FAILURE OF LOW-DOSE ASPIRIN TO AFFECT ENHANCED EXTRAPLATELET PROSTANOID BIOSYNTHESIS *IN VIVO* IN FAMILIAL ADENOMATOUS POLYPOSIS PATIENTS

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Introduction: Experimental evidences support a chemopreventive effect of low-dose aspirin against colorectal cancer. Clinical studies were performed to assess the effect of aspirin in Familial Adenomatous Polyposis(FAP), a dominant, autosomal disease caused by mutation in the Adenomatous Polyposis Coli (APC) gene, resulting in the development of numerous adenomatous polyps at a young age mostly in the distal colon. However, their findings are quite uncertain. Thus, we performed a biomarker study to elucidate the role of cyclooxygenase(COX)-isozymes in prostanoid biosynthesis in FAP and how aspirin acts, in this setting.

Material and methods: We performed a clinical study in FAP patients (n=11, 46.6±13.3years, mean±SD) chronically treated with low-dose enteric coated aspirin (100 mg daily for 3months) to explore the extent of acetylation of COX-1and COX-2in colorectal polyps versus normal mucosa and platelets, using a liquid chromatography/tandem mass spectrometry (LC-MS/MS), that we recently developed, and to evaluate the impact of aspirin on: 1) indirect markers of COX-1activity *ex vivo* [serum thromboxane(TX)B_2production] and *in vivo* [urinary levels of 11-dehydro-TXB_2(TXM), a marker of the systemic biosynthesis of TXB_2, mainly of platelet origin]; 2) *in vivo* systemic biosynthesis of prostaglandin(PG)E_2, mainly of COX-2origin, by assessing urinary PGEM (tetranor PGEM), 3)COX-isozymes, TX-synthase, TXA_2receptor(TP), mPGES-1and 15-PGDH expression in colorectal polyps and normal mucosa; 4)COX-1, TX-synthase and TP expression in platelets.

Results: A virtually complete suppression of platelet COX-1activity was found, as assessed by measuring the extent of reduction of serum TXB_2production, in 10 FAP patients at 24h after the last dose of aspirin (98.34±1.17%, P<0.01versus baseline). One subject was excluded from the analysis because in treatment with aspirin at the enrolment. The extent of COX-1acetylation was significantly (P<0.01) higher in platelets [mean(range): 74.21(54.55-93.45)%, n=10] versus colorectal mucosa [rectum: 51.77(27.01-69.69)%, right colon: 54.50(34.95-69.70)%, left colon: 50.01(34.41-62.80)%, n=10] and polyps [49.56(30.45-63.76)%, n=9; for one patient, no polyps were available after aspirin administration]. Baseline levels of systemic TXA_2biosynthesis were significantly higher in FAP patients [mean (range):1.87(0.74-3.33)ng/mg creatinine, n=10] than in healthy subjects [0.38(0.10-1.00)ng/mg creatinine, n=24] and in patients undergoing colorectal cancer (CRC) screening presenting adenomas/adenocarcinomas [0.36(0.17-0.79)ng/mg creatinine, n=19] assessed in one our previous study. Low-dose aspirin administration caused a significant reduction of the enhanced level of TXM in FAP (74.2±7.16%). Interestingly, the values detected after aspirin treatment were comparable to those measured in healthy subjects and in patients with sporadic adenomas treated with low-dose aspirin. Drug administration did not affect protein expression in colorectal biopsies and platelets of FAP patients. Moreover, COX-2was undetectable both in polyps and normal colorectal mucosa using both Western blot and LC-MS/MS. Baseline urinary levels of PGEM were significantly higher in FAP patients as compared to a group of healthy subjects, and they were not affected by low-dose aspirin administration.

Discussion and conclusions: In FAP patients, an increased systemic biosynthesis of TXA_2via COX-1pathway was detected. Moreover, enhanced TXA_2biosynthesis was only in part of platelet origin. Low-dose aspirin incompletely acetylated colorectal mucosal COX-1which may translate into limited reduction of TXA_2biosynthesis. Enhanced PGE_2biosynthesis *in vivo* was not affected by low-dose aspirin. In conclusion, high persistent levels of residual TXA_2biosynthesis together with enhanced PGE_2biosynthesis insensitive to low-dose aspirin may play a role in the limited capacity of low-dose aspirin to counteract adenomas growth in FAP patients.