Inhibition of Japanese encephalitis virus infection by diethyldithiocarbamate is independent of its antioxidant potential

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Inhibition of Japanese encephalitis virus (JEV) infection is dependent on the host immune response. However, the exact mechanism by which diethyldithiocarbamate (DDTC) exerts its therapeutic effects remains unclear. The present study was conducted to investigate the role of DDTC in the inhibition of JEV infection. DDTC was administered at various doses (10–100 µmol/kg) to mice infected with a lethal dose of JEV (10^5 LD50, i.c.). The low dose of DDTC (10 µmol/kg; i.p.) significantly prolonged the average survival time and delayed the progression of the disease. The low dose also provided >80% survival in sub-clinical (10^2 LD50, i.c.) JEV infection. Administration of DDTC to JEV-infected mice enhanced the induction of nitric oxide synthase (iNOS) activity in brain and level of serum tumour necrosis factor-α (TNF-α). We have recently demonstrated that DDTC-mediated inhibition of JEV is believed to involve the augmentation of protective role of MDF as evidenced by the observation that pretreatment with anti-MDF antibody significantly decreased the AST of mice and together with the inhibition of iNOS activity. Interestingly, DDTC alone did not stimulate iNOS and TNF-α in mock-infected normal mice. These results show that DDTC may have a possible therapeutic role during JEV infection.

Keywords: Japanese encephalitis, diethyldithiocarbamate, DDTC, host defence, nitric oxide synthase, TNF-α, drug, ANTIED

Introduction

Japanese encephalitis (JE) remains one of the major health hazards of epidemic proportions with significant mortality rate in India and all of South-East Asia. An estimated population of 30 million is at risk of JE infection. The mortality rate is 25–40%, and 60–70% of survivors have severe neurological sequelae (WHO, 1998). Clinical manifestations of the disease are fever, headache, vomiting leading to convulsions and altered sensorium. Protection against Japanese encephalitis virus (JEV) is provided both by humoral as well as cell-mediated responses (Mathur et al., 1983; Tandon et al., 2002). Previous studies in our laboratory have revealed production of neutrophil chemotactic macrophage-derived factor (MDF) on JEV infection (Khanna et al., 1991). JEV-stimulated MDF induces neutrophil respiratory burst and degranulation (Khanna et al., 1994). Recently we have seen that MDF induced reactive oxygen and nitrogen intermediates, inhibits JEV replication and thus protects against lethal effect of JEV (Srivastava et al., 1999; Saxena et al., 2000, 2001). The presence of circulating MDF in JE confirmed patients have also been observed (unpublished data). The demonstration of the inhibitory role of certain antiviral compounds in ongoing, serious, acute and chronic infections has led to the increased interest in the development of new antiviral agents. The exact mechanism by which DDTC exerts its therapeutic effects remains unclear. The present study was conducted to investigate the role of DDTC in the inhibition of JEV infection.
Dithiodyldithiocarbamate (DDTC), a low molecular weight thiol first used clinically in 1957 for the treatment of nickel poisoning, was found to be both effective and safe (Sunderman et al., 1990). It has been described as an immunomodulator and modifier of diverse biological actions in human and animal model (Hersh et al., 1990). DDTC has also been shown to be effective in several disease conditions, such as, arthritis, endotoxic shock (Kishnani et al., 1999), chronic bronchitis, tuberculosis (Lemaitre et al., 1996) and also exhibit antiproteasome (Rehg et al., 1996), antifungal (Walker et al., 1997) and antiretroviral (Hersh et al., 1990) activity.

Based on these findings, the present study was planned to evaluate the therapeutic aspect of DDTC during JEV infection in mice, as a model. The data presented demonstrate the inhibition of JEV infection by DDTC, independent of its antioxidant potential.

