Clinical evidence of the relationship between aspirin and breast cancer risk (Review)

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Abstract. In the search for new therapeutic alternatives against cancer, either as a preventive treatment or for advanced stages, it is common to appeal to well-known drugs used for the treatment of other diseases that may interfere with the metabolic pathways involved in carcinogenesis. Non-steroidal anti-inflammatory drugs (NSAIDs) display anticancer activity through the inhibition of the COX-2 enzyme, triggering processes such as apoptosis, a reduction in proliferation and inhibition of carcinogenesis. Breast cancer is a neoplasm with the highest incidence and mortality rate among young women worldwide. Epidemiologic data have shown that drugs such as NSAIDs, particularly aspirin, reduce the relative risk of breast cancer. However, in the subgroup of responsive patients, dose, time and frequency of use have not yet been established. Here, we review the reports published during the last 10 years regarding the relationship between breast cancer and aspirin.

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1. Introduction

Worldwide, breast cancer (BC) is the leading cause of death in women due to malignant neoplasms. In Mexico, the incidence of this disease reported in 2008 was 13,939 new cases per 100,000 inhabitants (1). In two decades (1990-2010), the annual risk increased from 2 to 5%, being more frequent in the north and central part of the country (2). In 2010, the National Institute of Statistics, Geography and Informatics of Mexico (INEGI) reported that 19 of every 100 women enrolled into a medical service, presented with a mammary neoplasm, and 47% of BC deaths were in women with ages ranging from 45 to 64 (3). There are many risk factors for the development of breast cancer. For the Mexican population, the principal risk factors include reproductive factors (e.g. age at menarche and menopause, nulliparity or late pregnancy and non-breast-feeding women) and genetics (BRCA1 and BRCA2 genes) (3). In contrast, a diet rich in fruits and vegetables, low alcohol consumption and no smoking habit, significantly reduce the probability to develop this disease (2).

The treatment for BC in the primary stages (I and II) generally consists of surgical procedures, followed by chemotherapy (mainly anthracycline-type drugs) and/or radiotherapy (depending on the stage of the cancer), offering successful and even curative results in the majority of cases (4). Notwithstanding, the cardiotoxicity of anthracyclines is well documented; the secondary effects are mainly cardiomyopathy and cardiac syncope (5). Epidemiologic studies indicate that 50% of patients exposed to anthracyclines develop cardiac abnormalities after 10 to 20 years of chemotherapy; 40% of patients present with arrhythmia and 5% with heart failure (6). In addition, patients in advanced stages or with metastasis present a survival rate of 22% after 10 years of treatment (7).

In search for new alternatives to the conventional treatment and/or for the improvement of known therapies, aspirin has been one of the most attractive prevention strategy proposals. There is a long literature record, of over 20 years, concerning the study of aspirin and its effect on BC risk. An example of this

is the meta-analysis performed by Khuder and Mutgi (2001), where evidence gathered from 1980 to 2000 was analyzed (8). In this work, the results highlighted that women with estrogen receptor (ER)-positive (ER^+) tumors and who regularly consumed non-steroidal anti-inflammatory drugs (NSAIDs), presented a 22% reduction in BC risk [relative risk (RR) of 0.82; 95% confidence interval (CI), 0.75, 0.89]. In the case of cohort studies, they reported an RR of 0.78 (95% CI, 0.62-0.99), and for case-control studies an RR of 0.87 (95% CI, 0.84-0.91).

More recently, Agrawal and Fentiman (2008) reported that the use of NSAIDs decreased the risk of developing BC by 20% (9). The authors proposed that NSAIDs could be employed as coadjuvant or palliative treatment, together with hormonal therapies, in women diagnosed with BC. Nevertheless, a consensus regarding the ideal drug, the dosage, and administration time has not been reached.

Due to the controversial use of NSAIDs, particularly aspirin, and the risk of developing BC, in this review we carried out a descriptive revision of the free access epidemiologic studies from 2000 to 2012 listed in PubMed, using the following search key words: 'aspirin or acetylsalicylic acid and breast cancer', 'non-steroidal anti-inflammatory drugs', 'clinical studies' and 'epidemiological reports'. The main objective of this article was to analyze whether aspirin reduces the risk of BC in women, and the optimal conditions required to benefit from the use of this drug. In order to organize the article, it was divided into three main sections: i) epidemiologic studies, ii) aspirin as an inhibitor of metastasis and iii) aspirin, mechanism of action. Table I summarizes the articles consulted in this review.

2. Epidemiologic studies

In this section, 20 studies are reviewed, 12 of them are cohort studies and the rest correspond to case-control studies. Both categories comprise studies in which BC was assessed exclusively and cancer studies where BC information was included. These works are presented in a chronological fashion, from 2000 to 2012.

Cohort studies. Johnson and colleagues (2002) (10) carried out a cohort study in the US in order to evaluate the effect of aspirin (and/or drugs with aspirin in their formulation), NSAIDs, or drugs used in the treatment of arthritis, in a population of 27,616 postmenopausal women. Less than 4% of the women were diagnosed with BC (RR=0.80) after 6 years of monitoring. In the aspirin group, the multivariate-adjusted RR for BC was 0.71 (95% CI, 0.58-0.87), considering those women that consumed aspirin at least 6 times a week and independent of the BC stage. In comparison, for the rest of the NSAIDs under the same conditions, the adjusted RR value was 1.01 (95% CI, 0.83-1.25). One year afterwards, the Women Health Initiative (WHI) completed a study including 80,741 postmenopausal women (50-79 years of age) during 10 years (11). During this period, less than 2% of the participants were diagnosed with BC. Their results showed that the regular use of NSAIDs (for more than 5 years but less than 10 years), taking two or more tablets per week (doses over 100 mg, no further specification was given) reduced the incidence of BC by 21% (RR=0.79; 95% CI, 0.60-1.04). An increment of 7 percentage units was found in those women that had consumed NSAIDs for over 10 years (RR=0.72; 95% CI, 0.56-0.91). Aspirin reduced by 21% the risk of BC at doses higher than 100 mg/day; in comparison, ibuprofen was more effective diminishing the risk by 49%. Importantly, they found that NSAIDs had a major impact in women with characteristics such as high mass index, lack of exercise, late pregnancy, family history of BC and/or hormonal therapy. The authors did not mention whether the cancer cases were diagnosed *in situ* or whether they were invasive.

