PENTOXIFYLLINE AND ENALAPRIL AND ITS EFFECTS ON POLYCYTHEMIA INDUCED BY HYPOBARIC HYPOXIA IN MICE.

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RESUMEN: Pentoxifilina y Enalapril y sus Efectos Sobre la Policitemia Inducida por Hipoxia Hipobárica en Ratones.

Se estudió 70 ratones Swiss macho de 3-4 meses de edad, para evaluar los efectos profilácticos y terapéuticos del enalapril y la pentoxifilina en la policitemia inducida por hipoxia. Se dividió a los animales en dos grupos: grupo pentoxifilina (n=39) y grupo enalapril (n=31). Cada uno fue adicionalmente dividido en un grupo profiláctico que recibió el medicamento antes de la exposición a hipoxia hipobárica intermitente (IHH) y en un grupo terapéutico que recibió el medicamento luego de la exposición a IHH. Cada subgrupo tuvo su respectivo control. La exposición a IHH fue realizada a través de una cámara hipobárica que simulaba una altura equivalente a 4,500m, 22 horas por día. Se midió semanalmente peso y hematocrito. La evolución del peso corporal en el tiempo no mostró diferencias sustanciales entre los animales tratados y los controles en los grupos profilácticos y terapéuticos, tanto en los grupos pentoxifilina como enalapril. Hubo una disminución significativa del hematocrito a los 36 y 47 días del inicio de la profilaxis en el grupo pentoxifilina. En el grupo profiláctico enalapril los hematocritos fueron significativamente menores en los animales tratados. Concluimos que ambas drogas son efectivas cuando son usadas profilácticamente antes de la exposición a IHH. Se sugiere que las drogas podrían ejercer su efecto a través de un bloqueo parcial de la producción de eritropoyetina (EPO).

Palabras Claves: Pentoxifilina, Enalapril, Policitemia, Hipoxia, Ratones.

RÉSUMÉ: Effets de la pentoxifiline et de l'enalapril sur la polycytémie induite par hypoxie hypobare chez les souris.

L'étude a porté sur 70 souris Swiss mâles de 3-4 mois dans le but d'évaluer les effets prophylactiques et thérapeutiques de la pentoxifiline et de l'enalapril sur la polycytémie induite par hypoxie. Les souris ont été divisées en deux groupes : le groupe pentoxifiline (n=39) et le groupe enalapril (n=31), chacun d'eux subdivisé ensuite en un groupe prophylactique qui a reçu le médicament avant l'exposition à l'hypoxie hypobare intermittente (IHH), et en un groupe thérapeutique qui l'a reçu après l'exposition à l'IHH. Chaque sous-groupe a été soumis à un contrôle. L'exposition à l'IHH s'est effectuée au moyen d'une chambre hypobare simulant une altitude équivalente à 4 500 m, 22 heures par jour. Le poids et l'hématocrite ont été mesurés une fois par semaine. L'évolution dans le temps du poids corporel n'a pas montré de différences substantielles entre les souris traitées et les animaux de contrôle des groupes prophylactiques et thérapeutiques, aussi bien dans le groupe pentoxifiline que dans le groupe enalapril. Une diminution significative de l'hématocrite est apparue le 36e et le 47e jour après le début de la prophylaxie dans le groupe pentoxifiline. Dans le groupe enalapril les hématocrites ont été significativement inférieurs chez les souris traitées. Nous en concluons que les deux substances sont effectives lorsqu'elles sont utilisées de façon prophylactique avant l'exposition à l'IHH. On suggère que celles-ci pourraient exercer leur effet par blocage partiel de la production d'érythropoïétine (EPO).

Mots-clés : Pentoxifiline, Enalapril, Polycytémie, Hypoxie, Souris.

