Gastroenterologia Japonica
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Vol. 25, No. 2
Printed in Japan

Mechanism of tissue damage through free oxygen radicals during hepatic amoebiasis in guinea pigs

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Summary: The capacity of Kupffer cells and blood monocytes to release free oxygen radicals was studied by chemiluminescence (CL) response during hepatic amebiasis in guinea pigs. Gunea pigs infected with *Entamoeba histolytica* intramesenterically were sacrificed on days 0, 2, 5 and 8 post-infection. Hepatic lesions were graded I-IV. A significant increase in the CL response was observed from day 2 post-infection and it increased with the progress of infection. Maximum increase was observed on the 8th post infection day. Animals with grade III or IV hepatic lesions had significantly elevated CL response. The degree of hepatic lesions correlated well with the CL response ($P<0.01$). It is postulated that tissue damage during hepatic amoebiasis may be mediated through enhanced release of free exygen radicals by Kupffer cells and blood monocyte. *Gastroenterol Jpn 1990;25:265–269*

Key words: *blood monocytes; chemiluminescence; free oxygen radicals; hepatic amebiasis; Kupffer cells*

Introduction

Amebiasis is one of the major health problems throughout the world[1]. Ever since its discovery, efforts have been made to understand the mechanism of the pathogenesis of this infection. However the factors responsible for the development of amoebic liver abscess are still not clear. Recently the role of host cells in the pathogenesis of this infection has been proposed[2]. Studies of the sequence of development of amoebic liver abscess in hamsters showed that trophozoites of *Entamoeba histolytica* did not cause direct lysis of hepatocytes. The necrosis occurs due to the release of secretory products, i.e. free oxygen radicals, acid hydrolases etc. of the infiltrating macrophages and neutrophils.

In recent years, many workers have emphasized that elevated release of free oxygen radicals, by immunologically activated phagocytic cells, is the prime cause of tissue damage in a variety of diseases including acute and chronic liver diseases[3-5]. These free oxygen radicals are generally very reactive. Even though the cell has a considerable capacity to handle the inactivation of such reactive oxygen radicals, they may still give rise to toxic events such as lipid peroxidation, enzyme inactivation and DNA damage[6]. Their role in tissue damage during hepatic amehiasis is not known. Moreover, that Kupffer cells which are situated at the site of development of amoebic liver abscess are expected bo play an important role in its establishment, since they interact with the amoebic antigens or toxins in situ during amebic liver abscess. We have, therefore, studied the ability of Kupffer cells and blood monocytes to release free oxygen radicals and their correlation with the severity of lesions during hepatic amebasis in guinea pigs.

Received February 9, 1989. Accepted August 18, 1989.
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Materials and Methods

Entamoeba histolytica strain

A rivulent *E.histolytica* strain KN-20, isolated from the stool sample of a dysenteric patient and being maintained monoaxenically in the modified Boeck and Drbohlav medium was used.

Animals

Normal healthy guinea pigs of either sex, weighing 100-150g gms, 3-4 weeks old were obtained from Central Animal House of the Institute. Only pathogen-free animals were included in the study.

Emxperimental Design

The animals were divided into two groups of 48 animals each.

Group I

Each animal was given 2×10^5 amebae/0.1 ml of normal saline by intramesenteric route following leparotomy[7].

Group II

In these animals 0.1 ml of amebae free inoculum obtained from supernatant of culture was given by the same route. Twelve animals each were sacrificed on days 0, 2, 5 and 8 post infection.

Grading of hepatic lesions

Grading of hepatic lesions was done according to the method described by Jarumilinta and Maegraith[8] with slight modifications.

Grade 0: No gross lesions.

Grade I: One or two abscesses present, neither more than 2 mm in size.

Grade II: One abscess of 5-10 mm in size or two to three small abscesses of 2-4 mm in size each.

Grade III: One big abscess involving about half a lobe or two or three abscesses of more than 5 mm in size.

Grade IV: Large abscess involving more than half of the lobe or multiple lesions covering almost all the lobes of the liver surface with necrosis and 'pus'.

To confirm the amoebic infection histopathology of the infected livers was also done.

Isolation of Kupffer cells

Kupffer cells were isolated by pronase digestion technique[9] as described elselwhere in detail[10].

Separation of blood monocytes

Leukocytes were separated by Ficoll-hypaque density gradient[11]. Monocytes were separated by adherent techinique.

The viability and purity of these cells was always cheked[12,13]. For the further characterization of Kupffer cells perroxidase staining was also done[14].

Chemiluminescence (CL) response

The ability of Kupffer cells and blood monocytes to release free oxygen radicals was measured through chemiluminescence assay[10]. The plastic disposable cuvettes containing 4×10^6 cells were washed with Munimum Essential medium (MEM), without phenol red, supplemented with 25 mM HEPES buffer, pH7.4, (assay medium) to remove nonadherent cells. In each cuvette, 2 ml of assay medium was added and kept at 37°C in 5% CO_2 incubator till the CL response was measured. Stock solution of luminol prepared in IN NaOH was made in assay medium. 20 μl of this was added to each cuvette containing cells and the basal CL response (BCL) was measured. 10 μl of latex particles were added for the stimulation of cells and the peak CL response was measured. ΔCL response was calculated as follows:

ΔCL response =

Peak CL response-Basal CL response

Results were expressed as CL response in millivolts (mV).