Materials and methods

Animals

Inbred Swiss albino mice, aged 4–6 weeks, obtained from a mouse colony maintained in the Department of Microbiology, King George’s Medical College, Lucknow, India, were used to study the infection. They were housed in an air-conditioned room at controlled conditions (22 ± 2°C, 50 ± 20% relative humidity) on 12 h light/dark cycle and had free access to standard pellet diet and water. All the animals were treated as per the norms of the ethical committee.

Virus

JEV strain 78668A, isolated from the human brain (Mathur et al., 1983) was propagated in suckling mouse brain in Earle’s minimum essential medium (MEM). The infectivity titre was estimated in the brain tissue of mice at different time periods after inoculation of JEV intracerebrally (i.c.). A 10-fold serial dilution of each specimen was inoculated (i.c.) into groups of adult mice (at least 10), and had free access to standard pellet diet and water. All the animals were treated as per the norms of the ethical committee.

Preparation of MDF and anti-MDF antibody

Macrophage-derived factor (MDF) was prepared by splenic macrophages of JEV-infected mice as described in detail elsewhere by Khanna et al. (1991). Anti-MDF antibody was prepared as described by Sauma et al. (2000).

Briefly, anti-MDF antibody was prepared by injecting two doses of MDF (100 µg) emulsified in Freund’s complete adjuvant (Sigma Chemical Co., St Louis, Miss., USA) intramuscularly (i.m.) at the inner side of the flank and this dose in Freund’s incomplete adjuvant (Sigma Chemical Co., St Louis, Miss., USA) at interval of 3 weeks i.m. in Swiss albino mice. This was followed by three intradermal injections (60 µg proteins/100 µl) in the peritoneal cavity at weekly intervals without any adjuvant at four to five places and an intravenous (i.v.) injection (40 µg protein/mouse) 3–4 days before bleeding. Mice were bled and serum separated and inactivated at 56°C for 30 min. The optimal dilution of antibody, which abrogated MDF-induced chemotactic activity, was measured and antibody was stored at this dilution at ~70°C.

Assay of NO-synthase activity

NO-synthase (NOS) activity was assayed in brain by monitoring the conversion of L-[14C]-arginine to L-[14C]-citrulline (Bredt & Snyder, 1989). Briefly, 0.2 ml of brain cytosolic fraction (108000×g supernatant) was added to the enzyme-substrate mixture containing calmodulin (2 mg/ml), NADPH (0.2 mM), L-arginine (0.02 mM), L-[14C]-arginine (0.5 µCi), MgCl2 (2 mM) and CaCl2 (0.2 mM) in Hepes (100 mM, pH 7.2) in a total volume of 0.4 ml, and was incubated at 37°C for 30 min. For Ca2+-dependent NOS (constitutive NOS; cNOS) activity, ethylene glycol-bis (β-aminoethyl ether) N, N′-tetraacetic acid (EGTA; 1 mM) was added to the reaction mixture. For Ca2+-independent enzyme (NOS) activity, the reaction mixture was incubated with 1 mM EGTA and 1 mM NG-monomethyl-L-arginine (L-NMMA). The reaction was terminated by the addition of 2.0 ml of 20 mM Hepes (pH 5.5) containing ethylenediaminetetraacetic acid (EDTA; 5 mM) and the resulting mixture was run over AG50WX-8 (Na+ form) resin column. L-citrulline was eluted with two fractions of deionized water (2.0 ml) and the L-[14C]-citrulline radioactivity was measured by liquid scintillation beta counter.

The activity of cNOS was determined from the difference between the L-[14C]-citrulline produced from complete systems in absence of EGTA and the systems containing 1 mM EGTA. The activity of NOS was determined from the difference between the systems containing 1 mM EGTA and systems containing 1 mM EGTA and 1 mM L-NMMA.
Assay of TNF-α

TNF-α was assayed with a TNF-α ELISA kit (R&D Systems Inc., Minneapolis, Minn., USA). The concentration was determined by a standard curve prepared by using serial dilutions of the standard obtained from the manufacturer.

