Influence of metformin therapy on breast cancer incidence and prognosis

Đorđe Popović[1], Lazar Popović[2], Edita Stokić[1], Dragana Tomić-Naglić[1], Milena Mitrović[1], Branka Kovačev-Zavišić[1]

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[1]Clinic of Endocrinology, Diabetes and Metabolic Disorders, Medical Faculty Novi Sad, Serbia, [2]Oncology Institute of Vojvodina, Sremska Kamenica, Serbia

Correspondence to:
Dr. Đorđe Popović, Clinic of Endocrinology, Diabetes and Metabolic Disorders, Clinical Center of Vojvodina, Hajduk Veljkova 1-9, 21000 Novi Sad, Serbia
pitstop021@gmail.com

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SUMMARY

Diabetes mellitus type 2 and malignant diseases are among the most frequent causes of morbidity and mortality worldwide. Number of studies showed that breast carcinoma and other cancers are more frequent and have worse prognosis in patients with diabetes. The mechanisms of effect of type 2 diabetes to frequency and prognosis of breast carcinoma are complex and besides a direct effect of insulin resistance, hyperinsulinemia and hyperglycemia, they include the effect of accompanying obesity and anti-diabetic therapy. One of the anti-diabetic drugs that reduces frequency and improves prognosis of breast carcinoma is metformin. Anticancer effects of metformin are indirect (insulin dependent) and direct (insulin independent). Insulin-dependent and insulin-independent antitumor effect of metformin opens the door for implementation of this drug in therapy and prevention of breast cancer and other malignant diseases even in patients without type 2 diabetes. This paper presents literature data on mechanisms of type 2 diabetes impact to the risk of development and prognosis of breast cancer and the anticancer potential of metformin.

Key words: *Breast Neoplasms; Metformin; Diabetes Mellitus, Type 2; Comorbidity; Risk Factors; Prognosis*

Diabetes mellitus type 2 (DMT2) and malignant diseases are among the most frequent causes of morbidity and mortality worldwide. Breast carcinoma occurs in about 12% of women, and DMT2 in 7% of adult population and 15% of persons older than 60 (1). One fifth of post-menopausal women diagnosed with breast carcinoma also suffers from DMT2 which exceeds all mathematical expectations and points to pathogenetic correlation of these two diseases (2, 3). The DMT2 impact to frequency and prognosis of the breast carcinoma is the consequence of independent effect of hyperinsulinemia and hyperglycemia and the indirect effect of diabetes through obesity and antidiabetic therapy effect (3, 4). Metformin chloride is a biguanide oral antihyperglycemic drug which is used as the initial therapy in DMT2 and which, pursuant to some experimental and clinical data, reduces the frequency and improves the prognosis of breast cancer in women with DMT2 (5). Insulin-dependent and insulin-independent antitumor effect of metformin opens the door for implementation of this drug in therapy and prevention of breast cancer and other malignant diseases even in patients without DMT2 (6). This paper presents literature data on mechanisms of DMT2 impact to the risk of development and prognosis of breast cancer and the anticancer potential of metformin.

DIABETES MELLITUS AND BREAST CARCINOMA

Correlation of DMT2 with the incidence and prognosis of malignant diseases is confirmed by multiple epidemiological and clinical studies (2, 4, 7, 8). DMT2 increases two times the risk of liver, pancreatic, endometrial carcinoma occurrence, and for 20%-50%, it increases the risk of occurrence of colorectal, breast and urinary bladder carcinoma. There are no sure evidences that in DMT2 cases, the frequency of occurrence of the lung carcinoma is increased, while the prostate carcinoma is rarer in men with diabetes (9). Breast carcinoma occurrence is 1.2-1.8 times more frequent in women with DMT2 than in women without diabetes (9, 10). Besides this, DMT2 worsens the prognosis and increases the mortality of patients in most of malignant tumors including breast cancer (11). Meta-analysis of larger number of studies showed that women with breast carcinoma and DMT2 have 50% greater risk of death than women without diabetes and that the carcinoma diagnosis in women with diabetes is set in more advanced stages of the disease (12).

