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PHARMACOLOGICALLY ACTIVE CHEMICAL MEDIATORS IN *PLASMODIUM BERGHEI* MALARIA

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Cordeiro et al. (1976, 1977, 1983) reported that mice infected with *Plasmodium berghei* exhibit leucocytosis, thrombocytopenia and progressive pulmonary edema between the fourth and seventh day of the disease. Histologically, the edema was characterized as an intense alveolar and interstitial edema accompanied by a significant leucocyte infiltrate.

Weiss & Kubat (1983), in studies using fluorescence techniques, observed the presence of fine granular precipitates on the alveolar and vascular walls of the lungs of mice infected with *Plasmodium berghei.* These authors suggested that one of the possible causes of the pulmonary edema induced by *P. berghei* may be the release of vasoactive chemical mediators probably mediated by immune complexes.

The pioneering idea of an association of the inflammatory phenomenon with the physiopathological alterations occurring in malaria was advanced by Cannon (1941), and several studies have shown the release/participation of inflammatory chemical mediators in this endemic disease (Maegraith & Onabanjo, 1970; Aviado & Sadavongvivad, 1970; Martins et al., 1986; Cordeiro et al., 1984; Onabanjo & Maegraith, 1971; Tella & Maegraith, 1963).

The occurrence of pulmonary edema in man induced by *Plasmodium falciparum* was first described by Falconer in 1919, and later confirmed by other groups (Bergin, 1967; Brooks et al., 1968; Hall, 1977; Fein et al., 1978). Malarial pneumopathy is associated with high mortality rates, and has not yet been well clarified in terms of physiopathological signs and symptoms despite the large number of studies on the subject (Deaton, 1970).

The objective of the present study was to determine the involvement of pharmacologically active lipid derivatives, such as prostaglandins, leucotrienes and PAF-acether in the acute inflammatory process occurring in the pulmonary pathology of malaria in mice.

MATERIALS AND METHODS

Male mice of the Swiss-55 strain weighing 18-20g were used.

The animals were infected by ip injection of 10^7 red blood cells parasitized with a mouse-adapted strain of *P. berghei* described by Vincke & Lips (1948). Previous experiments had established that this particular procedure resulted in the death of 100% of the animals by the seventh day of infection. The parasitemia was determined in thin blood films stained with May-Grunwald-Giemsa and examined under the oil immersion objective.

Each day normal and infected animals were anaesthetized with Pentobarbital (30mg/kg i.p.) and then killed by ex-sanguination. The lungs were dissected free from the trachea and weighed. Significant changes in lung wet weight/body weight in relation to uninfected mice were considered to reflect pulmonary edema (Staub, 1974).

This work was conducted using four experimental groups:

Group 1 – Normal animals injected with saline, i.p.; **Group 2** – Animals inoculated with *P. berghei* only; **Group 3** – Animals injected with antagonists only (Indomethacin in three separate doses of 8mg/kg at 8 h intervals, i.p.; or BN 52021, one dose of 10mg/kg; or Nordihydroguaiaretic acid (NDGA), one dose of 50mg/kg); **Group 4** – Animals injected with antagonists as above and inoculated with the parasite one hour after the last dose of antagonists. Additional doses of 8mg/kg of Indomethacin or 10mg/kg of BN 52021 or 50mg/kg of NDGA were given each day from the 1st to 7th (the last day of infection) day to groups 3 and 4.

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Statistical analysis was performed using the Student t-test.

Drugs used: BN 52021 (3-(1,1-dimethyl-ethyl) hexahydro-1,4,7b-tryhydroxy-8-α-methyl 9H-1,7-α-(epoxymethanol)-1H,6H-ciclopenta (c) furo (2,3-b) furo (3,2:3,4) cyclopenta (1,2-d) furan-5,9,1,2 (4H)-trione) was kindly provided by Dr. P. Braquet (IHB – IPSEN – Institut for Therapeutic Research, Le Plessis Robinson France). Indomethacin and Nordihydroguaiaretic acid were obtained from Sigma.

All other reagents and strains used were purchased from Merck, Darmstadt, Germany.

