

Role of Linoleic Acid Hydroperoxide Preformed by Cyclooxygenase-1 or -2 on the Regulation of Prostaglandin Formation from Arachidonic Acid by the Respective Enzyme

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Summary Linoleic acid (LA) preincubated with cyclooxygenase (COX)-1 or -2 inhibited prostaglandin (PG) formation from arachidonic acid (AA) catalyzed by the respective enzyme, but LA without the preincubation did not. 13S-Hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE) a hydroperoxy adduct of LA inhibited PG formation catalyzed by COX-1 or -2. 13S-Hydroxy-9Z,11E-octadecadienoic acid had no effect on both COX-1 and -2 activities. These results suggest that 13-HPODE which is preformed from LA by COX reaction under normal physiological conditions can be a basal suppressor of PG formation from AA.

Key Words: linoleic acid hydroperoxide, cyclooxygenase-1, cyclooxygenase-2, prostaglandin, arachidonic acid

Introduction

Prostaglandins (PGs) are critical mediators of a variety of physiological and pathophysiological processes. The initial step in the biosynthesis of these bioactive lipids is accomplished by cyclooxygenase (COX), which catalyzes the conversion of arachidonic acid (AA) to PGG₂ and H₂. Two COX isoforms have been identified and are referred to as COX-1 and COX-2 [1]. COX-1 is ubiquitous enzyme and it appears to perform many homeostatic functions [2–4]. COX-2 is generally thought to be an enzyme synthesized in response to inflammatory stimuli [2–4]. There are lots of published data concerning the differential roles of COX-1 and -2 on the physiological and pathophysiological functions, but it is unclear about differences in the endogenous regulation of these two isoforms in the body.

Up to date, it has been widely accepted that linoleic acid (LA) is a competitive inhibitor of COX-1 activity [5–7].

Ringbom *et al.* [8] have found that LA is also an inhibitor of COX-2, and the inhibitory potency on the COX-2 is more than that on the COX-1. On the other hand, many investigators [9, 10] including us [11] have shown that LA is transformed into hydroperoxides by COX reactions. Laneuville *et al.* [12] have shown that both COX-1 and -2 prepared from COS-1 cells metabolized LA, and that 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE) was the main product of both reactions. There is little information concerning a difference of potency of 13-HPODE on the COX-1 and -2 activities. Therefore, we investigated the effects of LA without or with preincubation using COX-1 or -2 on the PG formation from AA by the respective enzyme.

Materials and Methods

Materials

COX-1 (isolated from ram seminal vesicles) and COX-2 (isolated from sheep placenta) were purchased from Cayman (Ann Arbor, MI). Sodium salt of LA was purchased from Sigma (St Louis, MO). 13-HPODE and 13S-hydroxy-9Z,11E-octadecadienoic acid (13-HODE) were purchased

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Table 1. Effects of LA without or with preincubation using COX-1 or -2 on prostaglandin formation from AA catalyzed by the respective enzyme.

Drugs	COX-1 activity	COX-2 activity
	% of control	
Control	100.0 ± 9.8	100 ± 7.1
5 μM LA ^a	88.3 ± 7.6	85.0 ± 6.1
5 μM LA ^b	25.5 ± 4.9*	4.8 ± 4.9*

10 units of COX-1 or -2 were incubated with 100 μM arachidonic acid (AA) for 2 min at 37°C. (a); Linoleic acid (LA) was added to the incubation mixture at $t = 0$. (b); LA was preincubated with COX-1 or -2 for 10 min prior to the incubation with AA: Values are means ± SE (n = 5). * $p < 0.01$, compared with control

from Cascade Biochem. Limited (Berkshire, England). 9-Anthryldiazomethane (ADAM) was obtained from Funakoshi Pharmaceutical (Tokyo, Japan). All other reagents were of analytical grade.

Assay of PG formation from AA by COX-1 and -2

Ten units of COX-1 or -2 were incubated with 100 μM AA in 0.5 ml of 0.1 M Tris/HCl buffer (pH 8.0) containing hematin (0.1 μM) and phenol (2 mM) as cofactors for 2 min at 37°C. 13-HPODE, 13-HODE or LA was added in the incubation mixture at $t = 0$. In Table 1, LA was also preincubated with COX-1 or -2 for 10 min prior to the incubation with AA. After incubation, the reaction mixture was extracted with 6 vol. of n-hexane:ethyl acetate (2:1, v/v). The aqueous phase was then acidified (approximately pH 3) with 2 N HCl and extracted with 6 vol. of ethyl acetate. PGE₂, D₂ and F_{2α} in the extracted lipid were simultaneously determined by an HPLC using ADAM for derivatization, as described in our previous studies [13, 14]. Peak heights were measured for the quantification of ADAM derivatives of PGs relative to the standard derivatives prepared from authentic PGs. The recoveries of PGs during extraction were between 90% and 99%.

Statistical analysis

Results are presented as mean ± SE. Statistical significance was calculated by Student's paired *t*-test.

