EFFECTS OF LIPOXYGENASE INHIBITORS ON EICOSANOID PRODUCTION IN HUMAN WHOLE BLOOD AND ISOLATED PLATELETS *IN VITRO*

Rosa Fullone[1], Melania Dovizio[1], Stefania Tacconelli[1], Mirko Zucchelli[2], Patrizia Ballerini[1], Paola Patrignani[1]

[1]Department of Neurosciences, Imaging and Clinical Sciences, Chieti - Italy, [2]Center of Research on Aging and Translational Medicine (CeSI-MeT), Chieti - Italy

Introduction: Cyclooxygenase(COX) and lipoxygenases(LOXs) are the major enzymatic pathways for the metabolism of arachidonic acid. COX generates prostanoids, including thromboxane(TX)A_2, and the minor products 15(R)-hydroxyeicosatetraenoic acid(HETE) and 15(S)-HETE. LOXs produce HETEs in the S-configuration. Platelets express only 12-LOX(p12-LOX) and COX-1. The aim of this study was to characterize the inhibitory effects of three commercially available LOX inhibitors [Esculetin(6,7-Dihydroxycoumarin), ML355(N-2-benzothiazolyl-4-[[(2-hydroxy-3-methoxyphenyl) methyl]amino]-benzenesulfonamide), CDC (Cinnamyl-3,4-dihydroxy-α-cyanocinnamate)] and the COX-1inhibitor aspirin (acetylsalicylic acid) on the generation of 12-HETEs and 15-HETEs and TXB_2in vitro in clotting human whole blood and thrombin-stimulated platelets.

Material and methods: Increasing concentration of LOX inhibitors and aspirin or vehicle(DMSO) were incubated with 250 ml-aliquots of human platelet suspension ($2x10^8$platelets/ml), stimulated with thrombin(0.2U/ml, for 30 min at 37°C). The same compound concentrations were incubated with one-milliliter aliquots of human whole blood, which was allowed to clot at 37°C for 60 min. In platelets supernatants and serum, the levels of different eicosanoids[12(S)-HETE, 12(R)-HETE, 15(S)-HETE, 15(R)-HETE and TXB_2] were assessed by chiral liquid chromatography-tandem mass spectrometry.

Results: In serum samples, the baseline values of 12(S)-HETE, 15(S)-HETE and TXB_2were 536.7±137.4, 27±7.4and 248.2±97.7ng/ml (mean±SD), respectively. 12(R)-HETE and 15(R)-HETE levels were lower than the S-enantiomers (34.1±22.54and 7.19±4.44, ng/ml, respectively, $P<0.001$). Serum 12(R)-HETE is formed non-enzymatically. CDC (up to 1mM) did not significantly affect the levels of 12(S)-HETE, 15(S)-HETE and TXB_2generated in serum. Differently, esculetin caused a concentration-dependent reduction of the serum levels of 12(S)-HETE, 12(R)-HETE, 15(S)-HETE and 15(R)-HETE while $TXB_2$was only marginally affected. ML-355was tested up to 260 μM (due to its limited solubility); the compound did not affect the generation of these eicosanoids in serum samples. Aspirin inhibited serum TXB_2levels with an IC_{50}(95% Confidence Interval, CI) of 17.47(10.70-28.34) μM without significantly affecting the levels of 12(S)-HETE and 15(S)-HETE. Aspirin reduced 15(R)-HETE levels with IC_{50} value of 11.84(5.42-26.74) μM. In thrombin-stimulated platelets, CDC inhibited 12-HETE and TXB_2generation with IC_{50} values of 12.12(7.29-19.88) μM and 8.86(4.85-15.64) μM, respectively; esculetin inhibited 12-HETE and TXB_2generation with IC_{50} values of 44.14(29.70-66.06) μM and 61.69(43.54-89.20) μM, respectively. ML-355caused only a marginal inhibitory effect (35.64±10.55%) on 12-HETE generation at 260μM.

Discussion and conclusions: Our findings evidence that many variables influence the effects of LOX inhibitors on eicosanoid production in different cellular systems. CDC, considered a potent and selective p12-LOX inhibitor, reduced both 12-HETE and TXB_2production in isolated platelets while did not affect eicosanoid levels in serum. Our data suggest an off-target effect on COX-1activity by CDC presumably due to its redox features which however may be countered by plasma antioxidants. Esculetin caused a preferential inhibition of p12-LOX activity versus COX-1activity in serum but in isolated platelets, it resulted equipotent in inhibiting the two pathways. This is possibly explained by its radical scavenging activity. ML355, considered first-in-class highly selective, orally bioavailable 12-LOX inhibitor, did not show any significant inhibitory effects in our cellular systems. In conclusion, the characterization of LOX inhibitors on eicosanoids generated in human whole blood is useful to have information on their enzyme selectivity, off-target effects and the possible influence of plasma components on their pharmacological effects.