PRODUCTOS NATURALES

Effects of octacosanol and triacontanol alcohols on cyclooxygenase and 5-lipoxygenase enzyme activities in vitro

Efecto sobre la actividad *in vitro* de las enzimas cicloxigenasa y 5-lipoxigenasa de los alcoholes octacosanol y el triacontanol

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ABSTRACT

Introduction: policosanol, a mixture of eight primary aliphatic alcohols purified from sugar cane wax, contains octacosanol as major component. D-002, a mixture of six primary aliphatic alcohols purified from beeswax, presents triacontanol as the main component. Although both substances are high molecular weight alcohol mixtures, they have different compositions and pharmacological effects such as their distinct effects on arachidonic acid metabolism enzymes; whereas policosanol inhibits cyclooxygenase (COX)-1, D-002 inhibits COX and 5-lipoxygenase (5-LOX) activities.
Objective: to study the effects of octacosanol and triacontanol, which are main components of policosanol and D-002, respectively on the COX and the 5-LOX enzyme in vitro activities.
Methods: triacontanol and octacosanol were suspended in a Tween-20/H2O (2 %) (0.6-5000 g/mL) vehicle. The effects of adding these alcohols on COX-1, COX-2 and 5-LOX enzymes activities were assessed in rat platelet microsomes, rat seminal vesicle microsomes and rat polymorphonuclear (PMN) preparations, respectively. Indomethacin (0.4µg/mL) was used as reference inhibitor of COX-1 and COX-2, and Lyprinol as 5-LOX inhibitor.
Results: octacosanol showed significant, marked (70% with highest concentration) (IC50=143.54 g/mL) and dose-dependent (r=0.991, p <0.001) inhibitory action on COX-1 activity. However, Triacontanol did not affect COX-1, but inhibited significantly, depending on dose (r=0.985, p <0.001) the COX-2 activity to 50 % with 1250 g/mL. In contrast, octacosanol did not change COX-2 activity. Indomethacin inhibited both COX-1 and COX-2 by 83 %. Octacosanol addition was

ineffective whereas triacontanol had significant, dose-dependent (r=0.978, p<0.001) and marked effect (79 %) on the 5-LOX activity (IC50=58.74 g/mL). Lyprinol inhibited 5-LOX by 89 %. The inhibitions induced by octacosanol and triacontanol were competitive.
Conclusions: *in vitro* addition of octacosanol and triacontanol caused differential effects on COX-1, COX-2 and 5-LOX enzyme activities. Whereas octacosanol markedly inhibited COX-1 activity and did not change those of COX-2 and 5-LO, triacontanol markedly inhibited 5-LOX activity, but had moderate effect on COX-2 and did not change COX-1 activity.

Keywords: Octacosanol, triacontanol, lipooxygenases, ciclooxygenases, policosanol, D-002.

RESUMEN

Introducción: el policosanol, mezcla de ocho alcoholes purificados de la cera de la caña de azúcar, contiene octacosanol como componente mayoritario. El D-002, mezcla de seis alcoholes alifáticos primarios purificada de la cera de abejas, presenta triacontanol como el componente mayoritario. Aunque ambas sustancias son mezclas de alcoholes de alto peso molecular, exhiben diferente composición y perfil farmacológico como son sus efectos sobre las enzimas del metabolismo del ácido araquidónico: mientras el policosanol inhibe la actividad de ciclooxigenasa (COX)-1, el D-002 inhibe las actividades de la COX y la 5-lipooxigenasa (5-LOX).
Objetivo: investigar los efectos del octacosanol y el triacontanol, principales componentes del policosanol y el D-002, respectivamente, sobre las actividades de las enzimas COX y 5-LOX *in vitro*.
Métodos: el policosanol y el triacontanol se suspendieron en vehículo *Tween-20*/H2O (2 %) (0.6-5000g/mL). Los efectos de la adición de estos alcoholes sobre las actividades de las enzimas COX-1, COX-2 y 5-LOX se evaluaron en microsomas de plaquetas de ratas, microsomas de vesículas seminales de ratas y en preparaciones de polimorfonucleares (PMN) de ratas, respectivamente. Se utilizó indometacina (0.4 µg/mL) como inhibidor de referencia de COX-1 and COX-2 y Lyprinol como inhibidor de 5-LOX.
Resultados: la adición de octacosanol inhibió la actividad de COX-1 de modo significativo, marcado (70 % con la concentración mayor) (CI_{50}=143.54 g/mL) y dependiente de la dosis (r=0.991, p <0.001). La adición de triacontanol, sin embargo, no afectó COX-1, pero inhibió de modo significativo y dependiente de la dosis (r=0.985, p <0.001) la actividad de la COX-2 hasta 50 % con 1250 g/mL. En contraste, el octacosanol no modificó la actividad de la COX-2. La indometacina inhibió COX-1 y COX-2 en un 83 %. Mientras la adición del octacosanol no fue efectiva, el triacontanol inhibió de modo significativo, dependiente de la dosis r=0.978, p <0.001) y marcadamente (79 %) la actividad de la 5-LOX (CI_{50}=58.74 g/mL). El Lyprinol inhibió la 5-LOX en un 89 %. Las inhibiciones inducidas por el octacosanol y el triacontanol fueron competitivas.
Conclusiones: la adición in vitro de octacosanol y triacontanol produjo efectos diferenciales sobre las actividades enzimáticas de COX-1, COX-2 y 5-LOX. Mientras el octacosanol inhibió marcadamente la actividad de COX-1, sin afectar COX-2 y 5-LOX; el triacontanol inhibió marcadamente 5-LOX, pero moderadamente COX-2, y no cambió la actividad de COX-1.