The mechanisms of DMT2 effect to frequency and prognosis of breast carcinoma are complex and besides a direct effect of insulin resistance, hyperinsulinemia and hyperglycemia, include the effect of accompanying obesity and anti-diabetic therapy.

INSULIN RESISTANCE, HYPERINSULINEMIA AND HYPERGLYCEMIA

Resistance of insulin receptors in tissues, especially in liver, skeletal muscles and the fat tissue is the first step in development of DMT2 and it precedes it for a long time. The insulin resistance consequence is hyperinsulinemia, which in time becomes insufficient for regulation of glucose metabolism due to which hyperglycemia occurs.

Receptors related to insulin activity and insulin growth factors 1 and 2 (IGF1, IGF2) are insulin receptors A (IR-A) and B (IR-B), a receptor for IGF1 (IGF1-R) and a hybrid receptor composed of parts of IR and IGF1-R. IR-B receptors are expressed in different tissues, especially in liver, skeletal muscles and adipocytes and are responsible for metabolic effects of insulin (13). On the other hand, IR-A receptors are expressed in fetal and tumor cells and their activation by insulin or IGF2 stimulates proliferation, neoangiogenesis and metastatic potential, and reduces apoptosis (14, 15). IGF1, through IGF1-R, IR-A and hybrid receptors increases the mitogenic and proliferative activity of normal and most of the cancer cells (16). Linkage of ligands to insulin, IGF and hybrid receptors, activates two signaling pathways, MAPK (mitogen-activated protein kinase), responsible for mitosis, proliferation and apoptosis, and phosphatidylinositol 3-kinaza (PI3K/AKT) with activation of mTOR (mammalian target of rapamycin), responsible for synthesis of proteins and regulation of metabolism (17). Disorders in PI3K/AKT signaling pathway are often present in breast carcinoma and are the cause of resistance to chemotherapy, trastuzumab, tamoxifen and metformin (18). Activating mutation of PIK3CA subunit is

found in 20%-35%, mutation and loss of PTEN (phosphatase and tensin homolog) (negative regulator of the PI3K/AKT pathway) in 40%, and the overexpression of HER2 (human epidermal growth factor receptor 2) (activator of the PI3K/AKT pathway) in 30% of breast carcinoma (19-21). Hyperinsulinemia impacts the carcinoma risk not only by direct mitogen effect of insulin through insulin, IGF and hybrid receptors, but also indirectly, by increase of the level of the bio-available IGF1 and the sensitivity of IGF1-R to ligands as well as by the increase of hybrid receptor expression in the cancer cells (22, 23). The breast carcinoma cells pronouncedly express IR-A, IGF1-R and hybrid receptors due to which they are, contrary to the normal epithelial cells, very sensitive to proliferative and mitogenic effect of insulin and IGF1 (23). Indirectly, insulin, through changes of glycemia and other mechanisms, affects the activation of the AMPK (adenosine monophosphate kinase) signaling pathway.

Large number of experimental and clinical studies showed that DMT2 affects the risk and prognosis of breast carcinoma both accompanied with obesity or independently from the body mass of the patients. Meta-analysis of epidemiological studies showed that a high level of C-peptides and insulin in pre-diabetic phase in obese persons increases the risk of breast carcinoma and other malignant tumors, except prostate carcinoma (24). The conclusion of a similar meta-analysis is that women with DMT2 are at 20% greater risk of getting breast carcinoma than the women without diabetes (10). Gunter et al. (25) study and Nurses' Health Study (26) showed that DMT2 increases the risk of breast cancer development in post-menopausal women independently of obesity.