In another study carried out in the US, researchers found a protective effect of aspirin against cancer, decreasing mortality rates in both genders (12). The databases consulted were from the First and Second National Health and Nutrition Examination Study (NHANES I and II; 1971-1975 and 1976-1980, respectively) (12). However, the level of protection depended on the type of neoplasia, gender and patient age. The results showed a reduction in the mortality rate in men and women that were aspirin users (RR=0.88, 95% CI, 0.85-0.99). For women with breast and ovarian cancer, they found reduced mortality in those patients that used aspirin (RR=0.82, 95% CI, 0.49-1.36). In men, the use of aspirin reduced mortality by lung cancer (RR=0.69, 95% CI, 0.49-0.96). Nevertheless, they observed that when aspirin was ingested daily for a period longer than 5 years, the risk to develop bladder and central nervous system cancer was increased.

In California, US, an evaluation of BC risk and the use of NSAIDs was carried out in a population of 114,640 educators (13). Data were obtained from the California Teachers Study Cohort at baseline from 1995 to 1996, in a region with a high prevalence of BC (13). At the beginning of the poll, the participants were free of cancer. Six years later, 2,391 women were diagnosed with BC and were classified into two different groups: i) localized BC and ii) non-localized BC, including hormonal receptor-positive and -negative cancer. The tested drugs were aspirin, ibuprofen and acetaminophen. The last drug was included for comparative purposes, even though it is prescribed for pain relief. As a result of this cohort study, the researchers found no association in BC risk between daily and no regular use of aspirin and ibuprofen in combination with aspirin (RR=1.09, 95% CI=0.97-1.21 for NSAIDs; and RR=0.98, 95% CI=0.86-1.13 for aspirin); similar results to those previously reported for aspirin in 1996 (14). Concerning the hormone receptor groups, no statistically significant difference was observed for those patients with ER^+/progesterone receptor $(PR)^+$ breast cancer that were long-term aspirin users (RR=0.80, 95% CI, 0.62-1.03). Meanwhile, in the ER^-/PR^- BC group, the daily use of aspirin for longer than 5 years increased the risk of BC (RR=1.81, 95% CI=1.12-2.92). The same result was presented for patients with long-term daily use of ibuprofen; it was associated with an increased risk of BC (RR=1.51, 95% CI=1.17-1.95). Nevertheless, the authors could not validate their results, arguing that it could be a casualty.

Regarding the hormone receptor status, Kirsh and colleagues evaluated the effect of NSAIDs and aspirin, considering breast cancer risk according to ER^+ or ER^- status, PR^+ or PR^- status, smoking habit and arthritis, in women with incident BC (controls were randomly selected women) (15). The principal inclusion criteria for all participants were the daily use of drugs (for at least 2 months), as well as a dose of 325 mg for

the majority of aspirin users (78% of users). Users of NSAIDs presented a reduced risk of BC (OR, 0.76; 95% CI, 0.66-0.88); similar for ER^+/PR^+ (OR, 0.71; 95% CI, 0.60-0.84) and for ER^-/PR^- (OR, 0.80; 95% CI, 0.62-1.03). The authors concluded that NSAIDs are more effective for diminishing the BC risk when they are consumed for more than 7 years, regardless of whether the drug contains acetylsalicylic acid or not.

Terry and colleagues (2004) evaluated patients with hormone receptor-positive or -negative BC tumors who used NSAIDs and the risk of cancer vs. a control population free of disease; no age restriction was considered in this study (16). The research group found that consuming aspirin once a week for at least 6 months corresponded to an inverse association with BC risk (OR=0.80, 95% CI, 0.66-0.97) with respect to never-users. On the other hand, for ibuprofen users under the same conditions, no association with BC risk vs. never-users was found (OR=0.91, 95% CI, 0.71-1.16). Not surprisingly, acetaminophen was not associated with BC risk (OR=1.02, 95% CI 0.80-1.31; users vs. never-users). However, a reduction in risk was observed for users of aspirin with hormone receptor-positive BC tumors (OR=0.74, 95% CI, 0.60-0.93), vs. patients with hormone receptor-negative tumors (OR=0.97, 95% CI, 0.67-1.40). Aspirin decreased the BC risk, particularly in postmenopausal women for each experimental subgroup studied (ER^+/PR^+, ER^+/PR^-, ER^-/PR^+), except in patients with tumors negative for ER and PR, thus suggesting that it could have a chemopreventive effect in specific BC subtypes. One year afterwards, the same research group published an update of the aforementioned investigation, including 444 new cases, for a total of 7,006 patients with BC (17). The authors continued their investigation regarding the association between the regular use of NSAIDs and the risk of BC depending on hormone receptor status of the tumor. Their findings showed that the long-term use of NSAIDs diminished the risk of BC, independent of the drug and the hormone receptor status of the tumor (OR=0.78, 95% CI, 0.63-0.97 for regular use of NSAIDs; OR=0.86 for aspirin and OR=0.85 for ibuprofen).

A cohort study in Denmark with 29,470 individuals, compared cancer incidence after exposure to aspirin at three concentrations (75, 100 and 150 mg), against the expected cancer rates obtained from the country (18). During the 9 years of the study, 2,381 individuals developed cancer vs. the 2,187 expected [standardized incidence ratio (SIR)=1.09, 95% CI, 1.05-1.13]. Regarding BC, only 148 cases were registered in women and one in men (SIR=0.9, 95% CI, 0.8-1.1; SIR=0.6, 95% CI, 0.0-3.2, respectively) (18). Even though their work had several strengths, there was not enough evidence to support the use of low-dose aspirin as a chemopreventive agent in cancer. Harris and colleagues reported similar findings in the same year for aspirin at a dose of 150 mg; they did not differ in regards to the observed risk (11).