Assessment of average survival time

Animals were observed for their survival up to day 21 after treatments as described. Average survival time (AST) was calculated by counting the total number of deaths occurring each day and the figure was multiplied by the number of days the animals remained alive. The process of computation was continued until day 21, when all surviving animals were scored as having succumbed on that day. The AST of a group of animals is expressed as quotient obtained by dividing the total number of days mice were observed by the number of animals in the group.

Statistical analysis

The data were analysed using Student’s t-test and P-value (two tailed) of <0.05 was considered significant.

Results

JEV infection in mice

Following ic. inoculation with JEV (10\(^2\) LD\(_{50}\)), the mice appeared healthy up to day 3. On day 4 post-infection, ruffling of the fur and arching of the back occurred, which was followed by convulsions and paralysis of limbs on day 5. All the mice died by day 6. Mice appeared to be unaffected after i.p. inoculation of JEV.

Effect of DDTC on survival of JEV-infected mice

The effect of DDTC (at dose of 10, 50 and 100 µmol/kg) administered daily, on the survival of mice infected with JEV (10\(^2\) LD\(_{50}\), i.c.) is shown in Figure 1. The findings reveal that DDTC at a dose of 10 µmol/kg/day significantly prolonged (P<0.005) survival of JEV-infected mice as evident from the enhanced average survival time (AST, 8.86 ±0.6 days) compared to JEV controls (AST, 5.57 ±0.5 days).

Since 10 µmol/kg/day DDTC administration provided maximum survival of JEV-infected mice, the effect of DDTC, administered daily or on alternate days, on survival of JEV (10\(^2\) LD\(_{50}\), i.c.)-infected mice was studied. The data presented in Table 1 exhibit that administration of DDTC at 10 µmol/kg on alternate days was more effective as it slightly enhanced the survival of JEV-infected (10\(^2\) LD\(_{50}\), i.c.) mice (Table 1). Therefore, all successive experiments were performed at this dose and time duration.

Administration of DDTC (10 µmol/kg, alternate day) to mice infected with JEV at a sub-lethal dose (10\(^5\) LD\(_{50}\), i.c.) also prolonged the survival of mice as evident from the enhanced AST (10 ±0.4 days) compared to JEV-infected controls (AST, 17 ±0.6 days), and >80% DDTC-treated JEV-infected mice showed no mortality and remained alive after day 21 (P<0.02; Table 2).

Figure 1. Survival of JEV-infected mice treated with various doses of DDTC or placebo

Groups of mice (n=20) were infected with lethal dose of JEV (10\(^2\) LD\(_{50}\), i.c.), followed by administration with different concentrations of DDTC (10, 50 and 100 µmol/kg/day, i.p.) until the animal died. Control groups (n=20) consist of similarly placebo-treated JEV-infected mice or mice treated with different concentrations of DDTC alone. The survival rate of mice was monitored daily for 3 weeks.
Effect of DDTC on NOS activity during JEV infection

We have recently observed that NOS activity was significantly ($P<0.05$) enhanced in JEV (10**2** LD$_{50}$, i.c.)-infected mice. The enhancement in the NOS activity was mainly due to the induction of iNOS activity. The cNOS activity was not significantly altered (Saxena et al., 2001). Administration of DDTC (10 µmol/kg, alternate day) to JEV-infected mice caused significant ($P<0.02$) enhancement in the iNOS activity with the concomitant decrease in the cNOS activity compared to placebo. The total NOS did not change significantly ($P>0.05$). DDTC treatment alone did not produce any significant change in the NOS activity in normal mice (Figure 2).

