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EFFECT OF PIROXICAM, METAMIZOL, AND S-ADENOSYLMETHIONINE IN A MURINE MODEL OF EXPERIMENTAL TRICHOMONIASIS

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Summary:

Biological effects of piroxicam, metamizol, and S-adenosylmethionine (S-AMET) have been tested in NMRI mice infected intraperitoneally with *Trichomonas vaginalis*. An intraperitoneal treatment during ten preinfection days with piroxicam (10 mg/Kg/day), or metamizol (275 mg/Kg/day), but not with S-AMET (17 mg/Kg/day) induced a significant decrease of abdominal lesions and mortality, assessed by means of a pathogenicity index. The trichomonicidal activity of piroxicam, metamizol, and S-AMET was tested *in vitro* at the concentration of 300 µM, but found ineffective. These assays have shown the usefulness of the experimental trichomoniasis model for the study of the immunomodulating activity of synthetic drugs.

KEY WORDS : *Trichomonas vaginalis*, piroxicam, metamizol, S-adenosylmethionine.

Résumé : EFFETS PHARMACOLOGIQUES DU PIROXICAM, DU MÉTAMIZOLE ET DE LA S-ADÉNOSYLMÉTHIONINE DANS UN MODÈLE DE TRICHOMONASE MURINE

Les effets pharmacologiques du piroxicam, du métamizol, et de la S-adénosylméthionine (S-AMET) sur la réponse immunitaire ont été examinés dans un modèle de pathogénie expérimentale de Trichomonas vaginalis *(10^7 trophozoites inoculés par voie intrapéritonéale) chez la souris NMRI, par l'étude des changements histopathologiques sur les organes abdominaux et de la mortalité. Un traitement préinfection par voie intrapéritonéale pendant dix jours avec le piroxicam (10 mg/Kg/jour) ou le métamizole (275 mg/Kg/jour) induit une diminution significative des lésions abdominales et de la mortalité. La S-AMET (17 mg/Kg/jour) ne produit pas cet effet. L'activité trichomonacide du piroxicam, du métamizole et de la S-AMET (300 µM) a été examinée* in vitro, *mais ces médicaments ont été trouvés inactifs. Ces essais montrent l'intérêt de ce modèle expérimental de trichomonase pour l'étude de l'activité immunomodulatrice des médicaments.*

MOTS CLÉS : Trichomonas vaginalis, *piroxicam, métamizole, S-adénosylméthionine.*

Approximately 180 million women worldwide are infected with *Trichomonas vaginalis* every year. It is the most common cause of vaginitis and it is considered to be the most prevalent nonviral sexually transmitted disease agent (Heine & McGregor, 1993). This infection has been linked to various additional pathologic manifestations, including cervical neoplasia, atypical pelvic inflammatory disease, and tubal infertility. Actually, little is known about the possible mechanisms of pathogenesis of *T. vaginalis* infection. Among various animal models have been proposed for such a study, the murine intraperitoneal infection has been considered the most adequate (Teras & Roigas, 1966; Cavier *et al.*, 1972; Kulda, 1990). In our experience, the intraperitoneal inoculation of *T. vaginalis* into mice causes fibrinopurulent peritonitis with abscesses and necrotic foci in abdominal organs (especially pancreatic and hepatic) or phlegmonous inflammatory changes and production of ascitic fluid whose extent is proportional to the level of virulence of the inoculated strain (Nogal-Ruiz *et al.*, 1997). This *T. vaginalis* infection is also an experimental model to test the immunomodulating effect of drugs on the host immune response (Nogal-Ruiz *et al.*, 2003a). Besides, we have studied the effect of different drugs (immunosuppressors and immunostimulants) in this model (Nogal-Ruiz *et al.*, 2003b).

In this paper, we have assayed the potential immunomodulating effect of three drugs with different properties on the pathogenicity of *T. vaginalis* in our model of experimental trichomoniasis: (1) piroxicam as a nonsteroidal antiinflammatory drug (NSAID) that suppresses several inflammatory processes; (2) metamizol, a pyrazolone derivative, with analgesic, antipyretic and weak antiinflammatory properties (Levy *et al.*, 1995); and (3) S-adenosylmethionine (S-AMET), which is involved in the synthesis of polyamines, the methylation of lipids, nucleic acids, and proteins (Tabor & Tabor, 1984), and the transsulphuration pathway (Walker & Barrett, 1997).