Palabras clave: octacosanol, triacontanol, lipooxigenasa, ciclooxigenasa, policosanol, D-002.

INTRODUCTION

Policosanol is a mixture of eight primary aliphatic alcohols purified from sugar cane (*Saccharum officinarum*, *L*) wax, whose major component is octacosanol (C28) (60 -70 %), but that also contains C24, C26, C27, C29, C30, C32 and C34 alcohols.[1] D-002 is a mixture of six primary aliphatic alcohols (C24, C26, C29, C30, C32, C34) purified from the beeswax extracted from *Apis mellifera* L, wherein triacontanol (C30) is the most abundant (25-35 %).[2]

Despite both substances are mixtures of high molecular weight alcohols, they exhibit different compositions and pharmacological effects. While policosanol exhibits mainly cholesterol-lowering and antiplatelet effects,[3-12] D-002 have been shown to produce gastro-protective[13-17] and anti-inflammatory effects.[18-22] Also, experimental and clinical studies have shown that oral treatment with policosanol or D-002 produce antioxidant effects in vivo.[23-26]

Bearing in mind the antiplatelet and anti-inflammatory effects of policosanol and D-002, respectively, their effects on key enzymes of the arachidonic acid metabolism were investigated. Results demonstrated that policosanol inhibited cyclooxygenase (COX)-1, leaving unaffected COX-2 and 5-lipoxygenase (5-LOX) enzyme activities; D-002 inhibited markedly 5-LOX enzyme activity and produced a moderate inhibition of COX activity, but its effects on both COX isoforms were not tested.[27-29]

The inhibition of COX-1 by policosanol matches well with its antiplatelet effect in vivo.[7,9-12] In turn, dual inhibition of 5-LOX and COX may explain, at least partially, the mechanism whereby D-002 produce anti-inflammatory effects.[18-22]

Taking into account the different compositions of both substances we speculated that their respective effects on COX and 5-LOX activities should be exhibited by their most abundant components: octacosanol for policosanol, triacontanol for D-002. The demonstration of this assumption should reinforce our previous data.

In light of these issues, this study investigated the effects of octacosanol and triacontanol on COX and 5-LOX enzyme activities in vitro.

METHODS

ANIMALS

Male Wistar rats (180-200g) purchased from the Centre for Laboratory Animals Production (CENPALAB, Habana, Cuba) were adapted for 7 days to laboratory conditions: controlled temperature 25 ± 2 o C, relative humidity 60 ± 5 % and 12 hours light/dark cycles. Food (rodent pellets from CENPALAB) and water were provided *at libitum*. After a 12 hour fast rats were anaesthetized under ether atmosphere and sacrificed by exsanguination.

The study was conducted in accordance with the Cuban Guidelines for the laboratory animal's care and Good Laboratory Practices. An independent ethic board for animal use approved the protocol of this study.

MATERIALS

All chemicals were purchased from *Sigma-Aldrich Co*. (*St Louis, MO*), except 2, 2 azo-bis-2-amidinopropane hydrochloride (ABAP), which was obtained from *Polyscience* (*Warington, PA*).

Ultracentrifuge was from Beckman (*Beckman Instruments, Inc. Palo Alto, CA*) and Utrospec-Plus spectrophotometer from LKB (*Pharmacia LKB Biotechnology, Uppsala, Sweden*).

ADMINISTRATION AND DOSAGE

Octacosanol and triacontanol (*Sigma*) were suspended in 2 % Tween 20/water vehicle (0.6, 4.8, 19.5, 78.1, 312.5, 1250, 2500, 5000 μg/mL). Indomethacin (Cuban Pharmaceutical Industry –QUIMEFA-) was dissolved in 5 % sodium bicarbonate and Lyprinol (*Blackmores, Australia*) in *Tween 65*/water (2 %) vehicle.

PREPARATIONS FOR ASSESSING ENZYME ACTIVITIES

Effects of octacosanol and triacontanol on COX-1, COX-2 and 5-LOX enzyme activities were assessed in rat platelet microsomes, rat seminal vesicle microsomes and rat polymorphonuclear (PMN) preparations, respectively.

Each experiment was tested in triplicate and the results averaged; the concentration that gave 50 % inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the inhibitor concentration**.**

PREPARATION OF RAT PLATELETS MICROSOMES

The effects on COX-1 activity were assessed by using fresh microsomal preparations from rat platelets. In brief, venous blood samples were collected in tubes containing 38 % sodium citrate (9:1, v/v), which were centrifuged at 160 x g for 10 min at 10ºC. The supernatant was then centrifuged again at 2100 x g for 10 min at 10 ºC. The pellet was re-suspended in 50 mol/L Tris-HCl EDTA (pH 7.4) 1 mol/L EDTA and 2 % ammonium oxalate (1/20 v/v) and centrifuged at 2100 x g for 10 min at 4 ºC. The pellet was re-suspended again in the same Tris-HCl EDTA buffer, sonicated (3 cycles of 30 sec, sub-maximal potency) and centrifuged at 15000 x g for 20 min at 4ºC. Finally, the supernatant was centrifuged at 100 000 x g for 2 hours at 4 ºC. The pellet (platelets microsomal fraction) was re-suspended in 0.05 mol/L Tris/HCl buffer (pH 8.4) containing 0.01 % Triton X-100) 1:9 (p/v) and frozen at -20 ºC until use.[30]

