Chemoprevention is defined as the use of pharmacological or natural agents that inhibit the development of a disease. In the case of breast cancer, the main chemopreventive agents used are selective estrogen receptor blockers (SERMs) and aromatase inhibitors (AIs) (1, 2).

Cyclooxygenase 2 (COX-2) inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), metformin, and statins are drugs that have long been in clinical use. NSAIDs are used as anti-inflammatories, analgesics, and, in the case of aspirin, antithrombotics (3); metformin is used as an anti-hyperglycemic (4) and in the treatment of metabolic syndrome (5) and polycystic ovarian syndrome (PCOS); and statins are used for the primary and secondary prevention of cardiovascular disease by lowering cholesterol levels (6). Therefore, the safety profiles and adverse reaction profiles of these drugs are well understood. Thus, these drugs are good clinical candidates for further exploration of their mechanisms of action as applied to chemoprevention of breast cancer (7-9). Current studies regarding the novel application of these drugs have been critically analyzed with respect to their potential use in breast cancer chemoprevention. However, more research is needed to prove that these studies were adequately powered and thus are of good statistical significance, so that the use of these drugs can be considered as an option for chemoprevention of breast cancer in clinical practice.

Search strategy

The papers used as references in this review were identified using relevant keywords in related search engines such as Pubmed and Google Scholar, after performing a broader search regarding the chemoprevention of breast cancer and finding mentions of these drugs in other documents. The search terms used to identify these sources include “breast neoplasms,” “breast cancer,” “NSAIDs,” “aspirin,” “COX-2 inhibitors,” “COX2 inhibitors,” “cyclooxygenase 2 inhibitors,” “metformin,” “statins,” and “HMG-CoA reductase inhibitors.” The search results were then filtered, analyzed, and used as references in compiling this review.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Various epidemiological studies have been conducted to establish a relationship between the use of NSAIDs and the incidence of breast cancer (10-12). The results of these studies are inconsistent; however, this inconsistency is most likely due to the fact that tumors have variable molecular properties (13).

All NSAIDs, such as aspirin and ibuprofen, inhibit both cyclooxygenase enzymes, largely with little or no selectivity for either enzyme. Aspirin irreversibly inhibits cyclooxygenase, while ibuprofen and other NSAIDs are reversible inhibitors. A meta-analysis has been conducted, focusing mainly on the effects of aspirin and ibuprofen (14). This meta-analysis included 16 case-control studies, 18 cohort studies, 3 case-
control studies nested in well-defined cohorts, and one clinical trial, all of which were performed between 1966 and 2008, examining the association between the use of NSAIDs and the risk of breast cancer. The results of these studies were pooled and statistically analyzed separately for aspirin and ibuprofen. The conclusions of this meta-analysis were that aspirin use decreased the risk of breast cancer. It was also noted that high intake of aspirin did not strengthen this relationship. Similarly, use of ibuprofen led to a decrease in the risk of breast cancer; however, higher intake of ibuprofen did not strengthen this association (14).

This analysis also considered the effects that genetic polymorphisms of the COX-2 gene might have on these results; for example, the COX-2.847 mutation is associated with an even lower risk of breast cancer among patients using aspirin. Overall, NSAID use was associated with a lower risk of developing breast cancer.

More recently, the relationship of aspirin and ibuprofen use with the risk of breast cancer has been re-examined in the context of the different molecular subtypes of cancer (15). A total of 26,580 menopausal women aged 59 to 77 years were involved in this analysis. During follow-up through 2005, 1581 cases of breast cancer were observed. Estrogen receptor (ER) status was available for 1262 of these patients; 1060 were ER positive, and 202 were ER negative. Progesterone receptor status was available for 1237 cases; 910 were progesterone receptor positive, and 327 were progesterone receptor negative. The women were divided into groups based on frequency of NSAID intake. It was found that women who regularly took aspirin had an approximately 20% lower risk of breast cancer than those who did not. In this study, higher frequency of aspirin intake was associated with lower risk (15), which contrasts with the results from the 2008 meta-analysis. These inverse associations of aspirin use were observed for ER+, ER-, PR+, and PR- tumors, with the greatest correlations observed for ER+ and PR+ tumors. However, in this study, no association was found between intake of non-aspirin NSAIDs and risk of breast cancer. One major limitation of this study was that the type of non-aspirin NSAID used was not specified; this may have masked any association between the intake of particular non-aspirin NSAIDs, such as ibuprofen, and the risk of breast cancer (15). It was further suggested that this difference is due to the fact that aspirin is an irreversible inhibitor of COX-2, while non-aspirin NSAIDs are reversible inhibitors.