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MATERIALS AND METHODS

Parasites and culture

T. vaginalis strains C1-NIH (reference n° 30001) and JH31A n° 4 (reference n° 30326) from the American Type Culture Collection (ATCC, Maryland, USA) were grown at 37° C with 5 % CO_2 in Diamond medium (Trypticase-Yeast extract-Maltose) pH 6.2, supplemented with 10 % of heat inactivated equine serum, penicillin G (100 IU/ml), and streptomycin (100 µg/ml). Parasites are maintained in our laboratory by cryopreservation in liquid nitrogen.

Animals

NMRI female mice (25 g) were purchased from Charles River (Barcelona, Spain). They were housed in our laboratory in a temperature (22° C) and humidity controlled environment in plastic cages with wood shaving bedding under 12 h light-dark cycle. Throughout the experiments, mice were given to water and Panlab chow (Barcelona, Spain), ad libitum.

Drugs

Piroxicam (MW: 331,4; Feldene®, Pfizer S.A., Madrid, Spain), metamizol (MW: 311,4; Nolotil®, Europharma S.A., Madrid, Spain), and S-AMET (MW: 398,4; S.Amet®, Europharma S.A., Madrid, Spain) were purchased and suspended in sterile distilled water.

In vivo pharmacological effect

The first series of experiences was performed in order to determine the pharmacological effect of such drugs in a model of experimental trichomoniasis. Three experimental groups (n = 10) were treated ip, during 10 days before infection, with 10 preinfection doses of piroxicam (10 mg/kg/day), metamizol (275 mg/kg/day), and S-AMET (17 mg/kg/day), according to doses used in scientific literature of similar assays.

For infecting experimental groups, *T. vaginalis* strain C1-NIH (reference n° 30001) were inoculated ip with a single dose of 10^7 trichomonads in logarithmic phase from axenic cultures and resuspended in fresh medium. Beside, a control group was infected, but not treated. Mice died on the infection and killed under anaesthesia at day 15 postinfection were examined at necropsy for the presence of trichomonads and for gross-pathological changes in the abdominal cavity. Pathogenicity index (PI) was determined as previously (Nogal-Ruiz *et al.*, 1997). Briefly, mortality (0-50 points), ascites (0-6 points), and the gross damage produced to the peritoneum (0-10 points), spleen/pancreas/stomach (0-12 points), and the visceral (0-10 points) and diaphragmatic liver side (0-12 points) were quantified. The PI was then calculated as the arithmetic mean of the values for each parameter (Table I). The results showed were obtained from two independent experiments.

In vitro trichomonicidal activity

The *in vitro* trichomonicidal activity of piroxicam, metamizol, and S-AMET was assayed on the *T. vaginalis* strain JH31A n° 4 (reference n° 30326). The experiments were carried out in duplicate using glass tubes, containing 100,000 trichomonads/ml in a final volume of 2 ml. The drugs to be tested were dissolved

Pathological manifestations	**Values assigned**
Mortaligy	
(Days p.i.)	
3°	50
4°	44
5°	38
Every delay day	Discount 6 points
> 10°	6
Ascites	
< 1 mL	2
1-2 mL	4
> 1 mL	6
Peritoneum	
1-3 SN	2
Multiple SN and/or N1	4
Multiple N1 and/or N2	6
MN affecting pelvis	8
MN affecting intestine and kidneys	10
Spleen/Pancreas/Stomach	
1-3 SN and/or splenomegaly	2
Multiple SN and/or N1	4
N2	6
N3 affecting more than 1 organ	8
N4	10
N5 affecting liver and intestine	12
Visceral liver side	
1-3 SN	2
N1	4
N2	6
N3	8
N4	10
Diaphragmatic liver side	
1-3 SN and/or colour changes	2
N1	4
N2	6
N3	8
N4	10
N5	12

SN: Single necrosis; N1: Necrosis of 3-5 mm diameter; N2: Necrosis of 5-8 mm; MN: Multiple Necrosis; N3: Necrosis of 8-10 mm; N4: Necrosis of 10-15 mm; N5: Necrosis of more than 15 mm of diameter affecting the most organ surface.