Effect of DDTC on TNF-α during JEV infection

Groups of mice were inoculated with JEV (10**5** LD$_{50}$, i.c.) followed by treatment with DDTC (10 µmol/kg, alternate day) or placebo. Controls consisted of mice treated with normal mouse brain suspension or DDTC alone. The enhancement of TNF-α was performed on day 6 post-infection in sera obtained from JEV (10**5** LD$_{50}$, i.c.)-infected mice treated with DDTC (10 µmol/kg, alternate day) or placebo-treated JEV-infected mice. The data reveal that TNF-α was significantly ($P<0.02$) enhanced in JEV-infected mice (61.64 ±6.2 pg/ml) as compared to controls (<23.4 ±3.1 pg/ml) (Figure 3). DDTC treatment further augmented its level in serum (91.08 ±6.2 pg/ml; $P<0.05$) when compared with placebo-treated JEV-infected mice (61.64 ±6.2 pg/ml). DDTC alone did not stimulate TNF-α in mock-infected normal mice (<23.4 ±3.1 pg/ml).

Modulatory role of DDTC in MDF-mediated inhibition of JEV infection

We have previously demonstrated that the degradation of JEV is mediated by MDF, a low molecular weight polypeptide, secreted during JEV infection (Srivastava et al., 1999; Saxena et al., 2000, 2001). Therefore, we sought to ascertain the regulatory role of MDF in DDTC-mediated inhibition of JEV. Groups of mice were inoculated iv. with 200 µl anti-MDF antibodies (1:100 diluted) 24 h before JEV (0.025 ml of 10**-2** LD$_{50}$ JEV) inoculation (i.c.), followed by administration of DDTC (10 µmol/kg/alternate day, i.p.) or placebo. Control groups consist of similarly DDTC-treated JEV-infected mice or placebo-treated JEV-infected mice, or mice treated with DDTC or anti-MDF antibodies alone. The survival rate of mice was monitored daily for 3 weeks and brains of mice were titrated for virus. The findings presented in Table 3 reveal that anti-MDF antibody treatment significantly increased the disease progression in DDTC-treated JEV-infected mice (AST, 2.17 ±0.4 days; virus titre, 5.1 ±0.3 log$_{10}$ LD$_{50}$ per 0.025 ml) and in placebo-treated JEV-infected mice (AST, 1.89 ±0.5; virus titre, 5.4 ±0.4 log$_{10}$ LD$_{50}$ per 0.025 ml) as compared to only normal mouse brain suspension treatment (AST, 0 days; virus titre, 3.3 log$_{10}$ LD$_{50}$ per 0.025 ml).

### Table 1. Effect of DDTC on average survival time of JEV-infected mice

<table>
<thead>
<tr>
<th>Treatment(s)</th>
<th>No. of survivors on day 6 pi /total no. of mice (n=20)</th>
<th>Survival on day 6 pi (%)</th>
<th>AST (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mouse brain suspension</td>
<td>20/20</td>
<td>100</td>
<td>No mortality</td>
</tr>
<tr>
<td>JEV+placebo</td>
<td>0/20</td>
<td>0</td>
<td>5.97 ±0.5*</td>
</tr>
<tr>
<td>JEV+DDTC (10 µmol/kg/day)</td>
<td>4/20</td>
<td>20</td>
<td>8.86 ±0.6</td>
</tr>
<tr>
<td>JEV+DDTC (10 µmol/kg alternate day)</td>
<td>5/25</td>
<td>20</td>
<td>9.0 ±0.5*</td>
</tr>
<tr>
<td>DDTC (10 µmol/kg) daily or alternate day</td>
<td>20/20</td>
<td>100</td>
<td>No mortality</td>
</tr>
</tbody>
</table>

*P<0.001; pi, post-infection.

Groups of mice were inoculated with JEV (10**2** LD$_{50}$, i.c.), followed by administration of DDTC (10 µmol/kg, i.p.) or placebo, daily or alternate day. Controls consisted of mice treated with normal mouse brain suspension (mock-inoculation) or were treated with DDTC (10 µmol/kg, alternate day) alone. Mice were observed for survival for 21 days. AST was calculated as described in 'Materials and methods'.