The results of these various studies, although inconsistent and controversial, suggest that aspirin has chemopreventive potential; meanwhile, the chemopreventive potential of other NSAIDs remains to be clarified. Furthermore, in-depth examination of the relationship of NSAID consumption with various tumor subtypes, such as those in which COX-2 or epidermal growth factor receptor (EGFR) is upregulated, may shed light on the types of tumors that can be prevented with NSAIDs. These drugs would then be combined with other agents to achieve greater chemopreventive efficacy through combination treatment.

**COX-2 inhibitors**

NSAIDs not only inhibit COX-2, but also cyclooxygenase 1 (COX-1), which is responsible for regulating normal physiological functions; therefore, various adverse effects are associated with NSAID use. Side effects include stomach ulcers, nausea, vomiting, and prolonged bleeding after injury (16). For this reason, specific inhibitors of COX-2 have been developed. These include celecoxib and nimesulide, which are potent anti-inflammatory agents that lack the associated adverse effects (16). However, a different side effect profile was noted, mostly related to the cardiovascular system. This includes a higher predisposition to hypertension, atherosclerosis, and thrombosis, resulting in a higher risk of myocardial infarctions and strokes (17); these are conditions for which risk is already increased in peri- and post-menopausal females.

COX-2 is induced in inflamed tissue from its constitutively active isoenzyme, COX-1. It is involved in the metabolism of arachidonic acid into prostaglandins and thromboxanes specific to the inflamed tissue; this mediates local vasodilation, edema, pain, and fever (16).

Because prostaglandins are crucial in mediating inflammatory response and the associated pain, various inhibitors of the cyclooxygenase enzymes have been identified or developed; their main target is pain relief. Persistent inflammation may cause DNA damage, induce increased cellular proliferation to repair damaged tissue, and create an environment that is rich in cytokines and growth factors; all of these lead to tumorigenesis (18). Molecular links between cytokines and tumorigenesis have already been demonstrated for breast cancer and other conditions (19). In fact, COX-2 is highly overexpressed in numerous cancers, including breast, liver, colorectal, lung, and esophageal cancers (20, 21). Blocking persistent inflammation with NSAIDs and specific COX-2 inhibitors may therefore prove useful in the prevention of tumorigenesis.

Furthermore, a lower degree of prostaglandin synthesis leads to inhibition of the enzyme aromatase, which is responsible for the synthesis of estrogen. In fact, one of the major prostaglandins, PGE_2, specifically induces the promoter II region on the aromatase gene (22). COX-2 inhibition has been shown to prevent estrogen-induced breast tumor formation to a greater extent than ibuprofen (a non-selective NSAID); thus, it demonstrates selective chemopreventive potential for ER-positive tumors (22).

The effects of ibuprofen were compared to those of celecoxib, particularly in their ability to inhibit carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma-Aldrich; Darmstadt, Germany) in female Sprague-Dawley rats (22). These 50-day old rats were randomized into three groups, with 40 rats in each group. One group received powdered placebo with a standard diet, another group received 1500 mg/kg celecoxib with a standard diet, and the last group received 1500 mg/kg ibuprofen with a standard diet. After seven days, all rats were given an intragastric dose of 15 mg DMBA in 1.0 mL of sesame seed oil. The experimental and control diets were
Table 1. Biochemical and molecular associations between type 2 diabetes mellitus and breast cancer

<table>
<thead>
<tr>
<th>Biochemical Mechanisms (24)</th>
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<tbody>
<tr>
<td>Insulin</td>
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<td>Insulin-like growth factor-1 (IGF-1)</td>
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<td>Estrogens and androgens</td>
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<tr>
<td>Molecular Mechanisms (37)</td>
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<tr>
<td>Insulin Receptor (IR)</td>
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<td>Insulin-like growth factor-1 receptor (IGF-1R)</td>
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<td>Insulin receptor substrate-1 (IRS-1)</td>
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IGF-1: insulin-like growth factor-1; IR: insulin receptor; IR-A: insulin receptor isoform A (fetal); IR-B: insulin receptor isoform B; IGF-1R: insulin-like growth factor-1 receptor; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; IRS-1: insulin receptor substrate-1; PKC-zeta: protein kinase C-zeta