Hyperinsulinemia and DMT2 have a negative effect to breast carcinoma prognosis. Hyperinsulinemia in women with breast carcinoma increases total mortality for 31% and carcinoma-related mortality for 35%, and the correlation is especially expressed in women with developed DMT2, normal body mass, advanced stage of the disease and positive estrogen receptors (27). Women with breast cancer and DMT2 are up to two times at greater risk of death (28, 29) and greater risk of developing the distant metastases than women without diabetes (30).

Hyperglycemia in DMT2 induces insulin independent and energy inferior aerobic glycolysis, whose metabolites induce oxidative stress in a cell which is suitable for genetic instability and development of carcinoma (31, 32). The ARIC study (33) monitored the impact of the HbA1c level in women with and without diabetes to the cancer and cancer-related death risks: women without DMT2 with chronically increased glycemia are at greater risk of carcinoma and death than the normoglycemic women and women with well regulated diabetes. A long-term monitoring of over 3000 women with early stage breast carcinoma showed that the level of HbA1c higher than 7% increases the risk of death for 2.35 times, and for 26%, it increases the risk of disease recidive, but that it does not affect the remission duration (34). However, meta-analysis of four acknowledged studies in which intensive and standard therapies of DMT2 were compared, showed neither the differences in risks of malignant diseases' development, nor the differences in mortality of carcinoma patients (35). Epidemiological studies proved the effect of insulin resistance (36), hyperinsulinemia (27, 33, 37, 38) and hyperglycemia (33) in pre-diabetic period and DMT2, to the risk of breast carcinoma development and the risk of the patients' death. About 30% of women, including women with breast carcinoma, have asymptomatic non-diagnosed or late diagnosed DMT2 (39). This is the reason why the questions like: which glucose metabolism disorder mechanisms are dominant in development of breast carcinoma, what is the minimum of their duration, whether there are individual differences in these mechanisms, still remain open. The responses to the open questions are significant for selection of the anti-diabetic therapy, but also for assessment of correlation of the anti-diabetic therapy and the carcinoma development. Besides this, the accompanied effect of DMT2 and obesity exists in about 80% of women, which, in pathogenetic picture of most of the patients with breast carcinoma includes the mechanisms which connect obesity and cancer, relatively independently from the glucose metabolism disorder.

OBESITY

An excessive body mass and obesity most usually precede insulin resistance, DMT2 and other factors of the metabolic syndrome and a large number of studies showed that obesity increases the risk of carcinoma development, including breast carcinoma, especially in women in post-menopausal age (40-46). Also, obesity worsens the prognosis and increases the mortality in breast cancer patients (47-49). Obese breast cancer patients have larger tumor mass at the time of carcinoma diagnosis, shorter remissions and a greater risk of distant metastases development, than the patients with normal body mass (50, 51). Reduction of the excessive body mass, by physical activity or diet, improves the breast carcinoma prognosis (52, 53).

Obesity impact to the risk of development and prognosis of the breast carcinoma and other malignant tumors is very complex. Fat tissue is the endocrine organ in which macrophages synthesize inflammatory cytokines (IL-6, TNFα and other), and adipocytes, the adipocytokines (leptin, adiponectin) and estrogen in women in post-menopausal age (54, 55). IL-6 and other inflammatory cytokines, by activation of the STAT3 (signal transducer and activator of transcription 3) signaling pathway increase proliferation and invasiveness of malignant cells and suppress antitumor immunity (56). Adipocytes synthesize leptin, whose level positively, and adiponectin, whose level negatively correlate with the quantity of fat tissue and BMI. Both adipokines act through specific receptors and their effect is mutually dependant (57-59). Leptin, besides adipocytes, is synthesized by epithelial cells of the breast, ovaries, placenta, liver, muscles and other tissues and by activation of JAK2/STAT (janus kinase 2/ signal transducer and activator of transcription), PI3K/Akt, MAPK, Her2/neu signaling pathways, it increases the proliferative potential, survival and invasiveness of malignant cells. Besides this, leptin encourages synthesis of inflammatory cytokines in macrophages (60), activates proangiogenic factors and prevents apoptosis of endothelial cells (61, 62) and, by activation of aromatize in adipocytes, stimulates synthesis of estrogen (63). Expression of leptin receptors and a high level of leptin correlate with greater risk of breast carcinoma in post-menopausal age and worse disease prognosis (64-66). Adiponectin suppresses tumor growth and metastatic potential by activation of AMPK/mTOR signaling pathway and tumor suppressors LKB1 (liver kinase B1) and p53 (67, 68). Furthermore, it inhibits inflammation, angiogenesis and hyperinsulinemia (69). Low level of adiponectin, increases the breast carcinoma risk and worsens its prognosis (36, 70).