### Table 2. Effect of DDTC on average survival time of JEV (sub-lethal)-infected mice

<table>
<thead>
<tr>
<th>Treatment(s)</th>
<th>No. of survivors on day 21 pi /total no. of mice (n=20)</th>
<th>Survival on day 21 pi (%)</th>
<th>AST (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mouse brain suspension</td>
<td>20/20</td>
<td>100</td>
<td>No mortality</td>
</tr>
<tr>
<td>JEV+placebo</td>
<td>13/20</td>
<td>65</td>
<td>17 ±0.6 *</td>
</tr>
<tr>
<td>JEV+DDTC (10 µmol/kg alternate day)</td>
<td>17/20</td>
<td>85</td>
<td>20 ±0.4 *</td>
</tr>
<tr>
<td>DDTC (10 µmol/kg) alternate day</td>
<td>20/20</td>
<td>100</td>
<td>No mortality</td>
</tr>
</tbody>
</table>

*P<0.02; pi, post-infection.

Groups of mice were inoculated with JEV (10**5** LD$_{50}$, i.c.), followed by administration of DDTC (10 µmol/kg, i.p.) or placebo, alternate day. Controls consisted of mice treated with normal mouse brain suspension (mock-inoculation) or were treated with DDTC (10 µmol/kg, alternate day) alone. Mice were observed for survival for 21 days. AST was calculated as described in 'Materials and methods'.

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DDTC as an anti-JEV drug

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DDTC-treated JEV-infected mice (AST, 9 ± 0.6 days; virus titre, 2.3 ± 0.4 log₁₀ LD₅₀ per 0.025 ml) and placebo-treated JEV-infected controls (AST, 5.57 ± 0.5 days; virus titre, 4.7 ± 0.5 log₁₀ LD₅₀ per 0.025 ml). Mice treated with DDTC or anti-MDF antibodies alone showed no mortality and were still alive on day 21 and no virus was recovered from brain.

Effect of DDTC on replication of JEV

To evaluate the effect of DDTC on JEV replication groups of mice were treated with DDTC (10 µmol/kg, alternate day) following i.c. challenge with JEV (10² LD₅₀). Control mice were inoculated with JEV (10² LD₅₀; i.c.) alone. The virus titre in brain was significantly decreased in DDTC-treated JEV-infected mice (2.3 ± 0.4 log₁₀ LD₅₀ /0.025 ml) as compared to JEV-infected (4.7 ± 0.5 log₁₀ LD₅₀ /0.025 ml) controls on day 6 post-infection (P < 0.02).

Discussion

Japanese encephalitis is a serious CNS disease, with high mortality, for which there is no specific anti-JEV drug available today. DDTC is being recognized as an immunomodulator and has been associated with enhanced macrophage and natural killer cell activity (Hubner et al., 1991). Its antiviral role has been studied in HIV infection, where it might affect the metal-dependent tat gene product of HIV (Frankel et al., 1988), inhibit HIV expression and reverse transcriptase activity in peripheral blood lymphocytes, or restore immune functions (Lang et al., 1988). In the present study we have demonstrated for the first time that in vivo (i.p.) administration of DDTC protects mice against the lethal challenge of JEV as it significantly delayed the mortality with increased survival time, thus providing evidence of its therapeutic value in JEV infection. The molecular mechanism of action of DDTC-mediated inhibition of JEV infection remains to be investigated. DDTC is a lipophilic drug and has been shown to penetrate the blood-brain barrier as determined by ³⁵S-labelled drug isotope imaging (Hacker et al., 1982). Thus, it appears that during JEV

Figure 2. Effect of DDTC on NOS activity in brain of JEV-infected mice

Groups of mice were inoculated with 10⁵ LD₅₀ of JEV i.c., followed by administration of DDTC (10 µmol/kg, alternate day, ip.) or placebo. Controls consist of mice that either received normal mouse brain suspension (mock-inoculation) or were treated with DDTC alone. Brains were collected on day 6 when maximum NOS activity has been observed in JEV infection, and inducible nitric oxide synthase (iNOS), constitutive NOS (cNOS) and total NOS activity were assayed as described in 'Materials and methods'. Each bar represents mean ± SEM of eight independent values.

