J. Pharm. Pharmacol. 1991, 43: 140–142
Communicated September 8, 1990

Effect of nifedipine on *Leishmania donovani* infection in-vivo and in-vitro: chemiluminescence responses of peritoneal macrophages and neutrophils

N. K. GANGULY*, S. SODHI, N. KAUL*, S. KAUR, N. MALLA, R. C. MAHAJAN, *Departments of Parasitology and *Experimental Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India*

Abstract—After peritoneal macrophages had been exposed to different concentrations of nifedipine (10–120 ng mL^{-1}) there was a significant increase ($P<0.001$) in the percentage of *Leishmania donovani* infected macrophages compared with controls. Parasite load was also significantly increased ($P<0.001$) in nifedipine-treated, *L. donovani* infected, BALB/c mice, compared with untreated, infected mice, post-inoculation. Peak chemiluminescence responses were significantly depressed ($P<0.001$) in nifedipine-treated infected mice compared with untreated mice post-inoculation. It is suggested that availability of intracellular calcium is a factor in the defense mechanism of inflammatory cells in *L. donovani* infections.

Amastigotes of *Leishmania donovani* survive destruction by macrophages by excreting a carbohydrate-containing substance, excreted factor (EF) which has anti-β-galactosidase activity for mouse peritoneal macrophages (El-on et al 1980; Eilam et al 1985). EF also has the ability to bind Ca^{2+} thus elevating intracellular calcium in infected macrophages, so that the parasite is maintained in a microenvironment with a high Ca^{2+} concentration. This high concentration of Ca^{2+} bound to the EF within the phagolysosomes might increase the stability of the amastigote membrane (Carafoli & Crompton 1978) thus regulating the proliferation of amastigotes (Rasmussen & Goodman 1977; Rebhun 1977).

Another parasiticidal mechanism in *L. donovani* infections is the production of oxygen free radicals in inflammatory cells such as macrophages and neutrophils. The production of free radicals and other neutrophil responses such as chemotaxis, degranulation and phagocytosis depends on the changes in Ca^{2+} influx across the membrane (Irita et al 1986) suggesting that Ca^{2+} might be regulating the production of these toxic anions.

Ca^{2+} governs the optimal functioning of most of the immune and non-immune cells (Fanta & Drazen 1983). The calcium channel blocking drugs are known to affect the metabolism of macrophages and neutrophils (Wright et al 1985; Irita et al 1986).

For this reason we have examined the effect of the calcium channel blocking drug nifedipine on the conversion of *Leishmania donovani* promastigotes to amastigotes in macrophages and on the parasite load. In addition, the respiratory burst of macrophages and neutrophils obtained from nifedipine treated *L. donovani*-infected, BALB/c mice has been compared with untreated infected animals.

Materials and methods

Parasite. Leishmania donovani, strain RMRI 68, maintained by serial subcultures in modified NNN medium (Rao et al 1984) and by intracardiac inoculations in hamsters and mice, was used for the inoculation of an inbred strain of BALB/c mice, 18–20 g.

Drug. Nifedipine, obtained from Torrent Drug and Chemicals Pvt. Ltd, India, was dissolved in a minimal volume of ethanol and the final volume was made in 0·9% NaCl. Animals were dosed orally (0·015 mg kg^{-1} day^{-1}) for 15 days.

Course of infection. The mice were inoculated with 10^7 promastigotes (obtained from NNN medium) by intracardiac inoculation (Sodhi et al 1989). Animals ($n=5$) were killed on days 0, 7, 14 and 21 post-inoculation. The parasite load was monitored by impression smears of liver stained with Giemsa (Stauber 1958).

Experimental groups. One group of animals received nifedipine orally for 15 days and then were inoculated with *L. donovani*. The other group was inoculated with *L. donovani* but not given the drug.

Chemiluminescence responses of macrophages and neutrophils. Peritoneal macrophages were harvested by the method of Stuart et al (1978) in McCoy's medium (Hi-media). 10^6 cells mL^{-1} were allowed to adhere in cuvettes in McCoy's medium with 10% foetal calf serum (FCS, Sera-Lab) for 45 min at 37°C in a CO_2 incubator. The medium was then replaced with minimum essential medium (MEM) without indicator.

For isolation of neutrophils, heparinised blood was subjected to density gradient centrifugation on a Ficoll hypaque (lymphoprep) column (Cheung et al 1984). 10^6 cells mL^{-1} were incubated in MEM at 37°C for 10 min.

Chemiluminescence responses of macrophages and neutrophils were measured in a luminometer. Values were recorded as integrated counts min^{-1} using latex as a non-specific stimulant.