In 2008, a Danish research group published another work in which participants were selected from the database of the Danish Diet, Cancer and Health Cohort (1993-1997) (19). A group of 28,695 Danish women between 50 and 64 years of age completed a questionnaire. In this study, other aspects related to risk were evaluated, such as frequency and duration of use, in addition to the presence of hormonal receptors. At the end of the survey (7.5 years), only 847 women were diagnosed with BC. Nevertheless, their work disclosed that the use of NSAIDs, including aspirin, did not diminish the risk of BC under any of the conditions mentioned before. On the contrary, an increase in breast cancer incidence was found in NSAID users compared to non-users (RR=1.27, 95% CI, 1.10-1.45). In the case of aspirin users, an increment of BC incidence was noted (RR=1.38, 95% CI, 1.12-1.69) compared to NSAID users (not aspirin containing) (RR=1.25, 95% CI, 1.04-1.49).

Cook *et al* published similar results in 2005 (20). In this cohort study 39,876 healthy women participated and were randomly assigned to two groups: aspirin and aspirin placebo. Aspirin treatment consisted of a low concentration (100 mg), administered every other day for 10 years. For any cancer type (except non-melanoma skin cancer), the dose was not effective and no reduction in cancer risk was observed (n=2,865, RR=1.01, 95% CI, 0.94-1.08, P=0.87); in particular for BC, the values for a sample of 1,230 patients were RR=0.98 (95% CI, 0.87-1.09, P=0.68). Such a result could be related to the aspirin concentration used, since it has been observed that low doses of aspirin do not inhibit COX-2. In order to have a positive effect, aspirin must be ingested for longer periods. Likewise, the placebo group exhibited a similar behavior as the aspirin group.

In the same year, 2005, another research group found similar outcomes with almost twice as many patients (77,413 women) who were followed up for close to 10 years (21). Participants completed questionnaires from the Cancer Prevention Study II Nutrition Cohort (enrollments in 1992 or 1993; updates in 1997 and 1999). As in previous cohort study results, there was no direct association between current use of NSAIDs, with an average intake of 60 pills or more per month and BC incidence (NSAID users: RR=1.07, 95% CI, 0.96-1.21 as compared to NSAID non-users; aspirin users: RR=1.01, 95% CI, 0.84-1.20 as compared to non-users). Moreover, for those patients that reported a consumption of ≥30 pills per month for at least 5 years, the incidence of BC was not significant (RR=1.05, 95% CI, 0.88-1.26 for total NSAIDs; RR=0.88, 95% CI, 0.69-1.12 for aspirin).

Two years later, in 2007, with information obtained from the Cancer Prevention Study II Nutrition Cohort in the US, aspirin was evaluated in regards to long-term daily use and the cancer incidence at a dose of 325 mg/day in 10 different cancer types, including BC (22). Participants were diagnosed with cancer at the beginning of the enrollment in 1992-1993 and during follow-up until 2003. By 2003, 10,931 men and 7,196 women were diagnosed with a neoplasm. The incidence of cancer risk for men was associated with the use of aspirin for 5 years or longer. For women, on the contrary, the statistical difference was not significant, neither in general cancer nor in BC risk (BC: RR=0.83, 95% CI=0.63-1.10). In this study, it was demonstrated that high concentrations of aspirin were not associated with cancer risk, while previous research reported low doses and short-term aspirin administration (18,23).

In another cohort study carried out in 2007, which also evaluated aspirin and BC risk, the researchers focused on women smokers (22,507 patients) between 55 and 69 years of age (24). Notably, they found that the group of active smokers and aspirin consumers exhibited a reduction in cancer risk, differing with non-smoker or ex-smoker patients. This result could be attributed to the fact that smoking reduces the expression of COX-2 (25,26). However, in a subsequent study, no relationship between BC risk and smoking status was noted (26). Finally,

Bardia and colleagues (24) postulated that the use of aspirin could prevent the incidence of cancer by 4.7%, and mortality by 3.5 and 7.6% in coronary diseases. Even though the results were promising, the research had various weaknesses such as the lack of dose data, duration of treatment, and the criteria used for population sample selection (the research was carried out in postmenopausal and Caucasian women).

Another prospective study evaluated 35,323 women, who were enrolled in the Vitamins and Lifestyle study (VITAL, 2008), where information regarding the use of NSAIDs, lifestyle and BC risk factors was required (27). Participants that used a low dose of aspirin 4 times/week or more, for an average of 10 years, showed a decrease in BC risk (HR=0.65, 95% CI, 0.43-0.97) vs. non-users. Interestingly, frequent use and a high dose of aspirin was associated with an increase in BC risk (HR=1.26, 95% CI, 0.96-1.65). This study found a protective effect of NSAIDs for long-term users of low doses or moderate frequency of high doses, contrary to frequent users of high dose that showed an increased risk.

Gill and colleagues (28) published a cohort study in 2007 integrating three relevant facts associated with BC risk in women: hormone receptor status of BC, ethnicity and use of NSAIDs (aspirin, acetaminophen, ibuprofen, naproxen, and indomethacin). Mainly African-Americans, Caucasians, Japanese-Americans, Latinas and native Hawaiians, residents of Hawaii and California formed the multiethnic cohort even though other ethnicities were also documented. Data were collected between 1993 and 1996 by self-administered mail questionnaire; 98,920 women participated in this study. The results showed that aspirin was not associated with BC risk either for current or past users (HRR hazard rate ratio =1.05, 95% CI, 0.88-1.25; HHR=1.04, 95% CI, 0.84-1.27 for ≥6 years of use; respectively, compared with non-users); while, NSAIDs (other than aspirin) presented a protective action against the risk of BC in current users (HRR=0.70, 95% CI, 0.51-0.95, ≥6 years of use). Regarding ethnicity and hormone receptor status, the protective effect of NSAIDs (long-term use of NSAIDs other than aspirin) was found in Caucasian and African-American women, and in women with at least one positive hormone receptor. In conclusion, the results suggested a protective effect of NSAIDs against BC risk associated with time, ethnic group and hormone receptor status. The use of NSAIDs for longer than 6 years decreased the risk by 30%.