SUMMARY : To assess the prophylactic and therapeutic effects of enalapril and pentoxifylline on hypoxia-induced polycythemia in laboratory mice, 70 Swiss male mice of 3 to 4 months of age were studied. They were divided in 2 groups: pentoxifylline group (n=39) and enalapril group (n=31). Each group was further divided in a prophylactic group which received the drug before exposure to intermittent hypobaric hypoxia (IHH) and a therapeutic group which received the drug after exposure to IHH. Each subgroup had their respective control. Exposure to IHH was performed through a hypobaric chamber which simulated an altitude equivalent to 4 500 m above sea level, 22 hours a day. Weight and hematocrit were measured weekly. Time-course of body weight did not show substantial differences between treated and control animals in prophylactic and therapeutic groups in both pentoxifylline and enalapril groups. There was a significant hematocrit decrease 36 and 47 days after the beginning of prophylaxis in pentoxifylline group. In enalapril prophylactic group hematocrits were significantly lower in treated animals. We conclude that both drugs are effective when they are used prophylactically before exposure to IHH. It is suggested that the drugs might exert their effects through a partial blockade of erythropoietin (EPO) production.

Key Words: Pentoxifylline, Enalapril, Polycythemia, Hypoxia, Mice.

INTRODUCTION

Hypoxic polycythemia has traditionally been considered as an adaptive mechanism. Many studies have shown that, in humans and non-genotypically adapted mammals, chronic exposure to high altitude hypoxia may lead to excessive erythrocytosis (1-3), that, along with other clinical and laboratory features, is known as chronic mountain sickness (CMS). There is epidemiological evidence showing that aging at high altitude is a risk factor for the development of chronic mountain sickness, and it is associated with an increased blood red cell mass (4,5). It has been shown that clinical symptoms of CMS dramatically improve after descent to low altitudes or after isovolemic hemodilution (6,7). As blood volume remained unchanged in these studies, it seems that it is the decreased red blood cell mass which accounts for the clinical improvement. Therapeutic strategies aimed to decrease excessive erytrocytosis seem attractive approaches to CMS. Besides descent to low altitude and hemodilution, there have been attempts to decrease excessive erythrocytosis, in both humans and animal models

exposed to chronic hypoxia. Unfortunately, the development of an effective, acceptable therapy for CMS is still a remaining problem. One study performed in males with CMS and treated with medroxyprogesterone showed an unacceptable frequency of decreased libido (8). Likewise, pharmacologic blockade of erythropoiesis has been attempted with several drugs, including the α_1-adrenergic antagonist prazosin, the methylxanthines theophylline and pentoxifylline, and angiotensin converting enzyme inhibitors (ACEI) like enalapril (9,10).

In particular, erythrocytosis associated to renal diseases and to congestive cardiac failure has been assessed. Methylxanthines,particularly theophylline significantly reduced hemoglobin and hematocrit in post-renal transplanted patients who had developed polycythemia (11). It has also been reported that pentoxifylline reduces blood viscosity (12). In addition, enalapril has also been used in the treatment of post transplantation polycythemia with better results than those obtained with theophylline (13,14), and it also reduced the levels of EPO in patients with congestive cardiac failure (15) and the hemoglobin concentration in patients with renal diseases (16).

At our laboratory, the pharmocological blockade of the polycythemia induced by hypobaric hypoxia has been tested in two experimental studies in animals and in one study in humans. Yzaguirre showed a decrease in the hematocrit and a disminushed iron -59- labeled uptake with prazosin (9). Also, Huicho et al. tested two drugs: enalapril and theophylline. Enalapril decreased hematocrit two weeks after beginning of treatment, while theophylline had no effect in hematocrit compared with control group (10). Vargas et al. administered enalapril to 8 men and 6 postmenopause women with CMS and showed a decreased hematocrit, and an improvement of signs and symptoms of CMS (17).

CMS patients who move to lower altitudes show a substantial symptomatic relief, but there is recurrence of symptoms when they go back to high altitude. Thus the prevention and correction of polycythemia in CMS patients would be a more desirable aim than just the correction of preexisting polycythemia. We are not aware of drug studies showing a clear prophylactic effect on polycythemia. Thus we were prompted to evaluate both the prophylactic and therapeutic effect of enalapril and pentoxifylline on hypoxia-induced polycythemia in laboratory mice.