Figure 3. Effect of DDTC on TNF-α during JEV infection

Groups of mice were inoculated with JEV (10⁵ LD₅₀; i.c.) followed by treatment with DDTC (10 µmol/kg, alternate day) or placebo. Controls consist of mice that either received normal mouse brain suspension (mock-inoculation) or were treated with DDTC alone. The assay of TNF-α was performed on day 6 post-infection as described in 'Materials and methods'. Each bar represents mean ± SEM of five independent values.
Role of DDTC at different concentrations. At low dose of JEV, while the higher concentrations (>50 µmol/kg) administered on alternate days prolongs survival time of mice infected with lethal low-dose DDTC (10 µmol/kg) administered on alternate days. AST and virus titre was calculated as described in 'Materials and methods'.

Table 3. Role of DDTC in MDF-mediated inhibition of JEV infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of survivors on day 6</th>
<th>Survival on day 6 pi (%)</th>
<th>AST (days)</th>
<th>Virus titre in brain (log_{10}LD50/0.025 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mouse brain suspension</td>
<td>20</td>
<td>100</td>
<td>No mortality</td>
<td>0</td>
</tr>
<tr>
<td>Anti-MDF antibodies</td>
<td>20</td>
<td>100</td>
<td>No mortality</td>
<td>0</td>
</tr>
<tr>
<td>JEV+placebo</td>
<td>0</td>
<td>0</td>
<td>5.57 ±0.5</td>
<td>4.7 ±0.5</td>
</tr>
<tr>
<td>JEV+anti-MDF antibodies+placebo</td>
<td>0</td>
<td>0</td>
<td>1.89 ±0.5</td>
<td>5.4 ±0.4</td>
</tr>
<tr>
<td>JEV+MDF (10 µmol/kg) once weekly</td>
<td>0</td>
<td>0</td>
<td>2.17 ±0.4</td>
<td>5.1 ±0.7</td>
</tr>
<tr>
<td>JEV+DDTC (10 µmol/kg/ alternate day)</td>
<td>5</td>
<td>25</td>
<td>9.0 ±0.6 *</td>
<td>2.3 ±0.4 †</td>
</tr>
<tr>
<td>DDTC (10 µmol/kg) daily or alternate day</td>
<td>20</td>
<td>100</td>
<td>No mortality</td>
<td>0</td>
</tr>
</tbody>
</table>

*P<0.001; †P<0.005; pi, post-infection.

DDTC was shown to be safe, non-toxic and effective during the clinical trials in patients with HIV infection and in animal model of retrovirus-induced immunodeficiency disease (Hacker et al., 1990). Our data demonstrate that low-dose DDTC (10 µmol/kg) administered on alternate days prolongs survival time of mice infected with lethal dose of JEV, while the higher concentrations (>50 µmol/kg) were less effective. This may be due to the dual role of DDTC at different concentrations. At low concentrations DDTC works as an inducer of NO and TNF-α, while at high concentrations its role as an antioxidant is well recognized. Recently, dose-dependent multiple mechanisms of action of DDTC have also been reported by Ishiyama et al. (2000). Early studies on the mechanism of action of DDTC reveal that DDTC increases the response of T lymphocytes without alteration in B lymphocyte response (Lacomba et al., 1988) and induces IL-1 (Zucconi et al., 2002). Brewton et al. (1989) have also demonstrated in a pilot study of DDTC in patients with AIDS and the AIDS-related complex (ARC) that low doses of DDTC administered once weekly were effective as compared to higher concentrations. Our data further demonstrate that low doses of DDTC also significantly enhance survival (>80%) in JEV-infected mice at sub-lethal doses. Despite DDTC having a short serum half-life, the duration of the effect of a single dose in animal model is approximately about 7 days. The long duration of effect as compared to its short plasma life is unexplained (Hacker et al., 1982).