PREPARATION OF MICROSOMES FROM RAT SEMINAL VESICLES

The effects on COX-2 activity were assessed by using microsomal preparations from rat seminal vesicles. In brief, seminal vesicle slices were homogenized in 0.05 mol/L Tris/HCl buffer (pH 8.4) containing 0.01 % Triton X-100) 1:9 (p/v) with a potter. The homogenates were centrifuged at 15000 x g for 15 min and the supernatant was centrifuged again at 100000 x g for 1 hour, all operations being carried out at 4ºC, the pellet (microsomal fraction) was frozen at-20ºC until use.[31]

EFFECTS ON COX ENZYME ACTIVITY

COX activity was measured in accordance to Abad et al., 1994.[32] The reaction mixture contained 2 mmol/L arachidonic acid (AA); microsomal fraction (1 mg/ml); 5.8 mmol/L L-epinephrine and 0.05 mol/L Tris HCl) buffer(pH 8.4). Tubes

containing the vehicle, octacosanol or triacontanol (0.6, 1.2, 4.8, 19.5, 78.1, 312.5, 1250 and 5000 µg/mL), or indomethacin (0.4 µg/mL) (a COX inhibitor) were run in parallel. Each of these mixture reactions was pre-incubated with L-epinephrine for 4 min and then AA was added at 37 ºC. The changes of optical density (O.D.) at 480 nm were measured for 10 min in the spectrophotometer. The enzyme activity was expressed as the changes of O.D /mg of protein.

The concentration needed to produce a 50 % inhibition (IC_{50}) was calculated from the outline of the inhibition percentages as a function of the concentrations of the purported inhibitors (octacosanol or triacontanol).

The effects on COX reaction rates were assessed in front of increasing concentrations of AA (7.8, 31.2, 62.5, 125 and 250 mmol/L). Once the substrate was added, we measured the increase of O.D at 234 nm per min for 10 min in the spectrophotometer. COX activity was expressed as µmol of conjugated dienes/min/mg protein. The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control samples.

PREPARATION OF THE POLYMORPHONUCLEAR LEUKOCYTES (PMNL) CYTOSOLIC FRACTION

The effects of octacosanol and triacontanol on 5-LOX activity were assessed by using fresh preparations from the cytosolic fraction of rat blood PMNL.[30] In brief, venous blood samples were collected in tubes containing 10 % EDTA and then diluted with the same volume of saline solution (0.9 % NaCl) to 10 mL. Six (6) ml of diluted blood were gently layered over 3 ml of 14.1 % Nycodenz (density 1.077 g/ml, 20 ºC) prepared in 0.44 % NaCl and 5 mmol/L Tris HCl buffer (pH 7.2), and centrifuged at 800 x g for 30 min. at 20 °C. The mononuclear cells formed as band at the Nycodenz-plasma interface were then removed with a *Pasteur* pipette, washed with 50 mmol/L phosphate buffer/EDTA (pH 7.4) and centrifuged at 400 x g for 10 min. The pellet was washed again, re-suspended in the same buffer and used as the crude enzyme preparation.

PMNL were sonicated (3 cycles of 30 sec, sub-maximal potency) and centrifuged at 2000 x g for 10 min at 0 ºC. The supernatant was centrifuged at 100000 x g for 1 hour at 4 ºC, and then the cytosolic fraction was frozen at -20 °C up to use.

EFFECTS ON 5-LOX ENZYME ACTIVITY

The conversion of linoleic acid to 13- hydroperoxy linoleic acid was followed by the appearance of a conjugate diene at 234 nm on a UV/visible spectrophotometer.[33] In brief, the enzyme preparation (1ml) of the cytosolic fraction (50 µg of protein) dissolved in 50 mmol/L phosphate buffer/mmoL EDTA (pH 7) was pre-incubated for 5 min prior to add the substrate (250 µmol/L linoleic acid dissolved in ethanol). Parallel tubes containing the vehicle (2 % Tween-20/H_2O), octacosanol (0.6-5000 g/mL), triacontanol (0.6-5000 g/mL) and Lyprinol (50 g/mL) were run. Once the substrate was added, the increase of absorbance at 234 nm was measured every min for 10 min in the spectrophotometer. The enzyme activity was expressed as mol of conjugated dienes/min/mg protein.

The initial reaction rate was determined from the slope of the straight line portion of the curve and the percentage inhibition of the enzyme activity was calculated by comparing with the control samples.

The effects of octacosanol and triacontanol on the initial rate of 5-LOX reaction (Vmax) were assessed with increasing concentrations of linoleic acid (7.8, 31.2, 62.5, 125 and 250 mmol/L).