Results and Discussion

In the present study, when either COX-1 or -2 was incubated with AA, PGE₂ was mainly formed in both reaction mixtures (77–79%). Lesser amounts of PGD₂ and F_{2α} were also made by these two enzymes (PGD₂, 18–20%; PGF_{2α}, 3%). It has been reported that indomethacin inhibits both COX-1 and -2 activities, but NS-398 selectively inhibits COX-2 activity [15]. Our previous study [16] utilizing indomethacin and NS-398 has demonstrated that PGE₂

formations by the present assay conditions exactly reflect the activities of COX-1 and -2. Therefore, we evaluated the COX-1 and -2 activities by measuring the amounts of PGE₂ formed from AA.

Table 1 illustrated the effects of LA without or with preincubation using COX-1 or -2 on PG formation from AA catalyzed by the respective enzyme. Co-addition of 5 μM LA in the reaction mixture did not show any significant change in PG formation catalyzed by both enzymes. This inability of 5 μM LA is explained by the previous finding by Ringbom *et al.* [8], which showed that a competitive inhibition by LA on the COX-1 and -2 activities became clear at much higher concentrations (IC₅₀: 170 μM for COX-1; 94 μM for COX-2). In contrast, 5 μM LA preincubated for 10 min with COX-1 or -2 strongly inhibited the PG formation catalyzed by the respective enzyme, and the inhibition on the COX-2 (95% inhibition) was somewhat stronger than that on the COX-1 (74% inhibition).

It has been shown that LA is transformed into 13-HPODE by COX-1 and -2, and that 13-HPODE is further decomposed to form 13-HODE by enzymatic and non-enzymatic pathways [9–12]. To investigate the inhibitory effect of preincubated LA on COX-1 or -2 activity, the effects of 13-HPODE and 13-HODE on the enzyme activities were examined (Table 2). 13-HPODE inhibited PG formation catalyzed by both COX-1 and -2, and the concentrations required to inhibit the enzyme activities by 50% (IC₅₀) were 3.1 μM for COX-1 and 1.5 μM for COX-2. So, the inhibitory effect of 13-HPODE on the PG formation catalyzed by COX-2 was about two times stronger than that by COX-1. On the other hand, 13-HODE did not affect PG formation catalyzed by both COX-1 and -2 (IC₅₀, over 10 μM). We also confirmed that LA was rapidly converted into 13-HODE by COX-2; 13-HODE (1.2 nmol) was detected by HPLC-UV apparatus, when 100 μM LA was incubated with COX-2 (10 units) for 1 min (n = 2). Therefore, these findings indicate that 13-HPODE formed from LA during preincubation with COX-1 or -2 can be a potent inhibitor of PG formation from AA by the respective enzyme.

Table 2. Inhibition of COX-1 or -2-catalyzed prostaglandin formation from AA by 13-HPODE.

	COX-1	COX-2
	(IC ₅₀ values, μM)	
13-HPODE	3.1 ± 0.3	1.5 ± 0.2
13-HODE	>10	>10

10 units of COX-1 or -2 were incubated with 13S-hydroperoxy-9Z,11E-octadecadienoic acid (13-HPODE) or 13S-hydroxy-9Z,11E-octadecadienoic acid (13-HODE) at concentrations of 0.5–10 μM in the presence of 100 μM arachidonic acid (AA) for 2 min. Values are obtained from four dose-dependent curves on the COX-1 and -2 activities.

It is unclear about the mechanism by which 13-HPODE has an inhibitory effect on the COX-1 and -2 activities, and the effect on COX-2 activity is stronger than that on COX-1 activity. But, very low concentrations of hydroperoxides are required to activate COX, and in the absence, there is very little enzyme activity [17]. On the other hand, higher concentrations of these substances can inhibit COX [18, 19]. Since a discovery of COX-1 and -2 isoforms in living cells, Chen *et al.* [20] and Liu *et al.* [21] have reported that exogenously added peroxides decrease or abolish PGG₂-derived self-activation of COX-1 and -2, with a higher efficacy on the COX-2. Therefore, it seems likely that the inhibitory effect on COX-1 and -2 activities is ascribed to lipid hydroperoxide-derived inactivation mechanism, and COX-2 is more sensitive to lipid hydroperoxides than COX-1.

Excess amounts of PGs have various toxic effects in the body. Thus, it is thought that PGs produced by COX-1 being constitutively expressed and acting for homeostatic control are maintained at extremely low levels. Ringbom *et al.* [8] have shown that a competitive inhibition by LA on the COX-1 and -2 activities become clear at much higher concentrations (IC₅₀: 170 μM for COX-1; 94 μM for COX-2) *in-vitro*. However, we can consider that basal appearance of free LA *in vivo* is continuous upon physiological stimuli; namely free LA in physiological conditions is rather high compared to free AA because of a difference of total amounts of LA and AA *in vivo* [22, 23]. Therefore, it appears that 13-HPODE preformed by constitutively expressed COX-1 might be an important homeostatic suppressor of PG formation by this enzyme. Further, its stronger inhibition on the activity of COX-2 by 13-HPODE observed in the present study led us to speculate its protective role on excess production of PGs by COX-2 in early pathophysiological states.

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