Previously, DDTC has been known to exert antioxidant cytoprotective effects, as it interferes with the metabolism of glutathione. The present study revealed for the first time that DDTC augments JEV-induced iNOS activity in brain and protects the mice from the lethal effect of JEV in vivo independent of its antioxidant potential. Recently, Uchida et al. (2002) have also demonstrated that the antioxidant pyrrolidine dithiocarbamate caused inhibition of influenza virus, but not due to an antioxidant effect. We have recently shown that during JEV infection MDF enhance the expression of iNOS in brain (Saxena et al., 2001). MDF is a low molecular weight neutrophil chemotactic protein (~10 kDa) secreted during the JEV infection by activated macrophages (Khanuja et al., 1992). The in vivo studies suggest that MDF modulates the activation of neutrophils, regulates granulocytosis, increases capillary permeability, breaches the blood–brain barrier (Srivastava et al., 1999) and controls iron metabolism (Mathur et al., 1990). Recent studies revealed that MDF causes the degradation of JEV viral protein and nucleic acid (RNA) by inducing oxidative signals with generation of O2-, H2O2 and NO, and thus inhibits viral replication (Srivastava et al., 1999; Saxena et al., 2000, 2001). In the present study, anti-MDF antibody treatment significantly increased the disease progression in DDTC-treated JEV-infected mice. Thus, it appears that DDTC is playing an important role in association with MDF for controlling the infection and enhancing the survival during JEV infection. The cellular and molecular mechanisms that underlie the host defence are not well understood.

The mechanism of DDTC-mediated inhibition/prevention is believed to involve the induction and release of several cytokines including TNF-α, IL-1β and G-CSF (Kennedy & Borch, 1999). The present study shows the production of TNF-α in low concentration following JEV inoculation, which was augmented in DDTC-administered JEV-infected mice. It has been shown that TNF-α plays an important role in leukocyte movements during CNS inflammatory processes by influencing the production of chemokines (Sedgwick et al., 2000). TNF-α is released by monocytes/macrophages in response to infection as part of the host defence mechanism. Our previous studies have shown peripheral neutrophil leukocytosis along with inflammatory cell infiltration in various tissues. This effect is mediated by secretion of MDF and helps in...
anti-JEV host defence (Sitrasvata et al., 1999). TNF-α at a low concentration has been shown to have antiviral effect in ectromelia virus, vaccinia virus (Ruby et al., 1997), vesicular stomatitis virus and herpes simplex virus (Sieu, 1997). Thus, these results suggest that TNF-α may serve as a mediator of oxidative stress and its induced synthesis by DDTC may facilitate, at least in part, the inhibition of JEV infection. Bulger et al. (1998) have shown that DDTC enhances TNF-α production in LPS-stimulated rabbit alveolar macrophages, while Kishnani et al. (1999) have demonstrated that DDTC can inhibit overexpression of a number of cytokines during endotoxic shock and thus exerts multiple beneficial action. The exact mechanism involved in inhibition requires further elucidation.

Our recent data indicate that JEV-induced MDF is a key regulator of NO production (Saxena et al., 2000, 2001). Our earlier observations indicate that pretreatment of JEV-infected mice with i-NMMA, an inhibitor of NO, enhanced the mortality (Saxena et al., 2000), while administration of SNP (100 µM/mouse, ip., single dose), a NO donor to JEV-infected mice, significantly enhanced the AST with reversal in the JEV-induced anaemia (Saxena SK, unpublished data). NO in JEV infection inhibits viral RNA synthesis, viral protein accumulation and virus release from infected cells and is necessary for protective and non-fatal outcome (Lis et al., 1997). Although the exact mechanism of inhibition by DDTC is not known, collectively, our data suggest that DDTC may be a possible anti-JEV therapeutic agent as it provides inhibition of JEV infection probably by inducing the iNOS activity and TNF-α, and delays the progression of disease, though more work is needed to explore its role in JEV infection.

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References


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