Effect of nifedipine on in-vitro conversion of promastigotes to amastigotes in macrophages. Peritoneal macrophages, 10^6 cells mL^{-1} were suspended in RPMI-1640 with 10% FCS. The cells were allowed to adhere to sterilized coverslips in plastic petriplates (35 mm diam., Steriware) for 2 h at 37°C in a CO_2 incubator. The medium was replaced and after 24 h these cultures were exposed to various concentrations of nifedipine (10–120 ng mL^{-1}). This procedure was performed in triplicate. *L. donovani* promastigotes in the ratio of 5:1 were added to the macrophages and incubated after which the coverslips were dried, fixed and stained with Giemsa. The percentage of infected cells was calculated by microscopic examination and values were given as mean ± s.d. of triplicate cultures.

Results

Effect of nifedipine on parasite load. The parasite load was significantly greater ($P<0.01$) in nifedipine-treated, *L. donovani* infected mice compared with untreated mice on all post inoculation days (Fig. 1).

Chemiluminescence responses of macrophages and neutrophils. The peak chemiluminescence responses of peritoneal macrophages from nifedipine treated infected mice showed a significant reduction ($P<0.025$) when compared with untreated infected mice, on various post-inoculation days. No significant difference ($P>0.05$) in peak chemiluminescence was observed on day 21 (Fig. 2).

Peak chemiluminescence responses of neutrophils also

Correspondence to: R. C. Mahajan, Department of Parasitology, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India.

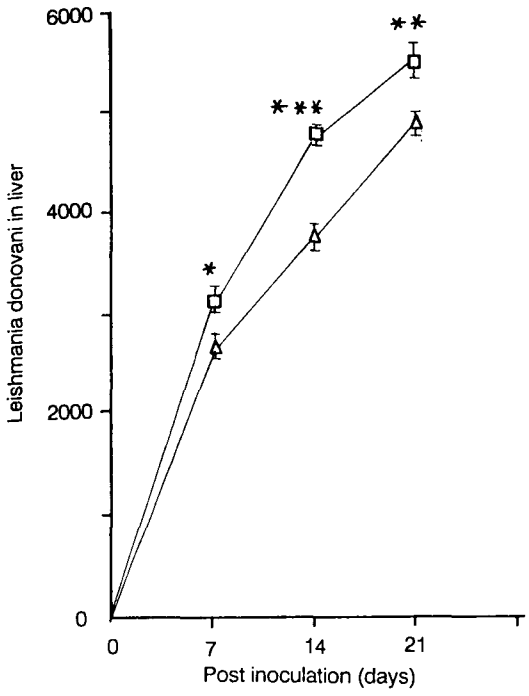

FIG. 1. Course of *L. donovani* infection in nifedipine treated (□) and untreated (△) *L. donovani* infected BALB/c mice. Each symbol represents mean ± s.d. of five mice. $*P<0.01$; $**P<0.005$; $***P<0.001$ compared with values from untreated infected mice.

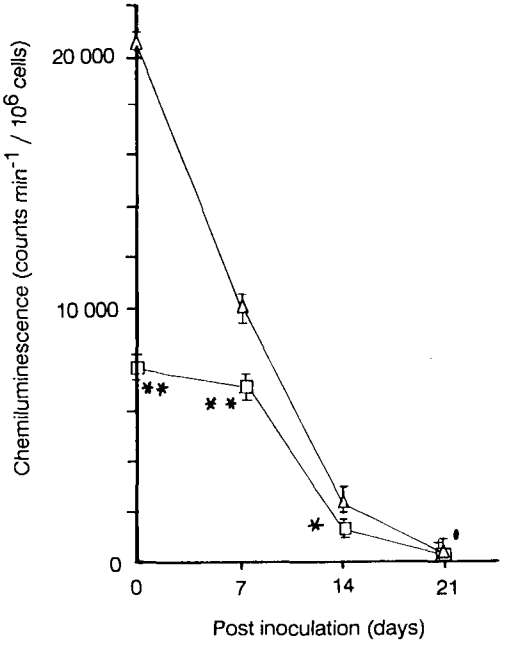

FIG. 2. Chemiluminescence responses of peritoneal macrophages from nifedipine treated (□) and untreated (△) *L. donovani* infected BALB/c mice. Each symbol represents mean ± s.d. of five mice. $*P<0.025$; $**P<0.001$, compared with values from untreated mice.

showed a similar trend, i.e. a significant reduction ($P<0.005$) in peak responses was observed in the nifedipine treated group compared with the untreated group post-inoculation (Fig. 3).