RESULTS

Figs. 1A and 1B show that pulmonary edema and parasitemia developed in parallel during the course of the infection in mice inoculated with *Plasmodium berghei.* Pulmonary edema increased progressively from the 4th day of infection, with the increase being significant from the 6th day on. A simultaneous dramatic increase in parasitized red blood cells was observed, reaching approximately 60-70% over basal values after the 6th day of disease.

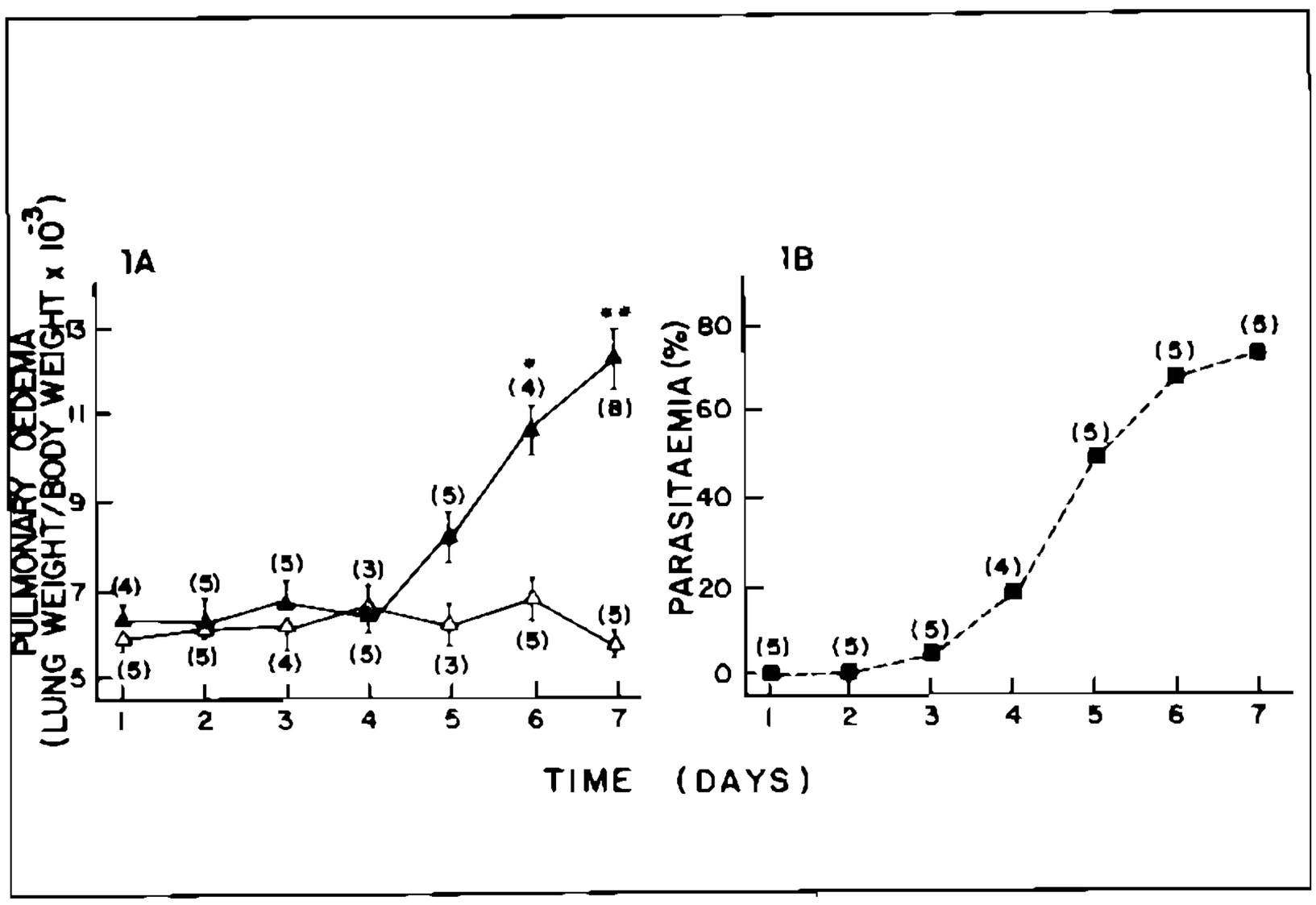

Fig. 1A: Variations of lung weight/body weight ratios (considered to reflect pulmonary oedema) on uninfected (△—△) and *P. berghei* infected mice (▲—▲) during the seven days of infection. Each value is the mean ± S.E.M. of individual determinations. Figures in parentheses refer to the number of animals tested.