In summary, according to the aforementioned studies, there is not enough evidence yet to support aspirin as a possible drug to lower BC risk. On one hand, some of the studies suggest that aspirin could be a preventive treatment over long periods of ingestion (longer than 3 years). However, the main problem with long periods of aspirin consumption is its adverse reactions, such as peptic ulcers and gastrointestinal hemorrhage. These secondary effects are important to consider by physicians before prescribing to women with the possibility to develop BC.

Case-control studies. In 2000, a study was published in the UK with data collected from 1993 to 1995 (29). In this study 12,174 recruited patients were divided into two large groups: i) gastrointestinal cancer (esophagus, stomach, pancreas, colon and rectum) and ii) no gastrointestinal cancer (bladder, breast, lung and prostate); negative controls consisted of healthy patients (34,934 individuals) (29). The effect of aspirin and other NSAIDs was evaluated after 36 months of regular use. No important statistical difference was observed for either group (OR=0.98, 95% CI, 0.89-1.07, for patients that received at least 7 prescriptions in the 13-36 months before cancer diagnosis). Similar results were obtained by a research group at Duke University, in North Carolina, US, who carried out a case-control study between 1996 and 2000. Their results showed an inverse relationship between invasive BC risk and the use of NSAIDs (OR=0.4, 95% CI, 0.3-0.6) (30). Contrary to the other two reports, the findings of a study carried out in 2001 in Canada (3,133 patients and 3,062 controls), showed a benefit to women who had ingested NSAIDs (31). A 24% reduction in BC risk was observed (OR=0.76, 95% CI, 0.66-0.88), when NSAID intake was daily and for at least 2 months; for those patients with longer periods of intake (more than 8 years) they exhibited improvement (OR=0.68, 95% CI=0.54-0.86).

In the effort to determine an optimal dose administration of aspirin, García-Rodríguez *et al* studied 3,708 patients who were divided into three experimental groups according to aspirin concentration: 75, 150 or 300 mg (32). In this case-control study, the lower dose was more effective (the relative risk decreased 33%) in comparison to the higher doses, where no significant differences were obtained (aspirin RR=0.77; NSAIDs (non-aspirin) RR=1.00; acetaminophen RR=0.76). Moreover, they reported that there was no correlation between time of intake and benefit (32).

The majority of the epidemiologic studies performed with aspirin are focused on dose-effect; few studies have consider time as a key factor in the chemopreventive effect of this medicine. However, Swede and colleagues reinvestigated this issue, testing aspirin with 1,478 patients diagnosed with BC and 3,383 free of disease. The authors found that time was an important variable in BC risk. Patients that consumed aspirin once a week for 1 year reported an RR of 0.84; and for those women consuming aspirin daily for 10 years or more the RR value was 0.74 (33).

A more specific study was performed evaluating genetic variation in the interleukin-6 (IL-6) gene, aspirin and BC risk in the southeast region of the US (34). Four experimental groups were included depending on their ethnic origin (non-Hispanic Caucasian women, native Hispanic-American women with BC, and the respective negative controls for each group). In the group of Hispanic-American women, factors including postmenopausal status, free of hormone use before the trial, and recent use of aspirin, significantly decreased the BC risk (OR=0.56, 95% CI, 0.33-0.96). The BC risk was not significant in the group of non-Hispanic Caucasian women. Furthermore, the genotype and the haplotype of the IL-6 gene modified the association between aspirin and BC significantly. Women that were not exposed to hormones presented a major benefit (P=0.06 for non-Hispanic Caucasian women, and 0.04 for native Hispanic-American women). According to their findings, IL-6 may interfere either in inflammation or estrogen pathways, due to its interactions with aspirin and the different associations of exposure to hormones of postmenopausal women. Furthermore, their data suggest that when estrogens are not present, the alleles associated with low IL-6 production levels and inflammation have a protective function. In addition, estrogen itself can modify levels of IL-6; thus the effect

can only be observed in the absence of estrogens. Therefore, it can be concluded that IL-6 could amend the relationship among estrogen, aspirin and BC risk.

3. Aspirin as an inhibitor of metastasis

Aspirin has not only been studied as a chemopreventive drug, but also as an inhibitor of metastasis. It is known that patients with BC have a 50% likelihood to develop metastasis after treatment (chemotherapy, radiation and/or surgery). The maspin protein (mammary serpin) and its mRNA are highly produced in normal breast epithelial cells (35), whereas their expression levels diminish in the presence of BC (36). Maspin protein is a tumor-suppressor serine protease, which is expressed in several tissues including mammary epithelia, prostate, epidermis, lung, and in the stromal cells of the cornea (35,36,61,62). This protein is of special interest as it is able to inhibit cellular invasion and metastasis and to act as a tumor suppressor (37,60).

Girish and colleagues proposed that aspirin corrects the production levels of maspin in human breast cells *in vitro* and in patients, regulating maspin to normal levels through the stimulation of nitric oxide (NO) synthesis independent of the insulin receptor (37). An epidemiologic example of this previously mentioned concept, is a study carried out in India, where the effect of aspirin and the incidence of metastasis in BC patients was determined (38). In this work, 35 women participated, and the age range was from 41 to 64 years. All of the women were diagnosed with BC and had been previously treated (chemotherapy, radiation and/or surgery). The patients consumed a dose of aspirin of 75 mg/70 kg body weight daily for 3 years. During this period of time, plasmatic levels of NO and maspin were measured, and a monthly surveillance of metastasis was performed by an oncologist and occasionally by biopsy. The control group consisted of 35 healthy volunteers whose plasma maspin levels were measured. The results showed that maspin levels were higher after 24 h of aspirin administration; such levels were maintained during the 3 years of treatment (initially maspin levels were 0.95±0.04 nM increasing to 4.63±0.05 nM at the end of treatment). Six patients developed metastasis, and the rest of the patients were, apparently, free of metastasis after 3 years. Researchers suggest that daily intake of aspirin in patients with previously treated BC may reduce the incidence of metastasis independent of the disease stage.