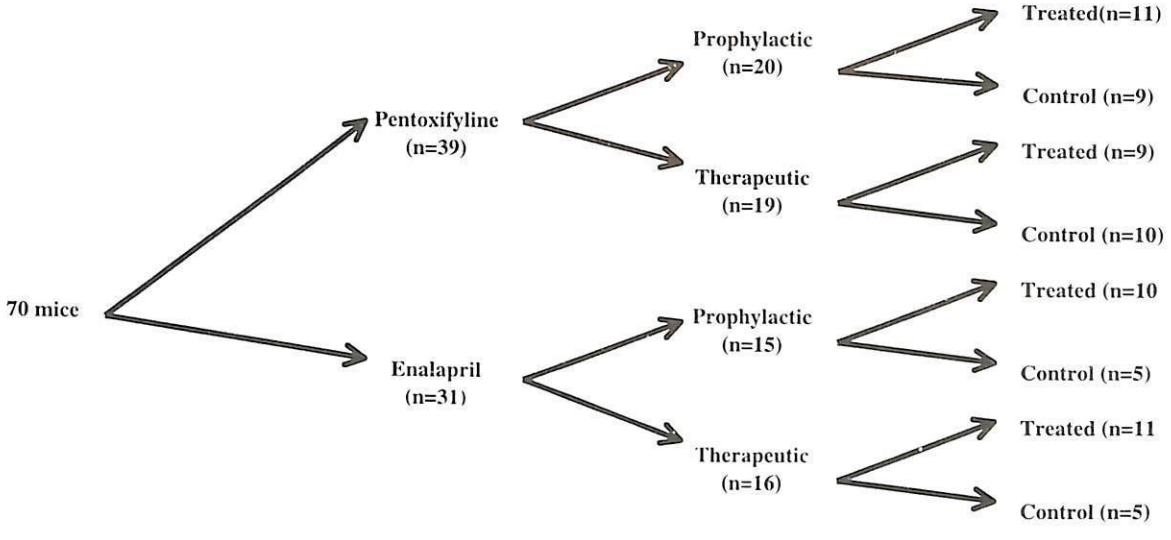
MATERIALS AND METHODS

Animals. 70 Swiss male mice of 3 to 4 months of age were divided in 2 groups:

Pentoxifylline Group (n=39). It was divided in a prophylactic group which began the drug treatment three weeks before exposure to intermittent hypobaric hypoxia (IHH) and continued until the end of the study, and a therapeutic group which began the treatment three weeks after exposure to IHH. Both groups had their respective controls.

Enalapril Group (n=31). It was divided in a prophylactic group which began the drug treatment four weeks before exposure to IHH and continued until the end of the study, and a therapeutic group which began the treatment five weeks after exposure to IHH. Both groups had their respective controls. A summary of the general, prophylactic and therapeutic experimental protocols are shown as diagram (figure 1).

Figure 1. Experimental Model



Drugs. Pentoxifylline 1,43 g/kg/day and enalapril 2,8 mg/kg/day were orally administered *ad libitum* in drinking water. Control groups received drinking water. The dose administered was calculated considering the mouse metabolic rate and on the base of the therapeutic dosis used in humans for each drug (18).

Exposure to IHH. It was performed in a hypobaric chamber equivalent to 4,500 meters above sea level, during 22 hours a day, as it was described before (19).

Measure of weight and hematocrit. They were measured weekly. Hematocrits were measured through the microhematocrit method.

Statistics. Unpaired *t* test was used for comparison of treated and control groups. $P<0,05$ was considered significant.

RESULTS.

Weight. Time-course of body weight did not show substantial differences between treated and control animals in prophylactic and therapeutic groups in both pentoxifylline and enalapril groups. (Tables 1 and 2).

Table 1. Body weight (g) in mice treated or not with pentoxifylline.

Days	0 (basal)	5	12	19	25	36	40	47
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	34,2±1,5	34,0±1,6	34,3±1,6	34,4±1,2	32,6±1,3	29,1±2,2	28,8±2,4	26,7±1,9
Treated	36,4±2,9	35,7±2,9	34,7±3,5	35,5±3,0	28,8±2,2	28,8±2,1	27,3±2,5	26,1±2,4
p	0,09	0,14	0,73	0,31	0,00002	0,08	0,14	0,7
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	35,8±3,7	34,4±3,0	34,0±2,8	31,8±3,4	30,7±2,8	29,3±1,3	28,3±1,0	27,6±0,8
Treated	38,2±3,6	34,9±3,1	35,0±3,3	34,9±3,7	31,3±3,4	26,3±3,2	26,6±3,3	29,5±3,3
p	0,21	0,74	0,49	0,09	0,22	0,67	0,49	0,77

Values are mean ± SD, SL = sea level, IHH = intermitent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 21.

