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Commentary

COX-2 and EGFR: Partners in Crime Split by Aspirin

Paola Patrignani*, Melania Dovizio

Department of Neuroscience, Imaging and Clinical Sciences, "G. d'Annunzio" University, Center of Excellence on Aging (CeSI), 66100 Chieti, Italy

The accumulating data from randomized clinical trials provide the rational to consider the potential role of daily aspirin use in colorectal cancer (CRC) prevention, and possibly other types of cancer (Thun et al., 2012). However, some questions require to be addressed before the recommendation for the prophylactic use of aspirin in the population: (i) what is the mechanism of the anti-tumorigenic effect of aspirin?; (ii) is the chemopreventive effect dose-dependent?; (iii) may daily low-dose aspirin affect other types of cancers in addition to CRC?; (iv) does the reduced risk of colorectal cancer by daily aspirin outweigh harm from aspirin-induced bleeding?

In this issue of *EBioMedicine*, Li et al. (2015) provide novel insights into the mechanism of action of aspirin in preventing CRC. They addressed the hypothesis that the drug normalizes the expression of epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase of the ErbB family implicated in the etiology of CRC (Normanno et al., 2006). Moreover, they explored the possibility that the effect of aspirin was dependent on its capacity to modulate cyclooxygenase (COX)-2 expression. The COX enzyme catalyzes the rate-limiting oxidative and peroxidative enzymatic steps in the biosynthesis of prostanoids which affect colorectal tumorigenesis *via* a number of distinct mechanisms (Patrignani and Patrono, 1851). They studied the expression levels of colonic EGFR and COX-2, in familial adenomatous polyposis (FAP) patients, sub-grouped on pathological disease stage, *versus* normal individuals. They found that EGFR and COX-2 proteins were overexpressed as compared to controls in premalignant and malignant lesions and that the two proteins were colocalized. Mechanistic studies performed in human colonic epithelial cells as well as in murine embryonic fibroblasts clearly showed that COX-2 overexpression triggers the activation of the c-Jun-dependent transcription factor, activator protein-1 (AP-1), which binds to the *Egfr* promoter, thus leading to EGFR accumulation. Interestingly, they found that FAP patients who were classified as regular aspirin users [if they reported taking two or more standard (325 mg) aspirin tablets per week within the previous 12 months] showed lower levels of EGFR and also COX-2.

Aspirin (acetylsalicylic acid) belongs to the family of nonsteroidal anti-inflammatory drugs (NSAIDs) which share common therapeutic and side-effects through the inhibition of COX-1 and COX-2 (Patrignani and Patrono, 1851). Aspirin is the only NSAID which causes an irreversible inactivation of COX-isozymes through acetylation of strategically located serine residues, *i.e.*, Ser529 and Ser516 in human COX-1 and COX-2, respectively. The drug is 60-fold more potent to inhibit platelet COX-1 than monocyte COX-2, *in vitro* (Dovizio et al., 2013). It has a short half-life (~20 min), due to a rapid hydrolysis to salicylic acid by plasma/tissue esterases and first pass hepatic metabolism. Its metabolite salicylic acid has a longer half-life (~2–4.5 h) but is not an efficient inhibitor of COX-isozyme activity. Aspirin is administered at low-doses (75–100 mg daily) for the prevention of atherothrombosis (Patrono et al., 2008). The administration of enteric-coated aspirin 100 mg/day is associated with systemic plasma concentrations of acetylsalicylic acid and salicylic acid in the μmolar range (*i.e.*, 4 μM and 40 μM, respectively) (Patrignani et al., 2014). Daily low-dose aspirin causes a complete suppression of platelet COX-1 activity which is associated with saturation of the antiplatelet inhibitory effect (Patrono et al., 2008; Patrignani et al., 2014). Due to irreversible COX-1 inactivation and the fact that platelets have limited capacity for de novo protein synthesis, the administration of low-dose aspirin every 24 h causes a complete and persistent inhibition of COX-1 in platelets associated with a limited and rapidly reversible inhibitory effect on COX-2 expressed in nucleated cells (Patrono et al., 2008). Consequently, persistent inhibition of COX-2 activity by aspirin requires the administration of higher and repeated daily doses (Patrono et al., 2008).

Based on clinical pharmacology data, the administration of two or more standard (325 mg) aspirin tablets per week used in the study by Li et al. (2015) seems to be incompatible with an inhibitory effect of the drug on COX-2-dependent prostanoids produced by nucleated intestinal epithelial cells. In contrast, this aspirin administration schedule might have indirectly down-regulated COX-2 expression in colonic epithelial cells through the inhibition of platelet function (Thun et al., 2012). In fact, platelet-derived products may regulate COX-2 induction in colorectal epithelial cells and stromal cells (Thun et al., 2012).

Li et al. (2015) verified the possible direct effect of aspirin on COX-2-dependent EGFR induction by performing experiments using epithelial cells isolated from intestinal polyps of $APC^{Min/+}$ mice which express high levels of COX-2 and EGFR *versus* and normal epithelial cells. These cells were treated *in vitro* with either aspirin (1–4 mM) or the selective COX-2 inhibitor celecoxib (10–40 μM) and a profound down-regulation of EGFR protein levels was found only at the highest concentrations of the two drugs. Differently from the data obtained in FAP patients, where aspirin was administered *in vivo*, the drug did not affect COX-2 protein levels *in vitro*. It is noteworthy that the concentrations of aspirin and celecoxib used *in vitro* were several folds higher than those reached at therapeutic doses in humans. These results may suggest the role of COX-independent mechanism in EGFR downregulation detected *in vitro* by high concentration of the drugs, plausibly through a direct inhibitory effect on the activation of AP-1 activity (Dong et al., 1997).

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* Corresponding author at: Department of Neuroscience, Imaging and Clinical Sciences and Center of Excellence on Aging (CeSI), "G. d'Annunzio" University, Via dei Vestini, 31, 66100 Chieti, Italy.

E-mail address: ppatrignani@unich.it (P. Patrignani).

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Previous studies have shown the possible interplay between COX-2 and EGFR. Thus, COX-2 derived prostanoids cause transactivation of the EGFR kinase cascade in colon cancer cells (Pai et al., 2002) whereas the activation of EGFR can stimulate COX-2 biosynthesis (Coffey et al., 1997). The study by Li et al. (2015) reveals a novel functional association between COX-2 and EGFR during colorectal carcinogenesis, and provides the rational for aspirin as an adjuvant treatment to improve the efficacy of EGFR inhibitors in CRC. Clinical studies should be performed to verify whether the coadministration of low-dose aspirin and possibly other antiplatelet agents, such as P2Y12 antagonists, may lead to overcome the resistance to EGFR inhibitors in cancer treatment.

Conflict of Interest Statement

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