DETERMINATION OF PROTEIN

The determination of protein was measured in accordance to modified Lowry method by Markwell et al., 1987.[34] Samples (10 μL) were added to 1 mL stock solutions of reagent (2.0 % Na_2CO_3, 0.4 % NaOH, 0.16 % sodium tartrate, 1 % SDS and 4 % $CuSO_4$-5H $_2O$), incubated for 15 min at room temperature, added to 100 μL of Folin-Ciocalteu phenol reagent, diluted 1:1 with distilled water and incubated for 30 min at room temperature. The 660 nm absorbance readings of the samples were measured on the spectrophotomer. The protein concentrations were determined from the patron curve of bovine albumin (1 mg/mL).

STATISTICAL ANALYSES

Comparisons among the groups were done by using the *Kruskal-Wallis* test, while paired comparisons between such groups were performed with the *Mann-Whitney U* test. Statistical significance was chosen at α= 0.05. Dose-effect relationship was assessed by using a linear regression and correlation test. Regression analysis was used to calculate IC_{50} values. Data were processed with the *Statistics Software for Windows* (*Release 4.2 Stat Soft Inc, Tulsa OK, US*).

RESULTS

Tables 1 and 2 summarize the effects on COX-1 and COX-2 enzyme activities, respectively. Octacosanol addition (0.6-5000 g/mL) produced a significant, dose-dependent ($r=0.991$, $p<0.001$) and marked (70 % with 5000 g/mL) inhibition of COX-1 activity, with an IC_{50}=143.54 g/mL. Triacontanol addition in the same concentration range (0.6-5000 g/mL), however, did not modify COX-1 activity. In turn, the addition of triacontanol (0.6-5000 ?g/mL) inhibited significantly and dose-dependently ($r=0.985$, $p<0.001$) COX-2 activity to about 50 % with 1250 g/mL (IC_{50}=1250 g/mL); while octacosanol (0.6-5000 g/mL) unchanged COX-2 activity. Indomethacin (0.4 μg/mL), produced a significant, marked and similar inhibition (83 %) of both COX-1 and COX-2 enzyme activities.

Table 1. Effects on COX-1 enzyme activity on rat platelets microsomes

Concentrations (µg/ml)	Enzyme activity (□OD/min/mg protein)	Inhibition (%)	Enzyme activity (□OD/min/mg protein)	Inhibition (%)
Control	0,352 ± 0,01	-	0,352 ± 0,01	-
Octacosanol			Triacontanol	
0,6	0,320 ± 0,03	9	0,347 ± 0,01	1
1,2	0,283 ± 0,01*	20	0,347 ± 0,01	1
4,8	0,267 ± 0,01*	24	0,347 ± 0,01	1
19,5	0,220 ± 0,01*	37	0,346 ± 0,01	2
78,1	0,184 ± 0,01*	48	0,346 ± 0,01	2
312,5	0,145 ± 0,01*	59	0,346 ± 0,01	2
1250	0,125 ± 0,01*	64	0,346 ± 0,01	2
5000	0,106 ± 0,01*	70	0,346 ± 0,01	2
Indomethacine (0,4)	0,059 ± 0,03**	83	0,059 ± 0,03**	83

(Mean ± SD) *p<0,05, **p<0,01 Comparison with the control (Mann Whitney U test)

Table 2. Effects on COX-2 enzyme activity of rat seminal vesicle microsomes

Concentrations (µg/ml)	Enzyme activity (□OD/min/mg protein)	Inhibition (%)	Enzyme activity (□OD/min/mg protein)	Inhibition (%)
Control	0,352 ± 0,01	-	0,352 ± 0,01	-
Octacosanol			Triacontanol	
0,6	0,347 ± 0,01	1	0,326 ± 0,03	7
1,2	0,347 ± 0,01	1	0,292 ± 0,01*	17
4,8	0,347 ± 0,01	1	0,274 ± 0,01*	22
19,5	0,347 ± 0,01	1	0,247 ± 0,01*	29
78,1	0,347 ± 0,01	1	0,219 ± 0,01*	37
312,5	0,347 ± 0,01	1	0,193 ± 0,01*	45
1250	0,347 ± 0,01	1	0,173 ± 0,01*	50
5000	0.347 ± 0,01	1	0,170 ± 0,01*	51
Indomethacine (0,4)	0,059 ± 0,03**	83	0,059 ± 0,03**	83

(Mean ± SD) *p<0.05, **p<0.01 Comparison with the control (Mann Whitney U test)

Octacosanol-induced and triacontanol-induced inhibitions of COX-1 and COX-2 activities, respectively, modified both kinetic parameters (Vmax and Km) (figures 1 and 2, Lineweaver-Burk plots). These inhibitions, therefore, were uncompetitive.