continued for 105 days before the experiment was stopped. The time of appearance of the first tumor in rats from each group was noted, while the size and location of the tumor was also assessed. At the end of the experiment, 127 palpable tumors were excised from the control rats (all adenocarcinomas) and 61 tumors were excised from rats treated with ibuprofen (all adenocarcinomas), while only 18 tumors were excised from rats treated with celecoxib (15 were adenocarcinomas, while 3 were non-malignant fibro-adenomas). Moreover, celecoxib was found to reduce the incidence of mammary cancer by 68%, tumor burden (tumors/rat) by 86%, and tumor volume by 81% compared to the control group. Only 13 of the 40 (32%) rats treated with celecoxib developed tumors, while all of the control rats (100%) developed tumors. Ibuprofen was also effective, but not as much as celecoxib; ibuprofen caused a 40% reduction in cancer risk, a 52% reduction in tumor burden, and a 57% reduction in tumor size. Moreover, the time for tumor development was prolonged with COX-2 inhibitor use. In the control group, the median time for detection of a tumor was 58 days after DMBA administration. In the celecoxib group, the median time was 95 days; in the ibuprofen group, it was 86 days. It was also noted that celecoxib and ibuprofen appeared to have no adverse effects on rat liver, kidneys, stomach, and intestines. In humans, however, there are concerns that drugs that inhibition of COX-2 can lead to severe cardiovascular adverse effects. Nonetheless, the use of COX-2 inhibitors in patients at low risk for heart disease appears to be safe (21). The results from this experiment show that celecoxib may be a very useful chemopreventive agent; they also support the role that COX-2 inhibitors, including the general NSAID ibuprofen, may play in reducing the risk of breast cancer.

Metformin

Diabetes has been associated with an increased risk of developing cancer; a recent meta-analysis involving 20 studies demonstrated the actual relationship between diabetes and breast cancer (23). This meta-analysis showed that women with diabetes have a 20% increased risk of developing breast cancer compared to non-diabetic women. A more recent meta-analysis suggested that diabetic women have a 23% higher risk of breast cancer, particularly menopausal women, while diabetes was also found to increase breast cancer mortality overall (24). Interestingly, the association between diabetes and breast cancer was strongest in Europe, followed by America, while it was non-significant in Asia (24). The biochemical and molecular associations between type 2 diabetes mellitus and breast cancer are outlined in Table 1 (25-42), while the structure, uses, outcomes, and adverse reactions of metformin are shown in Table 2 (4, 5, 38, 42, 43). Metformin increases the effectiveness of neoadjuvant chemotherapy in breast cancer patients (44). In one particular study,
the pathologic complete response (pCR), i.e., the absence of residual tumor at the time of surgery, was assessed for these patients (44). The difference in pCR between the non-diabetic group (16%) and the non-metformin group (8%) was significant, as was the difference between the metformin (24%) and non-metformin (8%) groups. In contrast, the difference between the non-diabetic (16%) and metformin (24%) groups, although numerically evident, did not attain clinical significance. This proved that the anti-proliferative characteristics of metformin impair tumor development. Moreover, because insulin use was twice as great in the non-metformin group compared to the metformin group (33% and 16%, respectively), it was observed that higher insulin levels were associated with decreased pCR (44). However, overall disease recurrence did not differ significantly between the groups, and both diabetic groups had worse overall survival than the non-diabetic group (45).

Various epidemiological studies have demonstrated a lower incidence of mortality from cancer in diabetic patients receiving low-dose metformin. A large meta-analysis (46) demonstrated that the overall cancer rate decreased by 31% in patients taking metformin compared to patients taking other anti-diabetic drugs. This difference was significant for pancreatic and liver cancer but was not significant for colon, breast, and prostate cancer.

This supports earlier results that showed that cancer incidence decreased by more than 50% in patients who had been taking metformin for over 4 years (47). In 2012, another meta-analysis confirmed the beneficial effects of metformin for decreasing the risk of cancer and reducing overall cancer mortality (48). The mechanisms underlying the action of metformin are complex and are far from fully understood. The beneficial effects of metformin may be indirect (through insulin), or it may directly affect the proliferation and growth of cells (45). Many mechanisms of metformin action have been proposed, as outlined in Table 3 (3, 45, 49-54).

