	504 ± 16·8[b]	258

All values are expressed as n mol malonyldialdehyde/g wet weight of tissue or/ml blood and represent the Mean ± S.D. of 6 rats.
[a]Formed after 2 h incubation of homogenates at 37°C.
[b]P value < 0·001 as compared to uninfected.

Table 2. Changes in liver enzymes and their reversal by metronidazole.

Enzymes	Uninfected	Uninfected drug-treated	Infected 3rd day	Infected 7th day	Infected drug-treated
Aminopyrene N-demethylase[a]	1·49 ± 0·099	1·59 ± 0·107	0·949 ± 0·089[g]	0·86 ± 0·082	1·42 ± 0·105
Aniline hydroxylase[b]	61·8 ± 5·4	64·2 ± 5·0	44·1 ± 3·3	40·6 ± 3·1	58·6 ± 5·0
Cytochrome P_{450}[c]	2·74 ± 0·16	2·62 ± 0·22	1·36 ± 0·11[g]	1·02 ± 0·12[g]	2·49 ± 0·18
Acid phosphatase[d]	0·115 ± 0·01	0·120 ± 0·012	0·171 ± 0·017[g]	0·189 ± 0·015[g]	0·135 ± 0·011
Acid ribonuclease[e]	0·021 ± 0·002	0·022 ± 0·0018	0·034 ± 0·003[g]	0·035 ± 0·004[g]	0·022 ± 0·002
Cathepsin B[f]	0·016 ± 0·0015	0·017 ± 0·002	0·033 ± 0·003[g]	0·034 ± 0·005[g]	0·017 ± 0·002

[a,b]Assayed in post-mitochondrial supernatant. [c]Assayed in microsomal fraction. [d,e,f]Assayed in total hoomgenate. Enzyme activities are expressed as units/mg protein and represent the Mean ± S.D. from 6 rats. One enzyme unit is defined as (a) n mol of formaldehyde formed/min/mg protein; (b) *p* mol of *p*-aminophenol formed/min/mg protein; (c) *n* mol/mg protein; (d) μ mol of *p*-nitrophenol released/min; (e) ΔA_{260}/min/mg protein, (f) μ mol of tyrosine released/min/mg protein, and [g]*P* values < 0·01 as compared to uninfected rats.

Table 3. Effect of metronidazole on lipid peroxidation in tissues.

Tissue	Uninfected	Uninfected drug-treated	Infected	Infected + drug-treated
Caecum 0 h	88 ± 7·2	86 ± 8·3	134 ± 11·7[b]	76 ± 6·7
2 h	146 ± 13·7	164 ± 14·3	252 ± 24·6[b]	146 ± 10·8
Lipid peroxides formed *in vitro*[a]	58	78	118	70
Blood 0 h	0·64 ± 0·038	0·67 ± 0·047	1·12 ± 0·078[b]	0·68 ± 0·052
Liver 0 h	208 ± 18·9	212 ± 17·4	294 ± 16·8[b]	196 ± 13·8
2 h	428 ± 22·4	438 ± 30·8	756 ± 42·8[b]	412 ± 23·4
Lipid peroxides formed *in vitro*	220	226	462	216
Kidney 0 h	162 ± 16·6	168 ± 13·4	236 ± 16·2	148 ± 13·7
2 h	286 ± 21·6	272 ± 17·4	482 ± 18·8[b]	258 ± 13·7
Lipid peroxides formed *in vitro*	124	104	246	110

All values are expressed as in table 1 and represent the Mean ± S.D. from 6 rats.
[a] Formed after 2 h incubation of tissue homogenate at 37°C.
[b] P value < 0·001 as compared to uninfected control.

caecum, liver and kidney homogenate to form lipid peroxides in *vitro* also increases. Since low redox potential is an essential requirement for the survival of trophozoites of *E. histolytica* (Singh *et al.*, 1973) they could survive in an environment of high lipid peroxides in infected caecum due to the presence of or induction of a powerful superoxide dismutase which has been reported to be present in *E. histolytica* (Weinbach *et al.*, 1980). These results suggest the possibility that lipid peroxides formed at the site of infection *i. e.* caecum are released into blood and transported *via* portal circulation to other organs particularly liver, causing secondary damage such as increase in the activity of lysosomal enzymes and decrease in the activity of drug-metabolizing enzyme and contents of cytochrome P_{450}. These biochemical lesions continuing over a prolonged period of caecal infection could make liver susceptible to amoebic infection.

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