Estrogens in women in pre-menopause are synthesized in ovaries during the menstrual cycle. In women in post-menopausal period, fat tissue expresses aromatase enzyme complex which converts androgens produced in the adrenal gland and ovaries in estrogens, and the aromatase activity is increased by the inflammatory cytokines and leptin (72). Furthermore, obesity and hyperinsulinemia reduce synthesis of estrogen transporter SHBG (sex hormone binding protein) thus increasing the quantity of bio-available estrogens (73). In breast cancer patients, the level of estrogen in circulation is elevated, and is extremely high in the breast tissue (74). Estrogens stimulate proliferation of carcinoma cells through specific estrogenic receptors (ERα) which, as well as IGF-R, activate the MAPK signaling pathway. In obese women in post-menopausal age, the frequency of ERα+ and PR+ (progesterone receptor positive) is increased in breast carcinoma, while the correlation between the body mass and the risk of HER-2+ carcinoma is most often negative in all age groups (75, 76). It seems that the expression of estrogenic and progesterone receptors is characteristic for an early phase of the breast carcinoma development because most of the advanced and metastasized tumors do not express these receptors (77, 78). Estrogen level effect to risk of development of breast carcinoma is extremely expressed. In accordance with the meta-analysis of nine prospective studies, the breast carcinoma risk is doubled in women with the increased estrogen level in blood (79). Tworoger et al. (80) found that the high level of estrogen increases the breast carcinoma risk in post-menopausal women, especially those with the elevated level of C-peptides and IGF1, which points to the common effect of estrogen and insulin in the breast carcinoma development. Large quantity of the fat tissue changes the pharmacokinetics of many drugs and reduces the therapy effect of the aromatase inhibitors (81).

The effect of hyperinsulinemia and obesity to development of breast carcinoma is interwoven and the questions whether their impact is independent, individual or different in various carcinoma stages still remains open. Goodwin et al. (82) found that hyperinsulinemia parameters (insulinemia, C-peptide, HOMA index) have a dominant prognostic effect in the first five years from the breast cancer diagnosis, and that the obesity parameters (BMI, leptin) have the primary role in the later stage of the disease. By the data analysis of the Women's Health Initiative registry, Chlebowski RT found that the breast carcinoma risk significantly depends on the level of insulin in women with normal BMI, the difference is at the statistically significant borderline in women with BMI 25-35 kg/m^2, and it disappears in women with BMI>35 kg/m^2 (37, 83). Further research within this segment may give the basis for selective application of the anti-diabetic drugs in prevention and treatment of the breast carcinoma in obese women, women with diabetes and women without any metabolic disorders.

ANTI-DIABETIC THERAPY

Besides human insulin and its analogue, biguanide (metformin-chloride), derivatives of sulfonylurea (glibenclamide, glipizide, glycvidon, gliklazide, glimepirid), alpha-glucosidase inhibitors (acarbose), thiazolidinediones (rosiglitazone, pioglitazone), 4-dipeptidil-peptidaze inhibitors (sitagliptin), GLP-1 analogues (exenatide and liraglutide), and very often their combinations, are used in the treatment of DMT2. Better knowledge of pathogenetic relation of hyperinsulinemia and the cancer actualizes the question of a direct or indirect effect of the anti-diabetic drugs to the frequency and prognosis of cancers of various localities. A logical presumption is that the exogenous insulin and the drugs which elevate the level of endogenous insulin increase the risk, and the drugs which reduce the needs for insulin and hyperinsulinemia, reduce the risk of cancer development.