*Statistically significant ($p < 0,01$), **Statistically significant ($p < 0,001$). Fig. 1B: Mean parasitemia of mice inoculated with 10^7 *Plasmodium berghei* – parasitized erythrocytes. Day 1 equal the 24 hours following inoculation. Figures in parentheses refer to number of animals tested.

Fig. 2 shows the interference of BN 52021, Nordihydroguaiaretic acid (Tappel et al., 1953) and Indomethacin with the *P. berghei* – induced pulmonary edema. Neither the PAF-acether antagonist – BN 52021 (Braquet et al., 1985) nor the dual inhibitor of cyclooxygenase and lipoxygenase-nordihydroguaiaretic acid – modifies the edema when compared with untreated – infected mice. In contrast, indomethacin significantly inhibited the development of pulmonary edema by 64, 55 and 60% on the 5th, 6th and 7th day of infection, respectively. Indomethacin, nordihydroguaiaretic acid, and BN 52021 did not change the parasitemia level of infected animals or the lung weight/body weight ratio of uninfected mice.

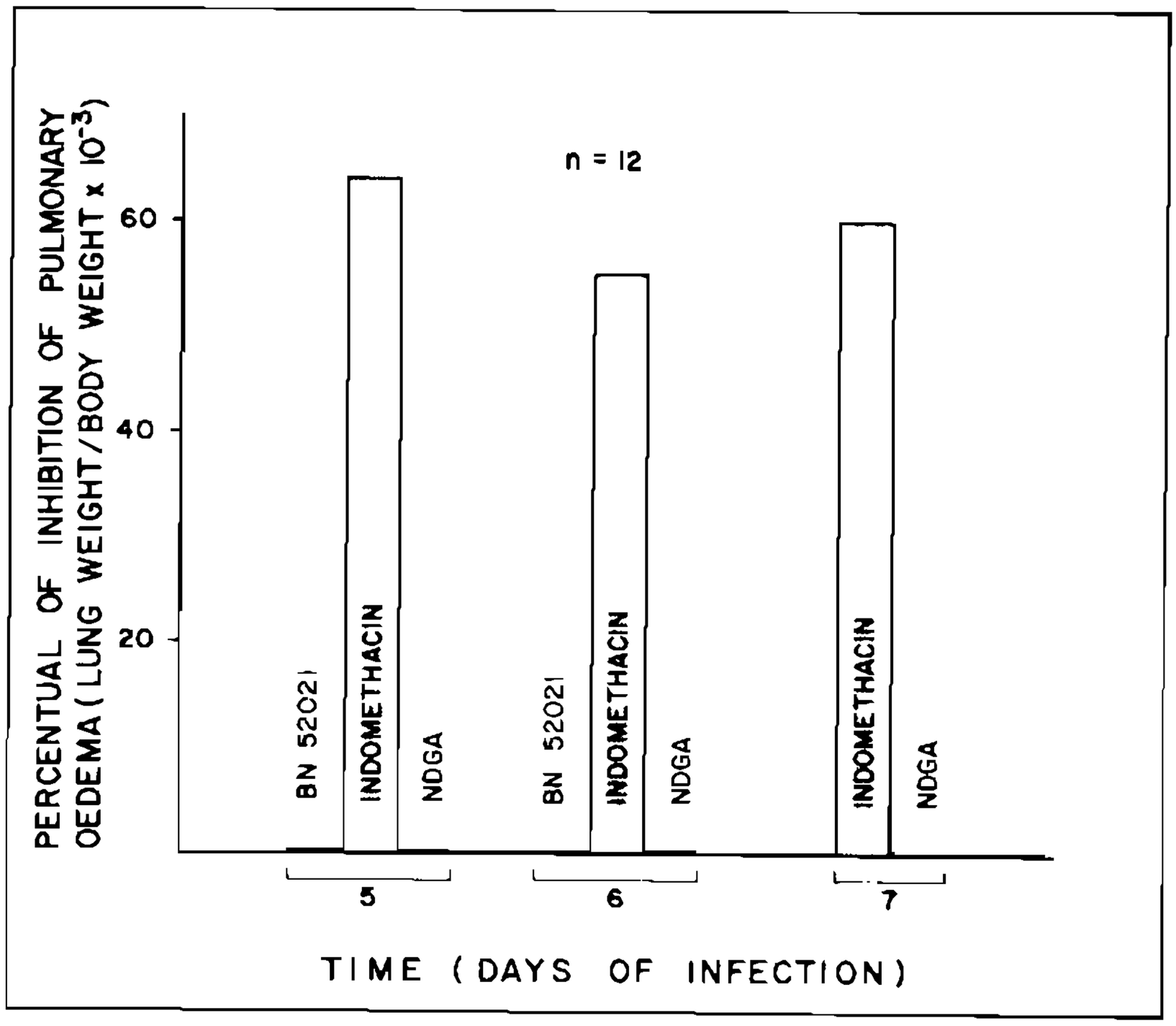

Fig. 2: Effect of BN 52021 (10 mg/kg, once daily), indomethacin (8 mg/kg, once daily) and nordihydroguaiaretic acid (NDGA) (50 mg/kg, once daily) in *Plasmodium berghei* induced pulmonary oedema observed in mice. Antagonists-treated mice showed no statistically significant differences of lung wet weight/body weight ratios from their respective untreated controls.

DISCUSSION

The present data confirm results obtained in previous studies which showed that mice infected with *Plasmodium berghei* exhibit drastic and significant pulmonary edema during the last days of infection.

In previous studies we demonstrated (data not shown) that mice infected with *P. berghei* show a clear impairment of pulmonary vascular endothelium selectivity for protein macromolecules in parallel with the development of pulmonary edema, suggesting that this phenomenon is associated with increased vascular permeability (Rangel et al., 1984). These data agree with those obtained by Weiss & Kubat (1983), who emphasized the importance of immune complexes in the potential production and/or release of inflammatory mediators. Among the main factors released in reactions of this type are vasoactive amines (histamine) and lipid derivates (prostaglandins, leukotrienes and PAF-acether).