Recently, a large study performed in the UK with 17,285 patients was carried out in order to elucidate the preventive effect of aspirin on distant metastasis comparing adenocarcinomas vs. other cancer types (39). Patients were divided into four groups according to the metastasis target: i) metastasis to distant tissues (secondary tumors, particularly to the liver, lung, bone, brain or other tissues distant to the primary tumor); ii) metastasis to any specific region; iii) local invasion; and iv) patients presenting rapid progression of disease and not enough clinical information recovered. After data analysis, the authors concluded that aspirin may help reduce the risk of distant metastasis in certain types of cancers from 30 to 40%, and in 50% of metastatic adenocarcinoma, using a low concentration (≤5 mg daily) and long delivery formulation. Additionally, it was observed that aspirin had a major impact on individuals with adenocarcinomas, particularly in those cases where curative surgery was performed (as in BC). The authors proposed that the anti-metastatic activity of aspirin is mediated by platelets, based on the knowledge that they participate in cancer development and metastasis (40), protecting tumoral cells during their travel through the bloodstream. Moreover, platelets activate a coagulation system allowing microthrombosis formation, thus facilitating the hosting of tumoral cells in target tissues (41). In addition, it is known that thrombocytosis is a common disease in many cancer types and it is used as a poor prognostic indicator (42).

Regarding aspirin and breast cancer metastasis, a research group at Brigham and Women's Hospital together with the Harvard Medical School, performed a prospective observation study in order to evaluate whether aspirin could decrease the risk of death from BC (43). The study consisted of 4,164 women (enrolled between 1976 and 2002; followed up until death or June 2006). According to their results, the use of aspirin reduced the risk of metastasis and BC death. The adjusted relative risks (stage of cancer, menopausal status, BMI, hormone receptor status) for distant recurrence were: for 1 day/week, RR=0.91 (95% CI, 0.62-1.33); 2-5 days/week, RR=0.40 (95% CI, 0.24-0.65); and for 6-7 days/week, RR=0.57 (95% CI, 0.39-0.82).

Evidence to date regarding the use of aspirin as an inhibitor of metastasis in patients diagnosed with cancer, suggests that aspirin delays the invasion of cancer cells to other tissues and in some cases prevents metastasis formation. The information indicates that regular consumption of aspirin and long periods of administration are required for a positive result.

4. Aspirin, mechanisms of action

NSAIDs can be classified depending on their mechanisms of action on the COX enzyme: classical NSAIDs (including aspirin) and COX-2 inhibitors (celecoxib and rofecoxib are mainly used). The molecular mechanism by which aspirin and other classical NSAIDs are capable of inhibiting COX-1 and COX-2, is by competing with arachidonic acid for binding to the active site of cyclooxygenase; this is a rapid and an irreversible union, followed by the covalent acetylation of serine 530 in COX-1, hampering the oxidation of arachidonic acid (Fig. 1) (44). After the same modification, COX-2 can still convert arachidonic acid into 15R-hydroxyeicosatertraenoic acid (HETE), instead of PGG2 (45). On the other hand, it is believed that specific inhibitors of COX-2 bind to valine 523, whereas in COX-1 access to this binding site is blocked by the isoleucine residue at the same position (46). This difference explains the different degrees of inhibition towards COX-1 and COX-2 that aspirin possesses.

As known, there are two major isoforms of the COX enzyme, COX-1 that is expressed constitutively, and COX-2, which is induced locally as part of an inflammatory cascade and during early tumor development (46,47). COX-1 performs positive homeostatic functions. It is antithrombotic when released to vascular endothelium and is cytoprotective when produced by the gastric mucous. Moreover, it provokes platelet aggregation and prevents inappropriate bleeding when it is thromboxane A2-dependent (48). On the other hand, COX-2, in addition to its action in tissular damage response, could be constitutively expressed in the brain, kidney, cells of the

Table I. Comparative table of studies associating aspirin or NSAID use and the risk of breast cancer.