Preclinical models show that metformin can lower the incidence of breast cancer (55). Metformin affects ER+ and ER- cell lines as well as human epidermal growth factor receptor 2 (HER-2) normal and abnormal cancer cell lines; it inhibits cell proliferation and causes cell cycle arrest at the G1 checkpoint, probably through reduction of cyclin D1 and E2F1 expression. It also inhibits MAPK, Akt, and mTOR activity in all of these cell lines. However, metformin does not induce apoptosis. Furthermore, at high doses, metformin was found to reduce HER-2 expression in cancer cells overexpressing HER-2; at lower doses, it was found to inhibit HER-2 tyrosine kinase activity (55).

As demonstrated in preclinical models, metformin at a low dose can inhibit the tyrosine kinase activity of the HER-2 receptor; also, a high dose of metformin can downregulate HER-2 (38). Thus, therapeutically combining metformin with the anti-HER-2 monoclonal antibody trastuzumab may be very efficient to eliminate stem/progenitor cell populations with amplified HER-2. This is particularly due to the fact that metformin can prevent resistance to trastuzumab treatment; this is very often mediated by high levels of IGF and insulin, which bind to their respective receptors and induce cellular proliferation, inhibition of apoptosis, angiogenesis, and metastasis. Metformin can decrease circulating insulin and IGF levels and can thus disrupt this alternative pathway for tumor development.

In the prevention setting, metformin can therefore regulate the rate of proliferation of tumor progenitor cells in premalignant lesions, thus preventing or delaying malignant tumor formation (38). Furthermore, by regulating proliferation of dormant cancer stem cells, metformin can also prevent recurrence of breast cancer; thus, it may be effective for secondary prevention of breast cancer.

Cancer is the second leading cause of death worldwide, while diabetes is the twelfth (48). Considering that the prevalence of diabetes is constantly increasing, the use of metformin as both an anti-diabetic drug as well as a chemopreventive agent for cancer will have numerous beneficial implications and positive results.

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**Table 2. Metformin: Structure, uses, outcomes, and adverse reactions**

<table>
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<th>Metformin</th>
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<tr>
<td><strong>Structure</strong></td>
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<td><strong>Uses</strong></td>
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<td><strong>Outcomes</strong></td>
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<tr>
<td><strong>Side effects</strong></td>
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| **Outcomes (37)** | Decreases circulating levels of cholesterol, LDLs, and triglycerides. |
| **Side effects (37, 42)** | Decreases circulating levels of cholesterol, LDLs, and triglycerides. |

PCOS: polycystic ovarian syndrome; LDLs: low density lipoproteins

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A proposed mechanism of action for statins is outlined in Figure 1 (59); this shows the mevalonate pathway on which statins (as HMG CoA inhibitors) act. This is also linked to various in vitro and in vivo studies, which are summarized in Table 4 (57-59). Statins can also interfere with microdomain formation in endothelial cells and inhibit oxidative stress pathways, both enzymatically and non-enzymatically. Furthermore, statins can upregulate endothelial nitric oxide synthase, eNOS, improving endothelial function (60).

In an in vitro study, the effects of statins on the cellular proliferation of breast cancer cell lines were studied, both alone and in combination with estradiol (57). The breast cancer cell lines, MCF-7 (ER+) and MDA-MB 231 (ER-), were cultured in the presence of the lipophilic statins atorvastatin, lovastatin, fluvastatin, simvastatin, and hydrophilic pravastatin, both alone and in combination with estrogen. The results showed that all statins, with the exception of pravastatin, significantly inhibited cellular proliferation in both cell lines after four days of culture; this association was dose-dependent. The inhibitory values...
Table 4. Effects of statins as outlined in Figure 1