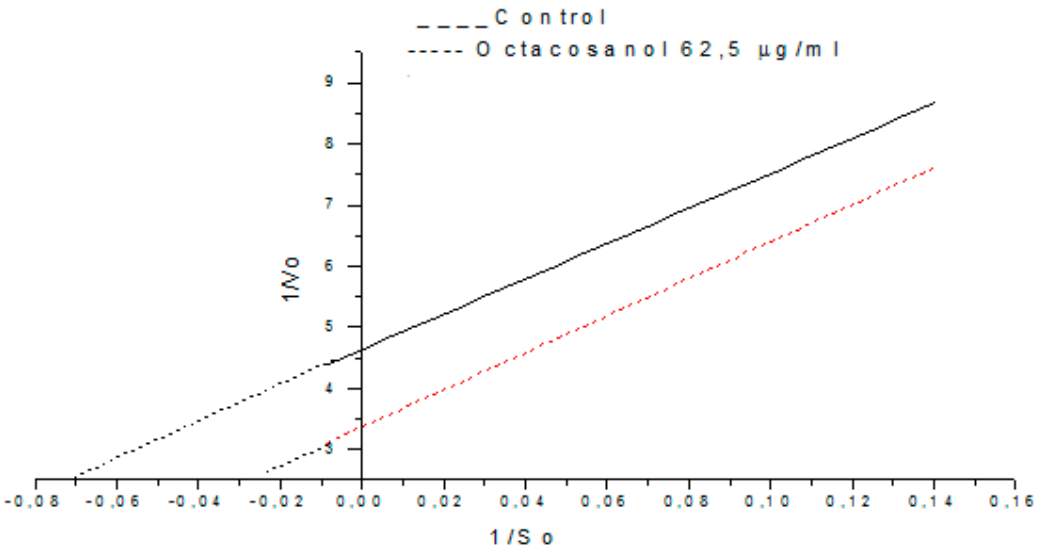

Fig. 1. Lineweaver-Burk plot (1/v0 versus1/[S]0), of the effect of Octacosanol (62.5 µg/ml) on the initial rate of the enzyme reaction measured in front of increasing concentrations of the substrate (arachidonic acid 7.8, 31.2, 62.5, 125 and 250 mmol/L). Octacosanol modified the values of both kinetic parameters Km (-1/Km, intercept with abscise axis) and Vmax (1/Vmax, intercept with the ordinate axis) of COX-1 enzyme activity.

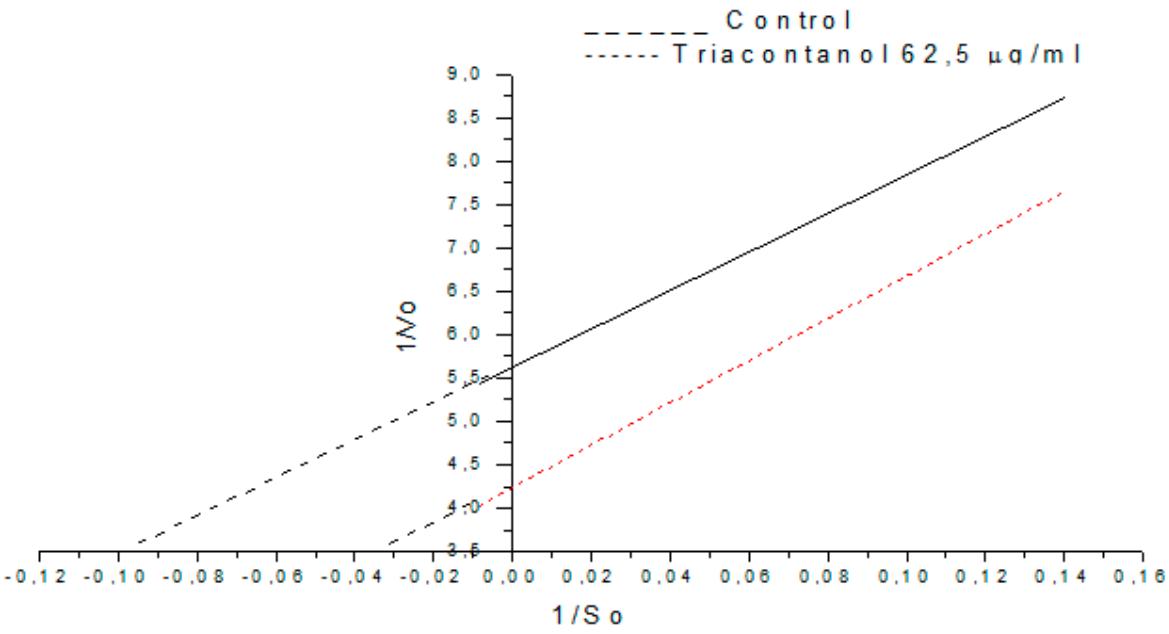

Fig. 2. Lineweaver-Burk plot (1/v0 versus1/[S]0), of the effect of Triacontanol (62.5 µg/ml) on the initial rate of the enzyme reaction measured in front of increasing concentrations of the substrate (arachidonic acid 7.8, 31.2, 62.5, 125 and 250 mmol/L). Triacontanol modified the values of both kinetic parameters Km (-1/Km, intercept with abscise axis) and Vmax (1/Vmax, intercept with the ordinate axis) of COX-2 enzyme activity.

Table 3 lists the effects on 5-LOX activity. While octacosanol addition was ineffective, the addition of triacontanol (0.6-5000 μg/mL) inhibited significantly, dose-dependently (r=0.978, p <0.001) and markedly (79 % with 5000 μg/mL) 5-LOX activity (IC_{50}=58.74 μg/mL). Lypirinol (50 μg/mL) significantly and markedly (89 %) inhibited 5-LOX activity.