Study	No. of patients	RR/OR/SIR	95% CI	Outcome	Authors, year of publication (ref)
Case-control	12,174 patients 34,934 control	RR=0.98	0.89-1.07	Effect of NSAIDs on 9 different cancers (gastrointestinal and not intestinal) Reduction in gastric cancers, 36 months of use previous to diagnosis of the disease	Langman *et al*, 2000 (29)
Case-control	3,133 patients 3,062 control	OR=0.76	0.66-0.88	OR=0.68 (95% CI, 0.54,0.86) for users of NSAIDs longer than 8 years	Cotterchio *et al*, 2001 (31)
Cohort-study	27,616 postmenopausal women	RR=0.71	0.58-0.87	Aspirin consumers at least 6 times/week	Johnson *et al*, 2002 (10)
		RR=1.01	0.83-1.25	NSAID consumers at least 6 times/week	
Cohort-study	29,470 participants (both genders) SIR calculated for both genders			150 mg of aspirin during 9 years	Friis *et al*, 2003 (18)
		SIR=0.9	0.7-1.1	Colon cancer	
		SIR=0.1	0.8-1.2	Rectal cancer	
		SIR=0.9	0.8-1.1	Breast cancer	
Cohort-study	80,741 postmenopausal women 1,392 confirmed cases of breast cancer	RR=0.79	0.60-1.04	NSAID regular use (2 or more tablets/week; 5-9 years)	Harris *et al*, 2003 (11)
		RR=0.72	0.56-0.91	NSAID regular use (10 or more years)	
		RR=0.51	0.28-0.96	Ibuprofen (long-term use)	
		RR=0.79	0.60-1.03	Aspirin (long-term use)	
Cohort-study	734,899 women enrolled 3,708 patients diagnosed with breast cancer			Daily doses of 75 mg of aspirin and 2,000 mg of paracetamol more effective dosages, compared to non-users	García-Rodríguez and González, Pérez, 2004 (32)
		OR=0.77	0.62-0.95	Aspirin (1 year or longer)	
		OR=0.76	0.65-0.88	Ibuprofen (1 year or longer)	
Case-control	1,442 cases 1,429 control users of aspirin, ibuprofen and acetaminophen	OR=0.8	0.66-0.97	Ever use of aspirin or other NSAIDs, once/week, 6 months or longer, ever vs. non-users	Terry *et al*, 2004 (16)
		OR=0.72	0.58-0.90	Frequent users (>7 tablets/week)	
		OR=0.74	0.60-0.93	Aspirin users with hormone receptor-positive tumors	
		OR=0.97	0.67-1.40	Aspirin users with hormone receptor-negative tumors	
Cohort-study	22,834 individuals	RR=1.07	0.84-1.35	Cancer mortality in women (aspirin users)	Ratnasinghe *et al*, 2004 (12)
		RR=0.82	0.49-1.36	Breast cancer mortality (aspirin users)	
		RR=0.90	0.73-1.13	Cancer mortality in men (aspirin users)	
		RR=0.98	0.84-1.14	Aspirin use and mortality both genders	
Case-control	1,478 breast cancer patients	OR=0.84	0.64-0.97	Regular use (1 tablet/week)	Swede *et al*, 2005 (33)
	3,838 control	OR=0.80	0.67-0.96	Occasional use	
Cohort-study	19,934 patients aspirin 100 mg 19,942 patients aspirin placebo	RR=1.01	0.94-1.08	Total cancer	Cook *et al*, 2005 (20)
		RR=0.98	0.87-1.09	Breast cancer (1,230 cases) No effect of aspirin was observed in reduction of risk, except lung cancer (n=205 patients; RR=0.78, 95% CI, 0.59-1.03)	

Table I. Continued.

Study	No. of patients	RR/OR/SIR	95% CI	Outcome	Authors, year of publication (ref)
Case-control	7,006 incidents of breast cancer	OR=0.78	0.63-0.97	Regular use of NSAIDs Regular use of aspirin OR=0.86	Zhang *et al*, 2005 (17)
Cohort-study	114,460 patients	RR=0.98	0.86-1.13	Breast cancer risk for daily vs. no regular use of aspirin	Marshall *et al*, 2005 (13)
	2,391 diagnosed with BC	RR=1.09	0.97-1.21	Breast cancer risk for daily vs. no regular use of NSADs	
		RR=0.80	0.62-1.03	Long term (≥5 years) daily aspirin users for ER/PR-positive BC	
		RR=1.81	1.12-2.92	Long term (≥5 years) daily aspirin users for ER/PR-negative BC	
Cohort-study	77,413 patients 3,008 cases of breast cancer			No incidence association was found with the use of NSAIDs or aspirin in either short- or long-time use (≥5 years)	Jacobs *et al*, 2005 (21)
		RR[a]=1.05	0.88-1.26	Long-time regular use of NSAIDs compared with no use (≥30 pills/month; ≥5 years)	
		RR[a]=0.88	0.69-1.12	Long-time regular use of aspirin compared with nonuse (≥30 pills/month; ≥5 years)	
Cohort-study	22,507 postmenopausal women	RR=0.84	0.77-0.90	Aspirin users compared with non-user cancer incidence	Bardia *et al*, 2007 (24)
		RR=0.87	0.76-0.99	Aspirin users compared with non-user cancer mortality	
	3,487 cases of cancer 3,581 deaths				
Cohort-study	98,920 women			Multi-ethnic cohort study (African-American, Caucasian, Japanese-American, Latina and native Hawaiian women)	Gill *et al*, 2007 (28)
		HRR=1.05	0.88-1.25	Current users of aspirin breast cancer risk ≥6 years of use	
		HRR=1.04	0.84-1.27	Past users of aspirin breast cancer risk for ≥6 years of use compared with non-users	
		HRR=0.70	0.51-0.95	Current users of other NSAIDs was protective for ≥6 years of use	
		HRR=0.90	0.62-1.30	Past users of other NSAIDs was not protective for ≥6 years of use	
Cohort-study	69,810 men	RR[a]=0.84	0.76-0.93	Overall cancer incidence in men (≥5 years of use)	Jacobs *et al*, 2007 (22)
	76,303 women at enrollment	RR[a]=0.86	0.73-1.03	Overall cancer incidence in women (≥5 years of use)	
		RR[a]=0.68	0.52-0.90	Colorectal cancer (both genders); long-term daily aspirin use, lower incidence	
	10,931 men and 7,196 women developed cancer	RR[a]=0.81	0.70-0.94	Prostate cancer; long-term daily aspirin use in men	
		RR[a]=0.83	0.63-1.10	Breast cancer; long-term daily aspirin use in women	
				Aspirin 325 mg/day	

Table I. Continued.