<table>
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<tr>
<th>Endothelial cells</th>
<th>Interfere with microdomain formation and inhibit oxidative stress pathways, both enzymatically and non-enzymatically. Statins can also upregulate endothelial nitric oxide synthase, eNOS, improving endothelial function (59).</th>
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<td><strong>In vitro breast cancer cells</strong></td>
<td>In an <em>in vitro</em> study, the effects of statins on cellular proliferation of breast cancer cell lines were studied, both alone and in combination with estradiol (56). The breast cancer cell lines, MCF-7 (ER+ cells) and MDA-MB 231 (ER-), were cultured in the presence of the lipophilic statins atorvastatin, lovastatin, fluvastatin, and simvastatin, as well as the hydrophilic statin pravastatin, both alone and in combination with estrogen. The results showed that all statins, with the exception of pravastatin, significantly inhibited cellular proliferation in both cell lines after four days of culture; this association was dose-dependent. The inhibitory values ranged from 10% to 90%, and the inhibition was greater in ER- cells. In ER+ cells, atorvastatin was a less potent inhibitor than the other statins. In the presence of estrogen, all statins equally inhibited cellular proliferation in ER+ cells. However, the statins were not completely successful in preventing cellular proliferation in the presence of stimulating estradiol (56). Further <em>in vitro</em> studies demonstrate the ability of the lipophilic statins to prevent cellular proliferation in tumor cell lines that are hormone receptor-positive and HER-2-negative (MCF-7), hormone receptor-negative and HER-2-positive (SKBr3), and double negative (MDA-231) (57). In <em>in vitro</em>, the hydrophilic statin pravastatin shows no inhibitory effect on any cell lines. In contrast, the lipophilic statins fluvastatin, lovastatin, and simvastatin show significant cell growth inhibition, particularly in cells with activated Ras and HER-2 pathways. Furthermore, response to statins appears to be associated with cellular levels of NF-κB, an anti-apoptotic mediator and transcription factor complex. Within four hours of statin treatment, all three cell lines showed significant reductions in cellular p-MEK1/2 levels; these are key factors in the Ras-Raf-MEK-MAPK pathway, which drives cellular proliferation. However, this decrease was transient, and the levels began to increase after 12 hours. In SKBr3 cells, levels of activated NF-κB decreased to approximately 70% after 48 hours, and AP-1 levels also decreased. Moreover, cyclin D1 levels were seen to decrease, thus halting the cell cycle, while the levels of apoptotic mediators, mainly caspases (57).</td>
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statins (132 cases) and 1.43% in the non-treatment group (122 cases). This difference was not found to be statistically significant. However, the trials included in this meta-analysis mainly focused on the effects of statins on cardiovascular disease; the dosages were set only in this regard, and follow-up was relatively short. The effects of long-term statin use and the incidence of breast cancer could not be identified, which may have affected the observed association between statin use and risk of breast cancer. Furthermore, this meta-analysis included the use of both lipophilic and hydrophobic statins; the latter cannot

Figure 1. Proposed mechanism of action of statins (59)
permeate the cell membrane and thus do not exert activity on cellular proliferation and motility. This may have also led to the calculated lack of association between statin use and risk of breast cancer.

More recently, a trial was performed to test the chemopreventive abilities of fluvastatin in women with diagnosed DCIS or stage 1 breast carcinoma. Patients were randomized to receive either 80 mg daily or 20 mg daily of fluvastatin for 3 to 6 weeks prior to surgery. The results showed that fluvastatin was most effective in patients with high grade (poorly differentiated) tumors. The proliferation of these tumors decreased by a median of 7.2%, while in low grade tumors, this decrease was only 0.3%; the difference between the two was statistically significant. Overall, tumor apoptosis increased in 38% of patients, remained the same in 41%, and decreased in 21%; high grade tumors showed an increase in apoptosis (68).

The preclinical, clinical, and epidemiological results show that statins can likely reduce the incidence of breast cancer and may have anti-tumor potential. However, controversy persists. Further investigation is still required to identify whether statin use is truly associated with reduced incidence of breast cancer, the magnitude of this association, and the class of patients in which it is most likely to be prevalent.

In conclusion, because the abovementioned drugs have different mechanisms of action than the endocrine drugs currently used as chemopreventive agents (mainly SERMs and AIs), they may be useful as chemopreventive agents in non-responders to conventional chemoprevention. This makes sense, especially when considering that these same drugs are also being used to treat concurrent, prevalent conditions such as diabetes and hyperlipidemia. However, further studies to evaluate the chemopreventive potency of these drugs compared to current chemopreventive strategies are required to clarify this controversial issue. These studies may associate these drugs with chemopreventive benefits in breast cancer and may also identify which patient groups are likely to benefit from this novel application of these drugs. The desired result would be the clinical implementation of these further studies, thus providing another mechanism of chemoprevention of breast cancer and reducing polypharmacy in the respective patients; thus, the instance of adverse drug reactions and drug-drug interactions would decrease.

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