Insulin and the insulin analogues are the first line therapy in treatment of diabetes type 1 while in DMT2, it is used in about 40%-80% of patients in the advanced stages of the disease (84). Maximal level of insulinemia is significantly greater after subcutaneous administration of the human insulin than during the physiological endogenous secretion of insulin which may amplify its carcinogenic effect. Besides this, insulin analogues, especially glargine, activate IGF1-R more than the human insulin, thus encouraging proliferation and mitosis of cell (85, 86). A German and Swedish study found a dose-dependent carcinoma frequency in patients treated with glargine, in relation to those treated with human insulin (87, 88). A Scottish study concluded that glargine generally does not increase the cancer risk except in case of the breast carcinoma, with reference that women treated with glargine were of older age and with more comorbidities (89). Analysis of data for about 20.000 Danish patients treated with insulins unexpectedly showed that the carcinoma risk was greater in patients treated with human insulin than those treated with glargine, except in case of breast cancer which is more frequent in the latter case (90). Suissa et al. found that the risk of carcinoma occurrence is 2.7 times greater only after five years of the glargine therapy in women who previously were treated with the human insulins (91). A five-year long prospective monitoring of patients treated with human NPH insulin or glargine did not prove any carcinogenic potential of the insulin analogue (92). The findings of the stated studies are the subject of discussions and the final response is expected from the prospective studies which are ongoing.

Sulphonylurea derivatives stimulate release of the endogenous insulin deposited in β-cells and, per some studies, increase the risk of cancer of various localizations and the cancer-related death (93, 94). Analogues GLP-1 (glucagon like peptide 1) of the intestinal hormone, suppress the postprandial hyperglycemia and increase glucose-dependent secretion of insulin. Oral dipeptidyl peptidase 4 (DPP-4) inhibits the enzymes which dissolve endogenous GLP-1, thus helping its effects. There are no sure proofs that the drugs from these groups, through increased secretion of the endogenous insulin, increase the risk of cancer, but their use in treatment of DMT2 is also relatively short (3).

Thiazolidinediones are agonists of PPARγ receptors (peroxisome proliferator-activated receptor γ) which, in the presence of insulin, act hypoglycemically. Some experimental data showed the anticancer effect of these drugs, but the meta-analysis of the clinical trials did not prove the effect of these drugs to the frequency of cancer (95). Nevertheless, the drugs from this group have been in use for a short period and the final evaluation of their anticancer effect should be expected from current clinical studies (96). Unlike thiazolidinediones, multiple experimental and clinical studies proved that metformin reduces cancer incidence, including breast carcinoma, and that it positively affects the course and the outcome of malignant diseases.

METFORMIN IN BREAST CARCINOMA

Metformin sulfate is biguanide derivative which is widely used in treatment of DMT2. Metformin is an efficient and a relatively safe drug accompanied by mild gastrointestinal problems with the most severe side effect of lactate acidosis, which occur extremely rarely, mostly in persons older than 80 and in patients with damaged function of liver, kidneys or heart (97). There are no more significant interactions with the drugs which are used in breast carcinoma treatment, and it reacts with some of them synergistically. Today, there are experimental and clinical proofs that metformin has the anticancer effect through insulin-dependent and insulin-independent mechanisms.

Seven years ago, Evans et al. proved that metformin reduces the cancer incidence for 24% in patients with DMT2 and that the anticancer effect depends on the therapy dose and duration (98). Earlier meta-analysis of clinical trials showed that metformin reduces the cancer risk in DMT2 for nearly one third as well as the patients' mortality (99). The newest analysis of twentyish epidemiological studies confirmed the anticancer effect of metformin in colorectal carcinoma, liver, pancreatic, lung and breast carcinoma (6). Systematic analysis of 439 statements and the meta-analysis of eight selected studies found that the risk of breast carcinoma in DMT2 is for 17% less in patients treated with metformin, i.e. 25% less in women who were taking metformin in the period longer than three years (5). Breast carcinomas which develop in patients treated with metformin are more often PR+ and rarely triple negative (100, 101).