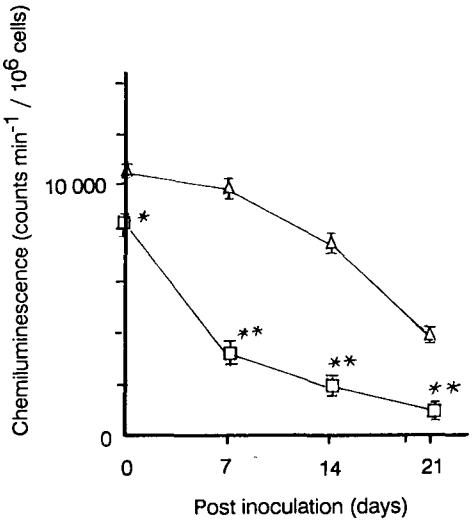

FIG. 3. Chemiluminescence responses of neutrophils from nifedipine treated (□) and untreated (△) *L. donovani* infected BALB/c mice. Each symbol represents mean ± s.d. of five mice. $*P<0.005$; $**P<0.001$, compared with values from untreated mice.

Table 1. Effect of nifedipine on the conversion of promastigotes to amastigotes in-vitro (n = 3).

Concentration of nifedipine (ng mL^{-1})	Infected macrophages (%)
0 (Control)	43·8 ± 2·1
10	50·0 ± 3·5*
20	57·0 ± 2·4***
40	60·0 ± 4·8**
50	66·0 ± 5·0***
75	72·0 ± 4·5****
100	80·0 ± 3·5****
120	80·0 ± 4·8****

$*P>0.05$; $**P<0.01$; $***P<0.005$; $****P<0.001$.

Effect of nifedipine on conversion of promastigotes to amastigotes in-vitro. Over the range 10–120 ng mL^{-1} there was a direct relation between the concentration of nifedipine and the number of infected macrophages. At 120 ng mL^{-1}, nifedipine, 80·0 ± 5% of the macrophages were infected which is highly significant ($P<0.001$) compared with control cultures (43·8 ± 2·1%) (Table 1).

Discussion

The blocking of calcium channels by nifedipine prevents lysosomal enzyme in the macrophages acting on the parasite (Eiferink 1982). Such conditions favour the survival of the parasite inside macrophages and hence the parasite load would be increased in nifedipine treated infected mice, as demonstrated in this study.

A reduction in oxygen free radicals as measured by chemiluminescence responses of macrophages and neutrophils in nifedipine-treated infected mice was observed. Peak chemiluminescence responses showed a depression after *L. donovani* infection, which indicates that the microbicidal function of phagocytic cells is impaired during *L. donovani* infection, which may result in the survival of parasites in immunocompetent cells.

The increase in the percentage of infected macrophages after in-vitro exposure to nifedipine, also suggests that nifedipine impairs the microbicidal functions of macrophages.

From these studies, it is clear that the availability of intracellular calcium is a factor in the defense mechanism of inflammatory cells in *L. donovani* infections.

References

Carafoli, E., Crompton, M. (1978) The regulation of intracellular calcium. In: Bronner F. Kleinzeller, A. (eds) Current Topics in Membranes and Transport Vol. 10, Academic Press, New York, pp 151–216

Cheung, K., Archibald, A. C., Robinson, M. F. (1984) Luminol dependent chemiluminescence produced by PMN's stimulated by immune complexes. Aust. J. Exp. Biol. Med. Sci. 62: 403–419

Eiferink, J. G. R. (1982) Interference of the calcium antagonist verapamil and nifedipine with lysosomal enzyme release from rabbit PMN's. Drug Research 32: 1417–1420

Eilam, Y., El-on, J., Spira, D. T. (1985) *Leishmania major* excreted factor, calcium ions and the survival of amastigotes. Exp. Parasitol. 59: 161–168

El-on, J., Bradley, D. J., Freeman, J. C. (1980) *Leishmania donovani.* Action of excreted factor on hydrolytic enzyme activity of macrophages from mice with genetically different resistance to infection. Ibid. 49: 167–174

Fanta, C. H., Drazen, J. M. (1983) Calcium blockers and bronchoconstriction (editorial). Am. Rev. Respir. Dis. 127: 673–674

Irita, K., Hujita, I., Takeshige, K., Minakami, S., Yositoke, J. (1986) Calcium channel antagonist induced inhibition of superoxide production in human PMN's. Mechanisms independent of antagonizing calcium influx. Biochem. Pharmacol. 35: 3465–3471

Rao, R. R., Mahajan, R. C., Ganguly, N. K. (1984) Modified media for in vitro cultivation of *Leishmania donovani* promastigotes-a comparative study. Bull. PGI. 18: 125–128