Table 3. Effects on 5-LOX enzyme activity of polymorphonuclear leukocytes cytosolic fraction

Concentrations (μg/mL)	Enzyme activity (□mol of conjugated dienes/min/mg protein)	Inhibition (%)	Enzyme activity (□mol of conjugated dienes/min/mg protein)	Inhibition (%)
Control	7,61 ± 0,14	-	7,74 ± 0,20	-
Octacosanol			Triacontanol	
0,6	7,55 ± 1,28	1	6,98 ± 1,01	9
1,2	7,55 ± 0,11	1	6,62 ± 0,57*	14
4,8	7,55 ± 0,53	1	5,96 ± 0,16*	23
19,5	7,55 ± 1,32	1	4,19 ± 1,22*	45
78,1	7,55 ± 0,05	1	3,15 ± 0,50*	59
312,5	7,55 ± 0,54	1	2,11 ± 0,22*	72
1250	7,55 ± 0,10	1	1,59 ± 0,36*	79
5000	7,55 ± 0,54	1	1,60 ± 0,37*	80
Lyprinol (50)	0,89 ± 0,01**	89	0,89 ± 0,01**	89

(Mean ± SD) *p<0,05, **p<0,01 Comparison with the control (Mann Whitney U test)

The inhibition of 5-LOX activity by triacontanol involved the modification of both kinetic parameters (Vmax and Km) (figures 3, Lineweaver-Burk plots), which indicates that such inhibition was uncompetitive.

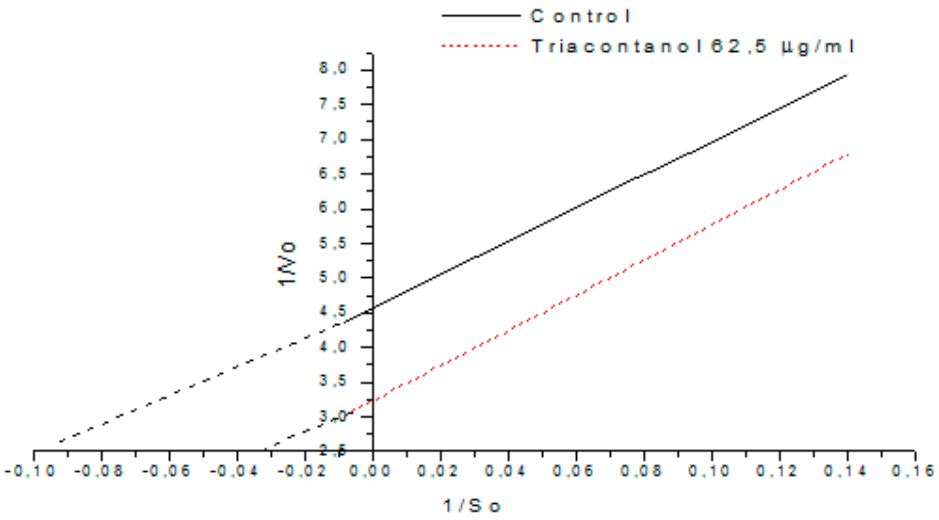

Fig. 3. Lineweaver-Burk plot ($1/v_0$ versus $1/[S]_0$), of the effect of Triacontanol (62.5 µg/mL) on the initial rate of the enzyme reaction measured in front of increasing concentrations of the substrate (linoleic acid 7.8, 31.2, 62.5, 125 and 250 mmol/L). Triacontanol modified the values of both kinetic parameters Km (-1/Km, intercept with abscise axis) and Vmax (1/Vmax, intercept with the ordinate axis) of 5-LOX enzyme activity.

DISCUSSION

The present results demonstrate that octacosanol addition (0.6-5000 g/mL) inhibits significantly, markedly (70 %) and dose-dependently COX-1 activity in fresh preparations of platelet microsomes, without modify COX-2 and 5-LOX activities in preparations of seminal vesicles microsomes and PMNL cytosolic fractions, respectively. By opposite, triacontanol (0.6-5000 g/mL) addition *in vitro* produced a significant, dose-dependent and marked (80 %) inhibition of 5-LOX activity in PMNL preparations, a moderate (50 %) inhibition of COX-2 activity in seminal vesicles microsomes, but failed to inhibit COX-1 activity.

The fact that indomethacin, a non-selective COX inhibitor,[35] and Lyprinol, 5-LOX inhibitor [36] have inhibited COX and 5-LOX activities by 83 and 89 %, respectively, confirm the validity of the assay in our laboratory conditions.

Our data agree with previous reports of the differential effects of apparently similar high molecular weight alcohols (octacosanol, triacontanol, hexacosanol) on different targets.[37-39] Besides, this study supports that the proportion of the most abundant alcohols in policosanol and D-002 (octacosanol and triacontanol, respectively) influence their pharmacological profile.

In such regard, the marked inhibition of COX-1 activity (70 %) induced with an IC_{50}= 143.54 µg/mL by octacosanol, is consistent with that reported for policosanol (78 % and IC_{50}=312.50 µg/mL)[27] and with the anti-platelet effects demonstrated for policosanol.[7-12]

Moreover, the lack of effects of octacosanol on 5-LOX activities here seen match well with the lack of effect of policosanol on this enzyme.[28]

On the other hand, D-002 has been shown to produce anti-inflammatory effects,[18-22] and to cause a dual inhibition of 5-LOX (81 %) with an IC $_{50}$ = 56.08 μg/mL and COX (57 %) activities with an IC_{50}=1408.48 g/mL.[29] In line with such data, the present study demonstrates that triacontanol inhibited 5-LOX (80 % and IC_{50} = 58.74 g/mL) and COX-2 (51 % and IC_{50}=1250 g/mL) activities, respectively. Although the differential effects of D-002 on both COX isoforms have not been described, the present data indicate that the effects of D-002 on 5-LOX and COX enzymes are coherent with those produced by its main component.