Study	No. of patients	RR/OR/SIR	95% (CI)	Outcome	Authors, year of publication (ref)
Case-control	1,527 NHW cases 1,601 NHW control 798 H/NA cases 924 H/NA control	OR=0.56	0.33-0.96	Recent aspirin use decreased risk of BC among postmenopause H/NA	Slattery *et al,* 2007 (34)
Case-control	3,125 BC cases	OR=0.80 OR=0.80	0.66-0.88 0.60-0.84 0.62-1.03	NSAIDs, reduced risk of BC ER^+/PR^+ ER^-/PR^- Acetylsalicylic acid and nonacetylsalicylic acid use associated with reduced risks	Kirsh *et al*, 2007 (15)
Cohort-study	28,695 women 847 breast cancer cases	RR^a=1.27 RR^a=1.38 RR^a=1.25	1.10-1.45 1.12-1.69 1.04-1.49	NSAID users vs. non-users, increase aspirin-only users, higher BC incidence than non-aspirin NSAIDs 7.5 years follow-up	Friis *et al,* 2008 (19)
Cohort-study	35,323 postmenopausal women	HR=0.65 HR=0.78	0.43-0.97 0.61-0.98	Low-dose aspirin, ≥4 days/week, at least 10 years. Decreased risk of BC vs. no use Moderate use of NSIADs, 10 years, ≥3 days/week	Ready *et al,* 2008 (27)
	4,164 women 341 BC deaths	 RR=1.07 RR=0.29 RR=0.36	 0.70-1.63 0.16-0.52 0.24-0.54	Aspirin associated with a decreased risk of BC death 1 day/week 2-5 days/week 6-7 days/week	Holmes *et al,* 2010 (43)
		 RR=0.91 RR=0.40 RR=0.57	 0.62-1.33 0.24-0.65 0.39-0.82	Aspirin associated with distant recurrence: 1 day/week 2-5 days/week 6-7 days/week	

NHW, non-Hispanic Caucasian women; H/NA, native Hispanic women; RR, relative risk; OR, odds ratio; SIR, standardized incidence ratio; [a]RR, rate ratio; HRR, hazard rate ratio; CI, confidence interval; NSAIDs, non-steroidal anti-inflammatory drugs; PR, progesterone receptor; ER, estrogen receptor.

pancreatic islet, ovary, gestational uterus and intestine (49,50). COX-2 is present in several cancer cell lines and is implicated in carcinogenesis, tumor growth, apoptosis and angiogenesis (51,52). As these isoforms are encoded in independent genes with different chromosomal localizations, their expression and regulation patterns differ. More recently, a third isoform, named COX-3, was identified as a COX-1 splice variant that may play an important role in fever and pain processes (53).

These enzymes are responsible for catalyzing the conversion of arachidonic acid to prostaglandin endoperoxide (prostaglandin H2) and thromboxanes; both COX-1 and COX-2 participate in maintaining physiological regulation in the stomach, platelets, kidneys and intestine (46). The principal side effects of aspirin and NSAIDs such as gastropathy, renal insufficiency and impaired vascular homeostasis among others, are due to a reduction in the appropriate prostaglandins in these organs (46). Prostaglandins are short-lived compounds acting as local mediators of continuous importance in normal cellular reactions, but they appear to be increased in several pathological conditions, particularly inflammation (46), and it has also been shown that the level of prostaglandins in various tumors is greater than that of normal tissues (54).

Reports from the past 15 years, have shown that COX-1 is localized in stromal cells adjacent to the tumor but not in tumor cells. In contrast, high levels of COX-2 are localized primarily in tumor cells but also appear in stromal cells (54). Such is the case of certain malignant human breast tumors that produce more prostaglandin-like material than do benign tumors or normal breast tissues (54).

Additional experimental data suggest that COX-2 can be induced by a mutation of the tumor-suppressor gene APC, subsequently increasing the expression of the nuclear transcription factor PPAR-δ (55). Induction of COX-2 expression

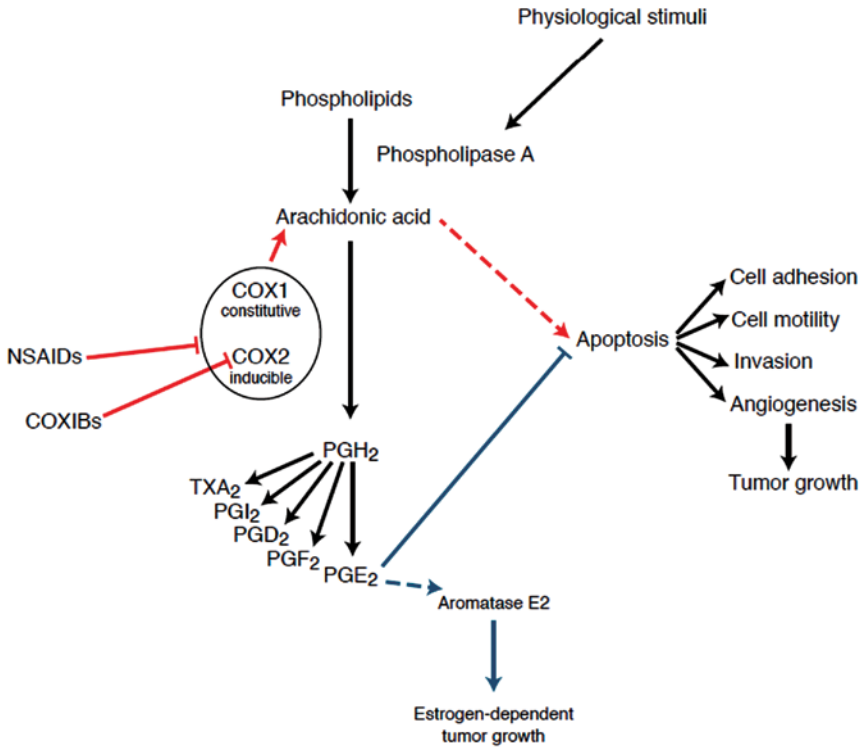

Figure 1. The mechanisms of action of aspirin. Prostaglandin synthesis via arachidonic acid and the possible effects of cyclooxygenase inhibitors as chemopreventive agents of breast cancer by the intracellular accumulation of arachidonic acid, which directly promotes apoptosis and attenuation of positive feedback for proliferation and survival. COX, cyclooxygenase; PG, prostaglandin; TXA2, thromboxane A2; NSADs, non-steroidal anti-inflammatory drugs; COXIBs, COX-2 selective inhibitors.