Rasmussen, H., Goodman, D. B. P. (1977) Relationships between calcium and cyclic nucleotides in cell activation. Physiol. Rev. 57: 421–509

Rebhun, L. I. (1977) Cyclic nucleotides, calcium and cell division. Internat. Rev. Cytol. 49: 1–54

Sodhi, S., Ganguly, N. K., Malla, N., Mahajan, R. C. (1989) Lymphokine mediated microbicidal activity of peritoneal macrophages from *Leishmania donovani* infected and drug treated BALB/c mice. Japan. J. Exp. Med. 59: 103–108

Stauber, C. L. A. (1958) Host resistance to the Khartoum strain of *Leishmania donovani.* Rice Inst. Pamph. 48: 80–96

Stuart, A. E., Hebeshaw, J. A., Davidson, A. E. (1978) Phagocytes in vitro. In: Weir, D. M. (ed.) Handbook of Experimental Immunology. Blackwell, Oxford, pp 31.1–31.30

Wright, B., Zeidman, I., Greig, R., Poste, G. (1985) Inhibition of macrophage activation by calcium channel blockers and calcium antagonists. Cell. Immunol. 95: 46–53

J. Pharm. Pharmacol. 1991, 43: 142–144
Communicated August 10, 1990

Concentration of ibuprofen in cervical mucus

I. G. SALAS-HERRERA, R. M. PEARSON, P. TURNER, *Clinical Pharmacology Department, Saint Bartholomew's Hospital, London EC1A 7BE, UK*

Abstract—Concentrations of an acidic drug, ibuprofen, in cervical mucus and serum have been measured by HPLC after oral administration in six healthy volunteers. After an 800 mg single dose of ibuprofen, the concentration reached in cervical mucus was less than 4% of that in serum. It is postulated that because ibuprofen is an acidic drug which is not subject to 'ion-trapping' in the acidic environment of cervical mucus, it is not concentrated in this secretion.

Studies on drug disposition in cervical mucus are important for at least two reasons. Firstly drugs may exert their therapeutic action through their concentration in the genital tract, for example metronidazole in the treatment of Trichomonas infections (Fleury et al 1977). Secondly, drugs may exert unwanted effects, for example reducing sperm motility and fertility. Propranolol has been shown to be concentrated in cervical mucus (Pearson & Ridgway 1985) and to reduce sperm motility in-vitro (Hong et al 1981).

Little is known, however, of those physicochemical properties of a drug which determine its penetration into the female genital tract. Basic drugs such as propranolol have been shown to concentrate forty fold in cervical mucus after oral administration (Pearson & Ridgway 1985). The disposition of acidic drugs in cervical mucus has not been studied. We have examined the concentration of a widely used acid drug, ibuprofen, in cervical mucus and serum with a view to identifying those factors influencing the secretion of drugs into cervical mucus. Ibuprofen is acidic (pK_a 5), moderately lipophilic and is 99% bound to serum proteins (Albengres et al 1988). It is readily and almost entirely absorbed after oral administration (Moffat 1986) and is inactivated and/ or eliminated by biotransformation, the liver fulfilling the main role in its total body clearance. Studies on its distribution in other bodily fluids have shown that the ratio of its concentration in synovial fluid compared with plasma was about 1·25 after a single dose with a similar value obtained at steady state (Netter et al 1989). In a case report, negligible levels ($< 0{\cdot}05$ $\mu g\ mL^{-1}$) of ibuprofen and its metabolites were reported in the breast milk of lactating women after 17 days of therapy with 400 mg tablets of ibuprofen twice a day (Weibert et al 1983).

Correspondence to: P. Turner, Clinical Pharmacology Department, St Bartholomew's Hospital, London EC1A 7BE, UK.

Materials and methods

Subjects and experimental design. Six healthy female volunteers, aged 21–23 (mean 21·3) years and weighing 57·2–72·0 (mean 65·2) kg, took a single dose of 800 mg (2 × 400 mg tablets) ibuprofen (Boots Co. plc) by mouth at 0800 h on the study day after overnight fasting. They attended on day 14 of the menstrual cycle when the cervical mucus is most prolific. None were using hormonal contraceptives or intrauterine devices. All were non-smokers, drug-free and abstained from sexual intercourse for two weeks before and during the study. None had significant abnormal findings on clinical examination, plasma biochemistry, urinalysis or was pregnant. All volunteers were informed of the design and aims of the study and gave written consent. This study was approved by the City and Hackney District Health Authority Ethics Committee.