Moreover, this study demonstrates that the inhibition of COX-1 induced by octacosanol, as well as the inhibitions of 5-LOX and COX-2 induced by triacontanol modifying both the affinity for the substrate (Km) and the maximal velocity of the enzymatic reaction (Vmax), so that all these inhibitions were uncompetitive, as occurs for those reported for policosanol and D-002.[27-29]

Bearing in mind the nature of the uncompetitive it is plausible to think that octacosanol and triacontanol may interact with some site near to, but not on the active enzymatic site, in a way that prevent the normal course of product formation.

In vitro addition of octacosanol and triacontanol produced differential effects on COX-1, COX-2 and 5-LOX enzyme activities. While octacosanol inhibited markedly COX-1 activity, unaffecting COX-2 and 5-LOX; triacontanol inhibited markedly 5-LOX, moderately COX-2, and unchanged COX-1 activity.

REFERENCES BIBLIOGRAPHICAL

1. Mas R. Policosanol. Drugs of the Future. 2000;25:569 – 86.

2. Mas R. D-002. Drugs of the Future. 2001;26(8):731-44.

3. Menéndez R, Arruzazabala ML, Más R, González RM, Amor A, et al. Cholesterol-lowering effect of policosanol on rabbits with hypercholesterolemia induced by a wheat starch-casein diet. Brit J Nutr. 1997;77:923-32.

4. Setnikar I , Senin P , Rovati LC . Antiatherosclerotic efficacy of policosanol, red yeast rice extract and astaxanthin in the rabbit. Arzneimittelforschung Res. 2005;55:312-7.

5. Mas R, Castaño G, Illnait J, Fernández L, Fernández JC. Effects of policosanol in patients with type II hypercholesterolemia and additional coronary risk factors. Clin Pharmacol Ther. 1999;65:439-47.

6. Wang Y, Kuanman KE, Wang Hia L, Jiao Y, Zhao X, Sun N, et al. Efficacy and safety of policosanol and pravastatin in treatment of hyperlipidemia in Chinese patients. J New Drugs Clin Res. 2008;2:124-9.

7. Arruzazabala ML, Carbajal D, Mas R, García M, Fraga V, et al.. Effects of policosanol on platelet aggregation in rats. Thromb Res. 1993;69:321-7.

8. Scazziota A, Pons S, Altman R. Efecto del policosanol sobre la función de las plaquetas en voluntarios sanos. Rev Iberoam Trombo Hemost. 1996;9:58-62

9. Valdés S, Arruzazabala ML, Fernández L, Más R, Carbajal D, Alemán C, Molina V. Effect of policosanol on platelet aggregation in healthy volunteers. Int J Clin Pharmacol Res. 1996; XVI (2/3):67-72.

10. Arruzazabala ML, Valdés S, Más R, Fernández L, Carbajal D. Effect of policosanol succesive dose increases on platelet aggregation in healthy volunteers. Pharmacol Ress. 1996;34(5/6):181-185.

11. Arruzazabala ML, Valdés S, Mas R, Carbajal D, Molina V, et al. Comparative study of policosanol, aspirin and the combination of policosanol-aspirin on platelet aggregation in healthy volunteers. Pharmacol Res. 1997;36:293-7.

12. Arruzazabala ML, Molina V, Más R, Fernández L, Carbajal D Valdés S, Castaño G. Antiplatelet effects of policosanol 20 and 40 mg/d in healthy volunteers and dyslipidemic patients. Clin Exp. Pharmacol. Physiol. 2002;29(10):891-7.

13. Carbajal D, Molina V, Valdés S, Arruzazabala ML, Mas R. Possible cytoprotective mechanism in rats of D-002 an anti-ulcerogenic product isolated from beeswax. J Pharm. Pharmacol. 1996; 48: 858-60.

14. Carbajal D, Molina V, Noa M, Valdes S, Arruzazabala ML, Aguilar A, et al. Effects of D-002 on gastric mucus composition in ethanol induced ulcer. Pharmacol Res. 2000;42:329-32.

15. Pérez Y, Oyárzabal A, Mas R, Molina V, Jiménez S. Protective effect of D-002, a mixture of beeswax alcohols, against indomethacin-induced gastric ulcers and mechanism of action. J Nat Medicine. 2013;32(1):113-117.

16. Rodríguez I, Illnait J, Terry H, Más R, Fernandez L, Fernández JC, et al. Efectos del Abexol ® (alcoholes de la cera de abeja) sobre síntomas gastrointestinales en sujetos de edad media y avanzada. Rev CENIC Cien Biol. 2009;40(3):147-54.

17. Illnait J, Terry H, Mas R, Fernandez L, Carbajal D, et al. Effects of D-002, a product isolated from beeswax, on gastric symptoms of patients with osteoarthritis treated with piroxicam: a pilot study. J Med Food. 2005;8(1):63-8.

18. Carbajal D, Molina V, Valdés S, Arruzazabala ML, Mas R. Anti-inflammatory activity of D-002: an active product isolated from beeswax. Prostagl. Leukotr. Essent. Fatty Acids. 1998;59:235-8.