Table I – Rating of pathological manifestations in mice infected by the intraperitoneal route with *Trichomonas vaginalis*.

in dimethyl sulfoxide (DMSO) and added to the cultures at a concentration of 300 µM in a volume of 4 µl, after 6 h starting the culture. In each experiment there were six control tubes, containing only the solvent, and six tubes for every concentration of the drugs to be tested. 24 and 48 h after incubation at 37° C, viable organisms were counted by using the Neubauer chamber. metronidazole (MW: 171,16; Sigma-Aldrich, Madrid, Spain) was used as reference drug at concentrations of 12, 6, and 3 µM. Cytocidal and cytostatic activities were determined in relation with controls as previously reported (Herrero *et al.*, 1992). Briefly, the cytocidal (% CA) or cytostatic (% ca) activities were calculated with respect to the growth rates (GR) as follows:

$$\% \text{ CA} = [1 - (GR_{drug}/GR_{control})]\ 100$$

GR being the relation between the number of viable *T. vaginalis* at 24 or 48 h, and the number counted at 0 h. If $GR_{drug}/GR_{control} < 1$ then it calculates % CA or, on the contrary, if $GR_{drug}/GR_{control} > 1$ then it calculates % ca.

STASTISTICAL ANALYSIS

Statistical analyses were performed using the non-parametric Kruskal-Wallis H and Mann-Whitney U tests. Kruskal-Wallis one way analysis of variance was used to test for differences among more than two independent samples. Mann-Whitney U allows assume that two independent samples come from populations having the same distribution. A probability $p < 0.05$ was considered indicative of statistical significance.

RESULTS

IN VIVO PHARMACOLOGICAL EFFECT

Fig. 1 shows the pharmacological effect of metamizol, piroxicam, or S-AMET in a preinfection treatment of 10 days on the pathological manifestations (a) and pathogenicity index (PI) of *T. vaginalis* (b) in a murine intraperitoneal model.
In this experimental trichomoniasis model, the lesions with the highest score are usually those found in spleen/pancreas/stomach, while ascites renders the lowest values. Only the liver lesions in both sides (visceral and diaphragmatic) have shown a significantly decrease after treatment with metamizol, piroxicam, or S-AMET. In this case, the parameter mortality contributes to PI so much as the abdominal lesions do. Analysis of variance by Kruskal-Wallis H test indicated that the three experimental groups of treatments show statiscally different PI. Preinfection treatment with piroxicam (10 mg/kg/day), or metamizol (275 mg/kg/day), diminishes significantly the PI compared with untreated group (control). But, PI of treated group with S-AMET (17 mg/kg/day) shows not significant difference when compared to the PI of control group, according to U Mann-Whitney test.

IN VITRO TRICHOMONICIDAL ACTIVITY

Trichomonicidal activity of piroxicam, metamizol, and S-AMET were tested *in vitro* at the concentration of 300 µM, but found ineffective (Table II). Only piroxicam exhibits a weak cytostatic effect (growth inhibition) around 40 %.

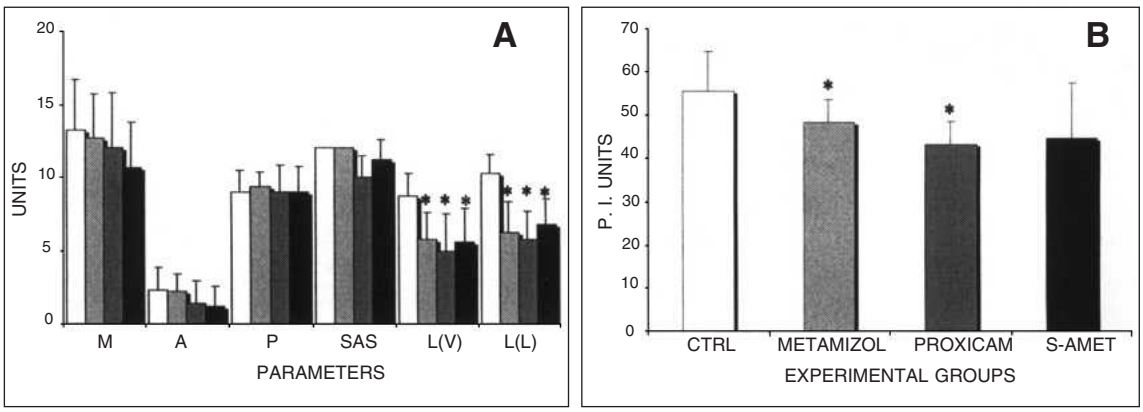

Fig. 1 – Values assigned to the pathologic manifestations (A) and pathogenicity indices (B) in mice treated with different drugs (metamizol, piroxicam, and S-adenosylmethionine) and infected with *Trichomonas vaginalis,* as expressed by the arithmetic means and the standard error (two independent experiments). The asterisk (*) shows significant differences when compared to the control group by Mann-Whitney U test ($p < 0.05$).
Abbreviations: M: mortality, A: ascites, P: peritoneum, S/P/S: spleen/pancreas/stomach, L(V): visceral liver side, L(D): diaphragmatic liver side, CTRL: control group.

