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SHORT COMMUNICATIONS

Further studies on the anti-thromboxane A_2 activity of monohydroxylated fatty acids

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Pro-aggregating activity of the mixture prostaglandin cyclic endoperoxides/thromboxane A_2 in blood platelets has been found to be inhibited by various lipoxygenase products of polyunsaturated fatty acids [1–5]. Indeed, most studies concerned human platelet aggregation induced by stable analogues of prostaglandin H_2 exhibiting a thromboxane A_2 (TXA_2) mimetic activity. Recently, we have also found a similar inhibition of TXA_2-induced vasocontracting activity of 12-HETE and 14-OH-22:6, the platelet lipoxygenase products of arachidonic and cervonic acids, respectively [6], and in a previous paper [3], we discussed the possible structure–activity relationships attached to various hydroxylated fatty acids.

In this report, the inhibition of platelet aggregation by some monohydroxylated fatty acids was investigated in the function of the stereochemistry of the hydroxyl group, and the geometry and place of the double bonds.

Methods

Twelve (R)- and 12(S)-HETE, 13(R)-hydroxy-octadeca-9Z,11E-dienoic acid (HODE) as well as 12(R)- and 12(S)-hydroxy-heptadeca-5Z,8Z,10E-trienoic acid (8Z-HHT) were chemically synthesized [7, 8]. Small amounts of 12(S)-HETE and 12(S)-hydroxy-heptadeca-5Z,8E,10E-trienoic acid (HHT) were also biosynthesized using platelets as the enzyme source [3], and 13(S)-HODE was obtained by soybean lipoxygenase treatment of linoleic acid (octadeca-9Z,12Z-dienoic acid) followed by $NaBH_4$ reduction [9]. According to the literature, the biosynthetic products were assumed to be S enantiomers.

Human platelets, prepared from volunteers who had not taken any drug for two weeks, were suspended into a Tyrode–Hepes buffer (THB) not containing albumin [10]. Platelet aggregation was studied with the turbidimetric method of Born [11], and was induced by U-46619 (range $2.8–3.5 \times 10^{-8}$ M according to the platelet batches) to reach around 70% aggregation. Each monohydroxylated fatty acid was added to the platelet suspension in ethanol (final concentration $< 0.5\%$) simultaneously with U-46619 in THB, and the dose of hydroxy derivative inducing 50% inhibition of the aggregation (IC_{50}) was determined from a dose–response curve.

Results and discussion

It is clear from Fig. 1 that the anti-aggregating effect of the monohydroxylated fatty acids relates to a dose-dependent response, as judged with 12(S)-HETE and 12(S)-HHT, and this pattern could also be observed with the other hydroxylated fatty acids tested in this set of experiments (results not shown). In our hands, only two other monohydroxy derivatives, namely 12(S)-OH-eicosa-5Z,8Z,10E-trienoic and 15(S)-OH-eicosa-8Z,11Z,13E-trienoic acids, exhibited a biphasic effect on U-46619-induced aggregation, with a potentiation below 5×10^{-7} M [12, 13]. It has been speculated that the potentiation might be related to the common 8-OH-octa-1Z,4Z,6E-trienoic backbone of both molecules.

Monohydroxylated fatty acids have been compared by the *t*-test or the Cochran's test in their potencies of inhibiting U-46619-induced aggregation on the basis of the IC_{50} observed. Results summarized in Table 1 reveal first that R enantiomers of 12-HETE and 13-HODE were significantly more active than S ones, and that the tendency was the same for 8Z-HHT ($P < 0.10$). In addition, similar observations could be made with both respective enantiomers of HHT (results not shown). This is totally new and unexpected but might be relevant to putative pathophysiological situations where R enantiomers would be produced as already described in psoriasis for 12(R)-HETE [14]. In our early work [3], we hypothesized the contrary based upon the observation that leukotriene B_4 or LTB_4 [5(S),12(R)-diHETE] was not able to inhibit platelet aggregation at the opposite of LTBx [5(S),12(S)-diHETE], the double lipoxygenase product of arachidonic acid. However, another important difference can be pointed out from the comparison of LTB_4 and LTBx. This relates to the geometry of double bonds. LTB_4 is a 6Z,8E,10E,14Z tetraene whereas the LTBx is a 6E,8Z,10E,14Z tetraene. This may well explain the different biological activity. As a matter of fact, 8Z-HHT appeared significantly more active on U-46619-induced aggregation than HHT, the only difference being the geometry of double bond at carbon number 8 (Table 1), indicating that the hydroxyl group at the α position of an E,Z conjugated double bond may be more active than when present at the α position of an E,E one. The 12-OH-10E,8E moiety in LTB_4 would then be responsible for the loss of inhibitory activity when compared to LTBx.

Finally, the inhibiting potency of hydroxylated fatty acids seems to increase with the number of double bonds. From Table 1 it can be seen that both enantiomers of 12-HETE (4 double bonds) are significantly more active than those of 13-HODE (2 double bonds). This agrees with our previous results [6] where the lipoxygenase product isomers of cervonic acid (6 double bonds) were found to be significantly more inhibitory than 12(S)-HETE.

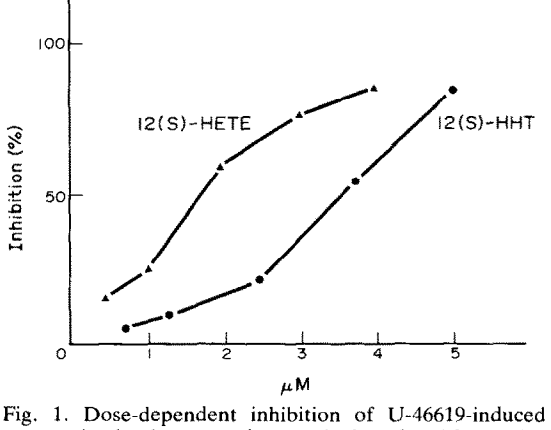

Fig. 1. Dose-dependent inhibition of U-46619-induced aggregation by the two main monohydroxylated fatty acids (12(S)-HETE and 12(S)-HHT) providing from arachidonic acid in platelets.

Table 1. IC_{50} obtained with various monohydroxylated fatty acids inhibiting U-46619-induced platelet aggregation

	IC_{50} (μM)
12(R)-HETE (N = 4)	0.75 ± 0.19 (A)
12(S)-HETE (N = 4)	1.48 ± 0.29 (B)
12(R)-8Z-HHT (N = 4)	0.91 ± 0.46 (C)
12(S)-8Z-HHT (N = 4)	1.26 ± 0.32 (D)
12(S)-HHT (N = 5)	3.46 ± 0.07 (E)
13(R)-HODE (N = 5)	2.76 ± 0.63 (F)
13(S)-HODE (N = 5)	4.73 ± 0.48 (G)

Results are mean ± SE. A vs B: $P < 0.01$; C vs D: $P < 0.10$; F vs G: $P < 0.02$; D vs E: $P < 0.05$; B vs E: $P < 0.02$; A vs F: $P < 0.05$; B vs G: $P < 0.01$.

To summarize, the present study pointed out two main features. First, the R enantiomers of the usual lipoxygenase products of polyunsaturated fatty acids may be more active than the lipoxygenase products themselves in antagonizing TXA_2-induced platelet aggregation. Second, the geometry of conjugated double bonds in the α position of the hydroxyl group influences the activity of the hydroxylated fatty acids, the E,Z geometry confering more antagonistic activity than the E,E one.

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