Anticancer effects of metformin are indirect (insulin dependent) and direct (insulin independent). Both ways of metformin impact are initiated by activation of adenosine monophosphate kinase (AMPK), caused by previous activation of LKB1 which are crossed at the level of TSC2 (tuberous sclerosis complex 2) and ended by regulation of the mTOR activity. LKB1 is a tumor suppressor and a fast activator of AMPK, and AMPK is a main cell energy sensor which reacts to energy stress and the increase of the AMP/ATP ratio (102). Insulin, through IR and IGF-R and PI3K/AKT signaling pathways, inhibits, and the activated AMPK stimulates phosphorylation and activity of the TSC2. Metformin, by activation of TSC2, reduces the activity of mTOR, thus reducing synthesis of proteins, proliferation, cell growth and angiogenesis, while insulin has the opposite effect (Figure 1) (103-105).

Insulin-dependent anticancer effect of metformin is mostly realized in liver, skeletal muscles, fat tissue and pancreatic β-cells. By activation of AMPK, metformin inhibits glyconeogenesis and synthesis of lipids in liver and the fat tissue, stimulates consumption of glucose and lipids in skeletal muscles and reduces production of insulin in pancreatic β-cells (106, 107). The end result is reduction of glycemia and hyperinsulinemia, while lower glycemia reduces the need for endogenous insulin. By reduction of hyperinsulinemia, the activation of IR and IGF1-R is reduced (induced by insulin and IGF1) and PI3K signaling pathway and proliferative and mitotic activity of the carcinoma cells is suppressed (108, 109). Insulin-dependent effect of metformin is very significant in breast carcinoma due to high expression of insulin receptors in cancer cells (110).

By activation of AMPK with the causal inhibition of the mTOR signaling pathway, a part of the insulin-dependent effects of metformin is realized in epithelial and cancer cells (111). Metformin may suppress mTOR