Results

In 52.08% animals hepatic lesions of Grade III or IV were present. The grades of hepatic lesions obtained on various post infection days varied. Even on the 2nd post infection day, Grade IV hepatic lesions were observed. On the 5th post infection day, a maximum number of animals had Grade IV hepatic lesions. On the 8th post infection day also, Grade IV lesions were present.

Table 1 Grades of hepatic lesions obtained on various post infection days

Grades of hepatic lesions	Post infection days*				Total animals (n=48)
	0	2	5	8	
0	12 (100)	—	—	—	12 (25)
I	—	2 (16.66)	1 (8.34)	1 (8.34)	4 (8.33)
II	—	1 (8.34)	2 (16.66)	4 (33.33)	7 (14.58)
III	—	5 (41.67)	4 (33.33)	4 (33.33)	13 (27.08)
IV	—	4 (33.33)	5 (41.67)	3 (25)	12 (25)

* On each post infection day 12 animals were sacrificed. Figures in parenthesis indicate percentage.

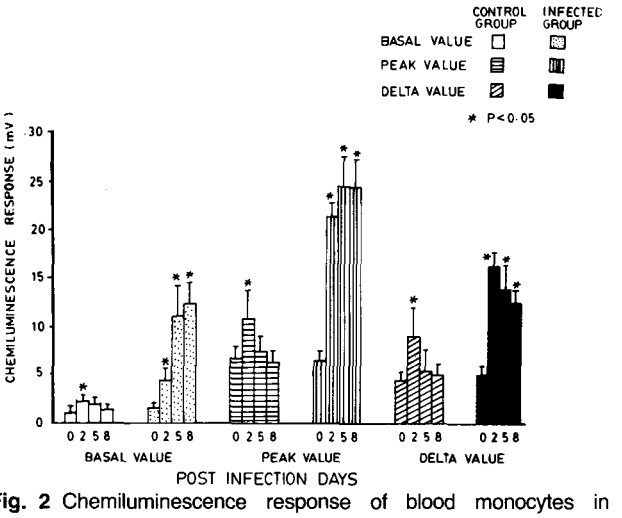

Fig. 2 Chemiluminescence response of blood monocytes in guinea pigs infected with *E. Histolytica* intramesenterically.

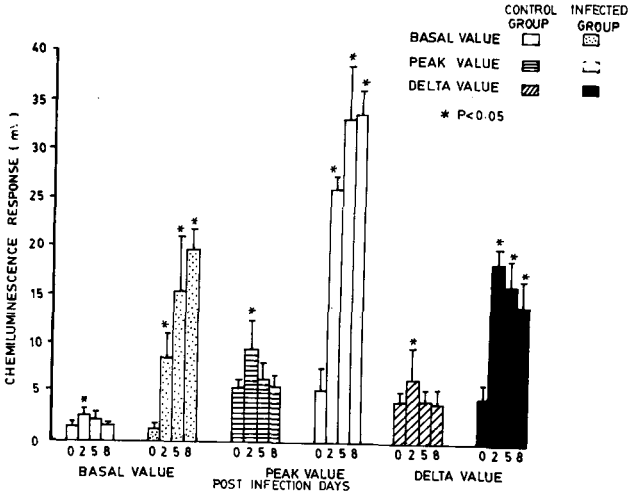

Fig. 1 Chemiluminescence response of kupffer cells in guinea pigs infected with *E. Histolytica* intramesenterically.

Hence, there was no correlation between the Grades of hepatic lesions and post infection days (**Table 1**).

Histopathology revealed development of well developed amebic liver abscesses from day 2 post infection. In control animals livers were normal both macroscopically and microscopically on all post infection days.

Chemiluminescence (CL) response

The CL response of both Kupffer cells and blood monocytes was significantly ($P<0.05$) elevated in the infected animals compared with controls (**Figs. 1, 2 and 4**). Both basal and peak CL responses were higher in the infected animals from day 2 post infection and they continued to increase with the progress of infection. Maximum increase was observed on the 8th post infection day ($P<0.01$) but it was not significantly higher than on the 5th post infection day ($P<0.05$). The ΔCL response was also significantly higher on day 2 post infection compared with day zero values. But afterwards, with the progress of infection, it showed a decreasing trend. In control animals, except on day 2nd post infection, no change in the CL response was observed compared with day zero values. This increase in CL response was much less compared with the increase observed in infected animals ($P<0.05$).