in tumors can also occur by lipopolysaccharide through the mitogen-activated protein kinase (MAPK) and protein kinase C-(PKC) pathways (56). Ceramide-stimulated activation of MAPK can also activate c-Jun N-terminal kinase (JNK), which in turn can lead to increased COX-2 gene expression in human mammary epithelial cells (57). In breast tissue, induction of COX-2 expression can trigger prostaglandin production, which indirectly stimulates cellular proliferation increasing the local biosynthesis of estrogen and increasing the expression of the aromatase gene (8,58). In humans, high levels of prostaglandins have been related with metastatic potential and reduced survival of patients (22). Such a process could be reversible when COX-2 is inhibited using NSAIDs with the purpose of reducing prostaglandin synthesis. For those women who present with tumors with positive hormone receptors, the use of aspirin could contribute to the suppression of aromatase activity, reducing the intra-mammary prostaglandin production, and thus, estrogen production (59). In particular, it has been shown that E2 prostaglandin (PGE_2) stimulates the transcription of aromatase, increasing the estrogen levels and, moreover, contributing to the progression of estrogen-dependent BC (60).

The induction and overexpression of COX-2 and its main product PGE_2 in human mammary tissue, have also been associated with aromatase-catalyzed estrogen biosynthesis (61). In addition, some epidemiologic studies have reported that hormone-receptor-positive breast tumors are more responsive to aspirin (62). The ability of aspirin and other NSAIDs to protect against breast cancer might vary according to hormone receptor status. For instance, in 2004, a case-control study, reported a reduction in risk only in women with hormone receptor-positive tumors, but not in women with hormone receptor-negative tumors (9). Moreover, COX-2 is related to the activation of carcinogens, mutagenesis, angiogenesis, inhibition of apoptosis and metastasis (53,63,64). In contrast, COX-2 inhibition may reverse these processes; aspirin and salicylates may also suppress NF-κB-related survival signaling by inhibiting IKKα activation, leading to apoptosis (45).

Molecular studies have also revealed a strong correlation between overexpression of COX-2 and other oncogenes, such as HER-2/Neu, in malignant breast tumors (65,66). A linkage between the expression of COX-2 and MDR1/Pgp 170 was shown by immunohistochemical analyses in human breast tumor specimens, and it is well known that overexpression of P-glycoprotein contributes to primary chemotherapy resistance (66,67).

Regarding the possible anti-metastatic effect of aspirin, it was demonstrated that the daily ingestion of aspirin in patients previously treated with standard therapies indeed reduces metastasis, assigning this effect to the maspin protein (38). Maspin is a serine protease inhibitor of 42 kDa synthesized abundantly in normal mammary epithelial cells, and it has been shown that the expression of this protein is reduced in patients with breast cancer. Thus, the lack of expression of maspin in breast cancer has been suggested to indicate the presence of an aggressive and metastatic tumor (36). Studies have revealed that ingestion of aspirin increases the levels of serum nitric oxide (NO) and maspin, both of which inhibited the growth of breast cancer cells *in vitro*, as well as invasion and metastatic processes in an animal model (9,38). This finding suggests

that aspirin participates in the restoration of maspin synthesis at any stage of the disease, and also that maspin presents beneficial effects at any stage of tumoral progression. It has been observed that maspin is reduced in BC, or even absent in invasive cancer; hence, there is a correlation between the protein synthesis and its reduction according to cancer type and stage (67). There is also evidence of maspin as an inhibitor of angiogenesis (68).

On the other hand, in 2008, Burnett and collaborators investigated the relationship between inflammation and angiogenesis and how it is regulated by aspirin. They proposed that aspirin alters macrophage regulation by inducing expression of IL-10 in MCF-7 cells, and suggested aspirin as a therapeutic strategy for modification of tumor-associated macrophages (TAMs), which can initiate both angiogenesis and invasion (69).

Finally, regarding the mechanisms of aspirin as an antitumor drug (70), Spitz *et al* discuss the use of acetylsalicylic acid (ASA) and salicylic acid (SA) in tumor cell viability and glucose metabolism. Both drugs modulate an important glycolysis-regulatory intracellular enzyme (6-phosphofructo-1-kinase, PFK) in a dose-dependent manner, and the inhibition occurs due to the modulation of the enzyme quaternary structure. The authors demonstrated that ASA, as well as its precursor SA, decreased the viability of the human breast tumor cell line MCF-7, diminishing its glucose consumption and PFK activity.

5. Conclusions

One of the most significant discoveries along the quest for novel cancer treatments and preventive therapies has been the COX-2 inhibitors. These drugs have been demonstrated to interfere directly with the carcinogenesis process, restoring apoptosis. The most representative medicine of the last 100 years is probably aspirin, a drug that is still currently under investigation. The COX-2 gene is overexpressed in breast cancer; furthermore, the presence of the COX-2 enzyme in tumoral tissue is directly proportional to the density of cancer cells. Moreover, some studies have revealed that high levels of prostaglandins are linked to unfavorable patient characteristics, such as the risk to develop metastasis and the reduction in survival rate, being good indicators for the correct diagnosis of disease stage.

In respect to the epidemiologic studies reported here, it is of great importance to homologate criteria in this type of research, for example, the time of administration of the drug, in order to truly assess the chemopreventive and anti-metastatic effect of aspirin. This issue should be considered because of the great variability existing in studies.

Evidence of the chemopreventive effect of aspirin cited in this review, suggests that it is likely to lower BC risk when administrated at doses greater than 100 mg/day for 3 or more years. Such information suggests that aspirin may start being favorable after a long period of regular use. However, this dosage poses adverse reactions, such as, an increase in gastric ulcer risk and gastrointestinal bleeding. Cancer is a multifactorial disease, and its behavior is constantly being investigated toward a greater understand and, at the same time, to propose less aggressive and more effective therapies or, when possible, chemopreventive alternatives. Aspirin could yield beneficial results for a specific group of risk patients, but who should be monitored carefully if there are any contraindications to the medicine. In conclusion, aspirin is a suitable alternative in this non-stop search for cancer prevention and treatment.

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