19. Ravelo Y, Molina V, Carbajal D, Arruzazabala Ml, Mas R, Oyarzábal A, et al. Effects of single oral and topical administration of D-002 (Beeswax Alcohols) on xylene-induced ear oedema in mice. LAJP. 2010;29:1451-4.

20. Ravelo Y, Molina V, Carbajal D, Fernández L, Fernández JC, Arruzazabala ML, et al. Evaluation of anti-inflammatory and antinociceptive effects of D-002 (beeswax alcohols). J Nat Med. 2011;65:330-5.

21. Ravelo Y, Molina V, Pérez Y, Oyarzábal A, Jiménez S, Mas R. Anti-oedema effects of D-002 and lyprinol on the carrageenan-induced pleurisy in rats. Int J Pharm Scienc Review Res. 2013;23(1):24-28.

22. Ravelo Y, Molina V, Oyárzabal A, Pérez Y, Noa M, Mas R, Jiménez S, Mendoza N. Effects of D-002 (beeswax alcohols) on lung leukocyte infiltration and lipid

peroxidation in rats with carrageenan-induced pleurisy. Int J Pharm Scienc Review Res. In press 2014.

23. Menéndez R, Fraga V, Amor AM, Mas R, González RM, Jiménez S. Oral administration of policosanol inhibits in vitro copper ion-induced rat lipoprotein peroxidation. Physiol Beh. 1999;67,1-7.

24. Menéndez R, Mas R, Amor AM, Mas R, González RM, Jiménez S. Effects of policosanol treatment on the susceptibility of low density lipoprotein (LDL) isolated from healthy volunteers to oxidative modification *in vitro*. Brit J Clin Pharmacol. 2000;50:255-62.

25. Menéndez R, Amor AM, González RM, Mas R, Pérez Y, Jiménez S. Inhibition of rat microsomal lipid peroxidation by the oral administration of D-002. Brazil J Med Biol Res. 2000;33:85-90.

26. Rodríguez I, Illnait J, Fernández L, Mendosa S, Más R, Fernández JC, et al. Comparative antioxidant effects of beeswax alcohols and grape seed extract in healthy persons. LAJP. 2010;29:255-62.

27. Pérez Y, Mas R, Oyarzábal A, Jiménez S, Molina V. Effects of policosanol (sugar cane wax alcohols) and D-003 (sugarcane wax acids) on cyclooxygenase (COX) enzyme activity *in vitro*. IJPSRR. 2013;19(2)4:18-23.

28. Pérez Y, Oyarzábal A, Mas R, Jiménez S, Molina V. Pérez Guerra, Y, et al. In vitro effects of policosanol (Saccharum officinarum L wax alcohols) on the 5-lipooxygenase enzyme. Rev Cubana Farm. 2014;48(1):139-145.

29. Pérez Y, Oyarzábal A, Ravelo Y, Mas R, Jiménez S, Molina V. Inhibition of ciclooxygenase and 5-lipooxygenase enzymes by D-002 (beeswax alcohols). Current Top Nutra Res. **In press** 2014.

30. Boyum A. In iodinated density gradient media. A practical approach. Ed. D. Rickwood; 1983;147-70.

31. Neeraja S, Ramakrishna B, Sreenath A S, Reddy G V, Reddy P R K, Reddanna P. Novel functional association of rat testicular membrane-associated cytosolic glutathione S transferases and cyclooxygenase *in vitro*. Asian J Androl. 2005;7(2):171-8.

32. Abad MJ, Bermejo P, Valverde S, Villar A. Anti-inflammatory activity of Hydroxyachillin a esquuiterpene lactone from Tanacetum microphyllum. Planta Med. 1994;60,228-230.

33. Tateson JE, Randall RW, Reynolds CH, Jackson Wp, Bhattacherjee P, Salmon JA , et al. Selective inhibition of arachidonate 5 –lipoxygenase by a novel acetohydroxamic acids: biochemical assessment in vitro and ex vivo. Br J Pharmacol. 1988;94:528-39.

34. Marxwell MA, Haas SM, Beiber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane lipoprotein samples. Anal Biochem. 1987;87:206-9.

35. Blanco FJ1, Guitian R,Moreno J, de Toro FJ, Galdo F. Effect of antiinflammatory drugs on COX-1 and COX-2 activity in human articular chondrocytes. J Rheumatol. 1999;26(6):1366-73.

36. Dugas B.Lyprinol inhibits LTB4 production by human monocytes. Allerg Immunol (Paris). 2000;32(7):284-9.

37. Fernández-Arche A 1, Márquez-Martín A, de la Puerta Vázquez R , Perona JS, Terencio C, Pérez-Camino C, Ruíz-Gutiérrez V. Long-chain fatty alcohols from pomace olive oil modulate the release of proinflammatory mediators. J Nutr Biochem. 2009;20(3):155-62.

38. Ohta Y, Ohashi K, Matsura T, Tokunaga K, Kitagawa A, Yamada K. Octacosanol attenuates disrupted hepatic reactive oxygen species metabolism associated with acute liver injury progression in rats intoxicated with carbon tetrachloride. J Clin Biochem Nutr. 2008;42(2):118-25.

39. Warren RP, Burger RA, Sidwell RW, Clark LL. Effect of triacontanol on numbers and functions of cells involved in inflammatory responses. Proc Soc Exp Biol Med. 1992;200(3):349-52.

Recibido: 18 de marzo de 2014
Aprobado: 19 de junio de 2014

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