Correlation between grades of hepatic lesions and chemiluminescence response of Kupffer cells and Blood monocytes

The correlaiton obtained between CL response and grades of hepatic lesions is depicted in **Figures 3 and 4**. A direct correlations was obtained between PCL, BCL and severity of hepatic lesions ($P<0.01$). The animals which showed Grade III or IV hepatic lesions had highly elevated CL response, whereas a negative correlation was ob-

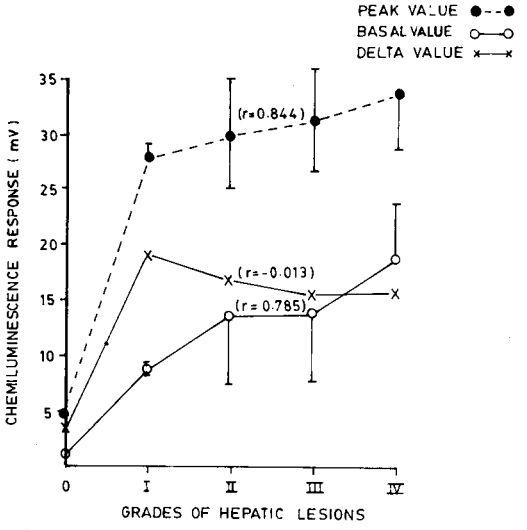

Fig. 3 Correlation between grades of hepatic lesions and chemiluminescence response of kupffer cells.

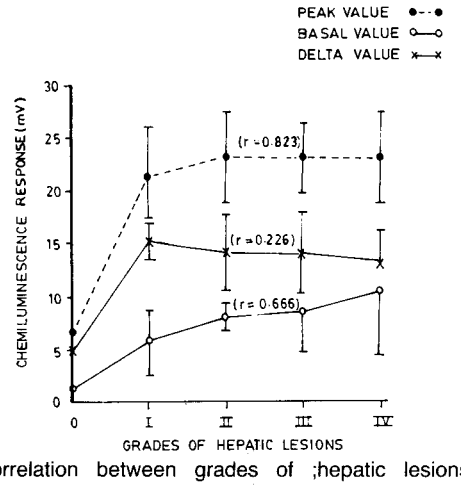

Fig. 4 Correlation between grades of ;hepatic lesions and chemiluminescence response of blood monocytes.

served between the DCL response and severity of hepatic lesions. However, it was not statistically significant ($P<0.05$).

Discussion

In this study we observed increased production of free oxygen radicals by Kupffer cells and blood monocyted during hepatic amebiasis. In infected animals, the basal CL response was also significantly elevated from day 2 post infeciton compared with controls and day zero reponse. Hence, the cells were already activated before their exposure to latex particles. The CL response of these cells was exceptionally increased in those animals who had Grade II or IV hepatic lesions. A direct correlation was observed between the CL response and grades of hepatic lesions. These findings suggest that the elevated release of free oxygen radicals by activated Kupffer cells and blood monocytes might be playing an important role in causing tissue damage during hepatic amebiasis in guinea pigs.

The elevated release of free oxygen radicals by phagocytic cells has been shown to cause hepatic injury in various liver diseases[15,17]. The mechanism of free oxygen radical-mediated tissue damage is through lipid peroxidation in which polyunsaturated fatty acids are broken down into saturated chains of fatty acids with consequent disruption of membrane integrity[18] and thus these reactions may initiate a cascade of events which lead to irreversible changes.

From this study, the type of trigger mechanism responsible for the activation of Kupffer celss/ blood monocytes cannot be specified. These may be engulfed by disintegrating amebae, amebic toxins, complement components, lymphokines, circulating immune complexes, etc., all of which have been shown to activate phagocytic cells[19]. Some of these factors have already been reported during amebiasis[20].

In addition, in this model of hepatic amebiasis, the inflammatory cell response consisted mainly of neutrophils in the early lesions and mononuclear cells in the advanced lesions[7]. The secretory products of infiltrated cells might also add to the tissue damage as hypothesized by Tsutsumi et al[2]. They pointed out that hepatic damage is mediated through the accumulation and subsequent lysis of infiltrated cells surrouding the amoebae. These leukocytes lyse as a consequence of contact mediated damage caused by the trophozoites. Recently in an ultrastructural study[21], they have the absence of direct contact between amebae and the hepatocytes, thus strengthening the view that hepatic damage might occur due to the release of certain secretions of host cells. Our results also

indicate the possible role of free oxygen radicals, released by activated Kupffer cells and blood monocytes during hepatic amebiasis as evidenced by the direct correlation of ability of these cells to release free oxygen radicals and severity of hepatic lesions.

In vitro studies by Ghadirian et al[22] showed that virulent strains of amoebae were less susceptible to the cytotoxic effects of oxidants. The strain used in the present study was also a virulent one. Hence, it seems that toxic effect of reactive oxygen radicals, generated by activated Kupffer cells, in vivo, on the parasite remains innocuous. Tsutsumi and Martinez Palomo[20] have also shown that inspite of being in close contact with the inflammatory cells the trophozoited of *E.histolytica* always survived.

On the other hand it has been shown that the oxidative metabolism of *E.histolytica* was increased following phagocytosis of erythrocytes[23]. This mechanism of amebae might also add to the pathogenesis of this infection.

The other important finding of this study is that amebae could not be demonstrated in advanced lesions, although abscesses and tissue necrosis were still there. This forms an attractive hypothesis for host cell mediated tissue damage during hepatic amebiasis in guinea pigs.

Hence, we conclude that elevated release of free oxygen radicals could be one of the important mechanisms of tissue damage during hepatic amebiasis in guinea pigs.

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