Drugs	Conc (µM)	Trichomonicidal activity percentages 24 h	48 h
Piroxicam	300	42.7	39.3
Metamizol	300	3.5	2.2
S-adenosylmethionine	300	2.7	0
Metronidazole	12	94.9*	99.2*
	6	92.4*	97.5*
	3	48.1	25.7

Table II. – *In vitro* cytostatic or cytocidal (*) activity of piroxicam, metamizol, and S-adenosylmethionine against Trichomonas vaginalis. Values are averages of two independent experiments with six replicates/concentration.

DISCUSSION

In a previous paper (Nogal-Ruiz *et al.*, 2003a), we have postulate that the development of abdominal lesions in our murine model of experimental trichomoniasis is compatible with a inflammatory phenomenon regulated by neutrophils (Rein *et al.*, 1980), macrophages (Landolfo *et al.*, 1980), and lymphocytes (Th1 cells) fundamentally, with lesser contribution of Th2 cells. Therefore, this may be a suitable experimental model to evaluate the immunomodulating effect of natural or synthetic drugs.

In this paper, we have administrated several drugs to know its pharmacological effect. An intraperitoneal treatment during 10 preinfection days with piroxicam (10 mg/Kg/day), or metamizol (275 mg/Kg/day), but not with S-AMET (17 mg/Kg/day), induced a significant decrease of abdominal lesions and mortality, assessed by means of a pathogenicity index previously established.

Piroxicam as NSAID exhibits such effects mainly through inhibition of the cyclo-oxygenase pathway of arachidonate metabolism, including prostaglandins, thromboxanes and leukotrienes (Vane, 1971), as well as the inhibition of the production of inflammatory cytokines (Chang *et al.*, 1990), and different membrane-associated processes (Abramsom *et al.*, 1989). In our experimental model, the mice treated with piroxicam have shown the down-regulation of cell-mediated immunity (destruction of infected cells by NK cells, cytotoxic T cells, and activated macrophages), which impedes the formation of gross necrotic foci in abdominal organs, especially in the liver.

On the other hand, metamizol as inhibitor of the cyclo-oxygenase enzyme (Lüthy *et al.*, 1983) produces an inhibition of the IL-4 release, and PGE_2 production. Also, this drug seems to induce an increase of IL-2 and IL-10 production. Such mechanism, inhibiting pro-inflammatory cytokine release, may be a pathway to explain its anti-inflammatory effect and the concomitant decrease of abdominal lesions in our model.

By contrast, assuming that it seems well based the arguments that attribute to S-AMET immunomodulating properties: a increase of S-AMET metabolic products (intracellular glutathione and plasmatic cysteine) has been correlated with higher percentages of $CD4^+$ T-cells (Kinscherf *et al.*, 1994) and the cytotoxic T-cells activation (Multhoff *et al.*, 1996), the minimum pharmacological effect observed in our experimental trichomoniasis model is not explained well. However, Thong *et al.* (1987) have indicated the potential participation of S-AMET in metabolic activation of *T. vaginalis* that would to balance the stimulation of host immune system. Having established the relation between S-AMET and different metabolic pathways, it is possible hypothesise that potential effect of the drug in the murine model has partially been masked by the activation of such pathways and its relation with pathogenic expression.

Likewise, *in vitro* trichomonicidal activity of piroxicam, metamizol, and S-AMET have shown an ineffective result at the concentration of 300 µM. Therefore, the pharmacological effects observed *in vivo* on the pathogenicity of *T. vaginalis* are due to the modulation of host immune response.

In conclusion, this work has shown the versatility of our murine model of experimental trichomoniasis for the evaluation of immunomodulating activity of synthetic products. Besides, in our experimental conditions, piroxicam and metamizol induce a significant decrease of the PI of the mice treated in experimental groups when compared to the untreated control group, and S-AMET treatment not affects significantly the PI because of the metabolic activation of *T. vaginalis*.

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