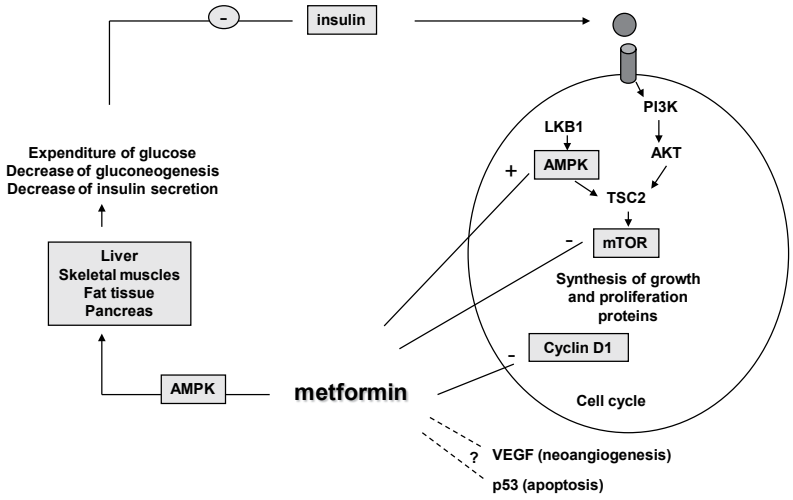

Figure 1. Mechanism of metformin action in breast carcinoma

expression independently from LKB1-AMPK-TSC2 basis by a direct inhibition of Rag-GTPase (Rag-guanosine triphosphatase) (112). Metformin, probably through mTOR, inhibits neoangiogenesis and inflammation by reduction of vascular endothelial growth factor (VEGF), TNFα (tumor necrosis factor α), hypoxia inducible factor α (HIFα) and plasminogen activator inhibitor (PAI-1) production (113). Inhibition of mTOR by metformin induces reduction of HER2 expression and apoptosis of carcinoma cells by activation of p53 tumor suppressors (114). By stimulation of AMPK pathway in adipocytes, metformin reduces aromatase activity and estrogen production (115). Furthermore, metformin, independently from the effects to mTOR, reduces the cyclin D1 expression and, thus, proliferation of carcinoma cells (116). It is possible that metformin, by its immunomodulatory effect or reduction of level of B12 vitamin, affects the malignant potential of carcinoma cells (117). In vitro, anticancer effect of metformin does not depend on expression of estrogenic, progesterone, HER2 receptors and expression of p53 suppressors (118). Also, metformin, by suppression of CD24, mucin-like adhesion molecule, reduces metastatic potential of triple negative breast carcinoma (119).

Resistance to anticancer effect of metformin may be the consequence of mutation of a gene line at PI3K signaling pathway, most frequently present in breast carcinoma, the absence of LKB1 and the reduction of OCT1 expression, OCT1 being a protein responsible for the drug influx into the cell (6).

Experimental and clinical studies showed that women with breast carcinoma and DMT2, treated with metformin, have better prognosis (6). By administration of metformin during neoadjuvant therapy of breast carcinoma, 24% of pathological complete remissions are achieved which is three times greater than in patients with diabetes who did not receive metformin, while 16% of remissions were achieved in women without DMT2 (and without metformin). A positive effect of metformin to the remission induction is independent from other prognostic factors but it does not result in longer survival of the patients (120). Baryaktar et al. found that metformin reduces risk of metastasizing, but that it does not affect the survival duration in patients with triple negative breast carcinoma (121). A study, in which standard neoadjuvant therapy with and without metformin,

including 3500 women with breast carcinoma, stages I and II, showed that metformin significantly reduces a disease recidive related risk (122). A trial of metformin implementation in a standard dose in obese women with breast carcinoma without DMT2 showed that metformin reduces the level of insulin for 22.4% and increases the insulin sensitivity for 25.6%, significantly reduces body mass, total and LDL cholesterol, without any severe side effects (108). The stated study encouraged implementation of metformin in adjuvant and neoadjuvant therapy of breast carcinoma in women without diabetes. Metformin relatively quickly reduces proliferative and increases apoptotic potential of carcinoma cells, joined with some other anticancer drugs or independently, especially in women with hyperinsulinemia (123-127).

Anticancer effect of metformin is proved in preclinical and clinical studies of breast carcinoma and other tumors, in women with and without DMT2. Further research is necessary in order to create basis for individual administration of metformin in breast carcinoma therapy depending on the dose and the dominant effect of metformin, possible resistance pathways, carcinoma characteristics, combination with other anticancer drugs and change of the patients' nutrition regimen and physical activity.

CONCLUSION

DMT2 through accompanying obesity, hyperinsulinemia and unhealthy habits increases the frequency of breast carcinoma and worsens its prognosis. The drugs which increase the level of endogenous insulin and some forms of exogenous insulin may potentiate harmful effects of diabetes to the carcinoma. Experimental and clinical data showed that metformin, a widely used antidiabetes drug, reduces the frequency of breast carcinoma and has the anticancer effect. Insulin-dependent and direct effects of metformin reduce proliferation and growth, quicken apoptosis of carcinoma cells and reduce metastatic potential of the tumor. Relative safety of administration and favorable effects of combining of metformin with the standard drugs, made this drug potentially useful in adjuvant and neoadjuvant therapy and prevention of breast carcinoma in women with DMT2 and obese women. New studies are necessary, which would make a solid basis for individual implementation of metformin in breast carcinoma depending on the carcinoma characteristics, individual characteristics of the patient and the dominant mechanisms of action of, and resistance to metformin.

Conflict of interest

We declare no conflicts of interest.

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