Table 2. Body weight (g) in mice treated or not with enalapril.

Days	0 (basal)	8	22	27	38	51	59	67
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	31,7±1,2	34,5±0,9	35,8±1,6	35,9±1,2	30,0±0,8	27,3±2,0	26,8±2,4	28,3±1,7
Treated	34,1±2,6	36,8±3,4	37,9±3,2	37,3±3,8	30,1±3,9	27,5±2,7	28,8±4,1	29,4±3,5
p	0,038	0,08	0,14	0,35	0,94	0,91	0,29	0,47
Days	0 (basal)	8	22	29	38	44	51	59
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	31,2±1,0	28,1±1,1	29,6±0,4	30,3±0,4	29,7±0,5	26,3±1,1	31,0±1,3	32,0±1,0
Treated	30,7±0,9	29,2±1,0	30,1±2,3	30,9±2,4	27,7±2,2	29,1±2,2	33,1±2,8	31,9±2,5
p	0,65	0,35	0,54	0,47	0,022	0,008	0,064	0,92

Values are mean ± SD, SL = sea level, IHH = intermittent hypobaric hypoxia.
*Received the drug since day 1, **Received the drug since day 34.

Hematocrit. There was a significant decrease 36 and 47 days after the beginning of prophylaxis in pentoxifylline group (Table 3 and figure 2). In enalapril prophylactic group hematocrits were all lower in treated animals as compared with control ones, except at 51th day of the study (Table 4 and figure 3).

Table 3. Hematocrit (%) in mice treated or not with pentoxifylline.

Days	0 (basal)	5	12	19	25	36	40	47
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	49,8±3,3	48,1±2,9	48,0±2,1	46,7±1,8	52,1±4,1	60,7±3,7	61,3±4,5	64,8±1,3
Treated	48,2±1,9	46,8±2,9	47,3±2,1	45,2±1,2	57,0±4,3	56,4±4,9	58,2±5,2	60,4±4,5
p	0,26	0,36	0,51	0,07	0,97	0,035	0,29	0,022
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	46,7±1,3	56,0±1,3	57,1±1,6	59,6±1,9	60,1±1,4	60,0±3,1	56,4±1,4	56,9±1,4
Treated	47,8±2,2	56,7±1,7	55,9±2,0	56,9±2,0	59,8±2,9	64,2±4,5	58,5±2,5	56,4±1,2
p	0,22	0,7	0,19	0,01	0,77	0,10	0,14	0,66

Values are mean ± SD, SL = sea level, IHH = intermitent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 21.

Table 4. Hematocrit (%) in mice treated or not with enalapril.

Days	0 (basal)	8	22	27	38	51	59	67
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	47,8±1,3	47,0±0,6	48,8±1,5	47,8±2,6	62,4±1,9	59,8±2,6	62,2±1,9	61,0±2,4
Treated	46,1±1,8	44,0±2,2	43,2±1,7	43,3±1,8	59,7±2,1	57,5±2,2	57,8±4,3	55,7±3,7
p	0,47	0,003	0,0002	0,02	0,05	0,17	0,025	0,024
Days	0 (basal)	8	22	29	38	44	51	59
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	47,3±1,5	61,4±2,8	59,4±0,5	61,6±2,4	61,7±1,7	61,8±2,8	57,2±1,2	58,8±2,4
Treated	47,6±3,0	60,1±3,2	58,1±2,5	60,1±1,6	63,0±2,3	61,0±2,2	55.8±2.0	58,5±2,0
p	0,81	0,47	0,14	0,3	0,29	0,6	0,13	0,81

Values are mean ± SD, SL = sea leve, IHH = intermittent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 34.

Therapeutic groups did not show significant differences in hematocrit values.