Martins et al. (1986) observed a significant increase in histamine in total blood and plasma of mice infected with *P. berghei*. However, treatment of the animals with antagonists of H1 and H2 receptors did not modify the development of edema, suggesting that no histaminergic mechanisms are involved in this process.

In the present study, animals infected with *P. berghei* and pretreated with the specific PAF-acether inhibitor, BN 52021, showed no changes in the development of lung disease, although Voelkel et al. (1983) have shown that this lipid produces significant pulmonary edema in rodents. These preliminary results involving the participation of PAF-acether in the phenomenon will be extended using other PAF-acether antagonists and different BN 52021 concentrations.

The ineffectiveness of nordihydroguaiaretic acid in inhibiting the development of pulmonary edema suggests that leucotrienes do not participate in this phenomenon. On the other hand, the powerful inhibition of pulmonary edema caused by indomethacin strongly suggests the participation of prostaglandins. Cordeiro et al. (1983) reported increased levels of this inflammatory lipid in the central nervous system of mice infected with *P. berghei*, together with a drastic leucocytosis. It is possible that stimulated cells, especially those of the leucocytic line, release prostaglandin-like substance which may produce permeability changes and leakage of serum proteins into the extravascular spaces of the lungs. A previous study by our group (Cordeiro et

al., 1984) showed that mice infected with *P. berghei* and treated with phenoxybenzamine, an adrenergic blocker, were significantly protected against pulmonary edema suggesting the involvement of catecholamines in this phenomenon. The possibility that sympathetic mediators act through prostaglandin release, as is the case for isolated cat spleens (Ferreira et al., 1973) cannot be ruled out.

The present data suggest that there is a potential participation of prostaglandins in malarial pulmonary edema and widen the spectrum of the study of this tropical disease using a pharmacological approach.

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