Figure 2: Pentoxifylline
Hematocrit. Prophylactic Group

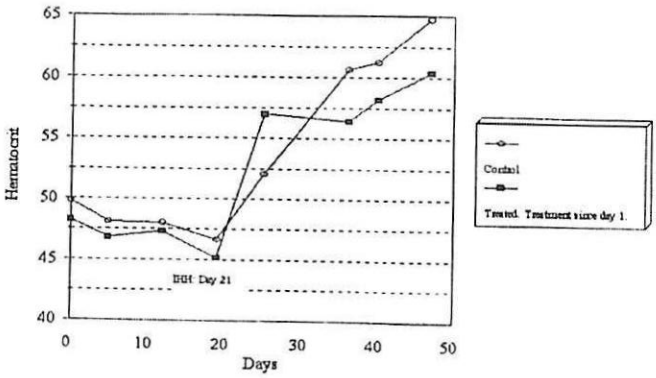
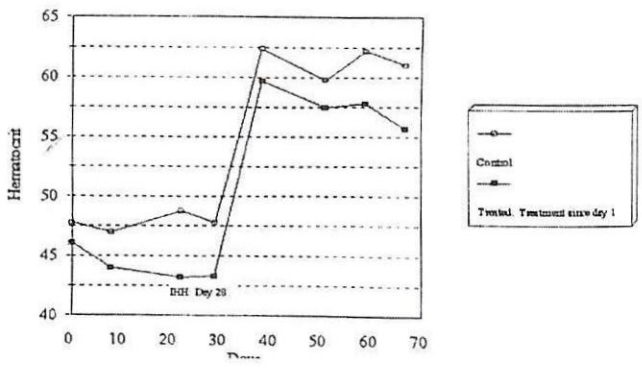


Figure 3: Enalapril
Hematocrit. Prophylactic group



DISCUSSION.

Our study showed, on a prophylactic base, a decreased level of hypoxic polycythemia in animals treated with pentoxifylline and enalapril. Therapeutic groups did not show differences in hematocrit values when compared with their respective controls. We must acknowledge that a definite conclusion on the clinical relevance of the role of these drugs in CMS associated polycythemia needs appropriate trials in humans. Also, a longer follow-up period seems necessary to ascertain whether or not the polycythemic response reaches a plateau.

Also, additional experimental studies addressed to elucidate the underlying mechanism of action of pentoxyfilline and enalapril in attenuation of hypoxic polycythemia are warranted. It has been shown, in conditions of exposure to hypobaric hypoxia, that serum erythropoietin reaches a maximum concentration within 6-24 h in rodents and within 48h in humans (20). In our study, the drugs attenuated the erythropoietic response when they were administered prior to hypoxic stress. It is a possibility that the drugs may have partially blocked erythropoietin production, well before EPO can exert their maximum effect on erythroid target cells.

Adenosine is a cellular messenger whose levels increase in hypoxia. Increased levels of adenosine lead to inforced EPO production. In our hypoxic model, it is likely that pentoxifylline may have inhibited erythropoiesis by means of a renal adenosine stimulus. We will briefly summarize some studies addressed to elucidate the cellular mechanisms of EPO production in hypoxia. We think that such an approach may help to the development of potential strategies of intervention in different phases of the erythropoietic response. In hypoxic conditions the increased degradation of ATP results in the formation of adenosine and other nucleosides (21), and also increases EPO production (22). Two types of adenosine receptor have been proposed.: A_1 receptors which inhibit adenilatecyclase, activated by nanomolar concentrations of adenosine, and A_2 receptors which stimulate adenilatecyclase, activated by micromolar concentrations of adenosine (23). Under hypoxic conditions micromolar concentrations stimulate A_2 receptors resulting in an increase of the levels of AMP_c, second messengers in the renal production of EPO (24). Pentoxifylline antagonizes the A_2 receptor, so it would produce a reduction in the levels of AMP_C and EPO.

At the target organ level, the relationship between the renin-angiotensin system and EPO would be angiotensin-II by means of a vasoconstrictive action in renal tissue thus yielding to tissue hypoxia, which is the stimulus for EPO production (25). It is known that enalapril increases renal blood flow (26), which in turn increases oxygen supply to renal tissue, and that would produce a reduction of renal EPO release. However, we must acknowledge that recent studies, especially in post renal transplanted polycythemia, point out the independence between erythropoeitin levels and the effect of ACEI on the hematocrit (14,16,27), so the exact enalapril mechanism remains unknown.

From our study it can be concluded that a prophylactic approach seems more promising for attenuation of the erythropoietic response to hypobaric hypoxia. Pharmacological intervention of different steps of erythopoietic process, at renal level and at early phases of erythroid target cells, may prove useful for identification of other useful alternatives to treatment of CMS.

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ACKNOWLEDGEMENTS

This work was partially financed by Consejo Nacional de Ciencia y Tecnologia.