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REVIEW ARTICLE

Antidiabetic pharmacology: a link between metabolic syndrome and neuro-oncology?

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Summary

One of the main topics of the annual meeting of the American Society for Clinical Oncology in 2011 were the results presented on breast cancer chemotherapy and concomitant administration of the oral antidiabetic metformin. The overall agreement was that current evidence is just enough to dramatically change the clinical practice of oncology, and in our case, brain cancer treatment, and that further research is needed to address the relationship between diabetes, metabolism, insulin analogues and neoplasia. Still, it is very interesting to explore the potentially beneficial effects of metformin in glioma chemo/immunotherapy and wait for results in the clinic.

In the current paper we present the cell and molecular aspects of the metabolic syndrome, metformin administration and cancer chemotherapy, with a special emphasis in neuro-oncology, since brain tumors are usually devastating diseases with an extremely high mortality within two years of diagnosis even when surgical, radiotherapeutic and chemotherapeutic interventions are applied.

Key words: cancer stem cell metabolism, chemotherapy, glioma, metformin

Background

Chemotherapy can effectively reduce the initial cancerous lesion, but the disease will usually ultimately relapse. In order to explain all the clinical data, the cancer stem cell hypothesis suggests that a tumor contains a small number of tumor-initiating, self-renewing cancer cells with stem-like characteristics within a population of non-tumor forming cancer cells. Unlike other types of tumor cells, cancer stem cells (CSCs) are resistant to conventional chemotherapy and have the ability to regenerate the initial tumor after treatment. Due to this observation, drugs that selectively target CSCs offer a great promise for cancer therapy, may it be chemo/ radio or immunotherapy, although none are known to this moment [1,2].

Brain tumors are usually devastating diseases with an extremely high mortality within two years of diagnosis even when surgical, radiological and chemotherapeutic interventions are applied [3]. Gliomas, the most frequent tumors of the central nervous system tumors, are treated by gross total resection when possible, correlated with a better clinical outcome and improved neurological functions. But because such tumors are very often infiltrative, total resection is difficult to achieve, which results in poor survival. Subsequent treatments with intravenously or intrathecally administered chemotherapeutic drugs have limited use because of the adverse systemic side-effects and poor blood-brain barrier penetration, despite state-of-the-art strategies such as gold-nanoparticles or targeted molecular therapy [4-6].

Recently, several epidemiological studies have reported that diabetes is correlated with an increased risk of breast cancer [7]. On the other hand, other data also suggest that treatment of diabetes with the biguanide metformin, a first-line drug used in patients with non-

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insulin dependent diabetes mellitus, is connected with a significantly decreased incidence of cancer when compared with other drugs such as sulfonylureas or insulin [8]. Thus, in this article we review the possible mechanisms of action of this old drug, which might exert a potentially important impact in neuro-oncology.

Cell metabolism

Cell physiology is regulated coordinately by multiple signals that include a wide variety of growth factors, availability of nutrients and intracellular ATP. When talking about cell signalling, several mechanisms of metformin's action have been proposed, out of which the most important one relates to the activation of the adenosine monophosphate-activated protein kinase (AMPK) pathway. Cell growth inhibition is partially abolished in the presence of small interfering RNAs against AMPK or AMPK inhibitors, thus demonstrating the pivotal role of this pathway [9,10]. Linear potteray culture (LKB1) expression is essential for the activation of AMPK by metformin as this drug does not inhibit cell growth in LBK1-null cells and confirms the requirement of functional LBK1 for metformin-induced AMPK activation.

Recent data show that AMPK is activated through LBK1 without an increase in the AMP/ATP ratio, but in the presence of increased reactive oxygen species (ROS) levels generated in hypoxic condition. Activation of AMPK is abolished when cells are treated with the antioxidant EUK-134 and in cells deficient in mitochondrial DNA, as hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in the AMP/ATP ratio [11] (Figure 1). Several studies suggest that metformin has an acute insulin-like effect independent of its ability to increase insulin binding, possibly by enhancing the activity of the glucose transporters. In a study on human adipocytes, metformin increased GLUT-4 protein content in the plasma membrane [12]. GLUT -1 mRNA is also expressed in human high grade gliomas [13]. Thus, the distribution of the glucose transporter in human brain tumors suggests that metformin increases the intracellular concentration of glucose in glioma cells.

In normoglycemic state, most intracellular glucose is metabolized via the energy-producing glycolytic pathway involving an initial phosphorylation by the enzyme hexokinase. Only a small percentage of glucose enters the polyol pathway but in contrast, under hyperglycemic conditions there is increased flux of glucose into the polyol pathway. While glucose has a high affinity for hexokinase, the affinity for aldose reductase is

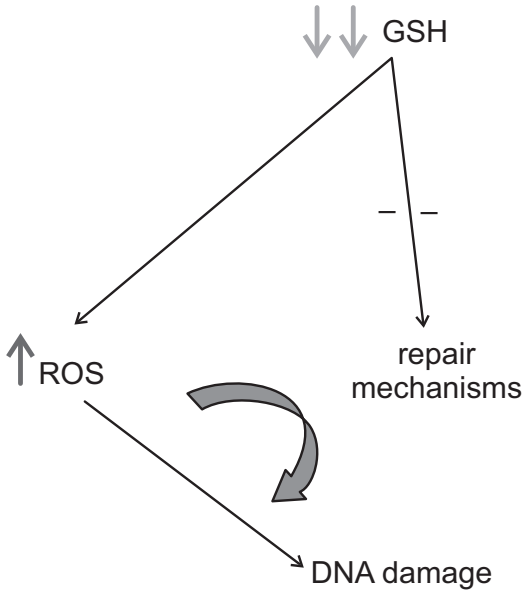

Figure 1. Any reduction in glutathione (GSH) increases cellular susceptibility to oxidative stress and also affects the DNA repair mechanisms. ROS: reactive oxygen species.

low. As a result, in the presence of high glucose concentrations, hexokinase becomes saturated and the polyol pathway becomes activated. The excess glucose is metabolized by the enzyme aldose reductase to sorbitol and eventually to fructose, in a reaction that uses NADPH (the reduced form of nicotinamide dinucleotide phosphate) as a cofactor. NADPH is also required by the enzyme glutathione reductase in a reaction that regenerates reduced GSH. This can compromise the recycling of glutathione disulphide (GSSG) to GSH, which in turn can compromise the conversion of hydrogen peroxide to water. Mitochondria produce superoxide ($O_2^{\cdot -}$) and impaired clearance of hydrogen peroxide (H_2O_2) can cause the production of superhydroxide ($^{\cdot}OH$) through the Fenton reaction [14-18] (Figures 2, 3).

Thus the ROS superhydroxide is able to activate AMPK, which leads to cell growth inhibition. Furthermore, GSH is one of the most important antioxidant mechanisms in the cell and any reduction in GSH increases cellular susceptibility to oxidative stress and also affects the repair mechanisms of DNA. Sorbitol is degraded slowly and does not readily diffuse across the cell membrane. The intracellular accumulation of sorbitol results in osmotic changes that potentially lead to cell damage. The increase in intracellular osmolarity, due to shunting of glucose into the polyol pathway and the consequent sorbitol accumulation, may lead to compensatory depletion of the endoneurial osmolytes taurine and myo-inositol in order to maintain osmotic balance [19].

The majority of the growth inhibitory effects of metformin are mediated through the inhibition of

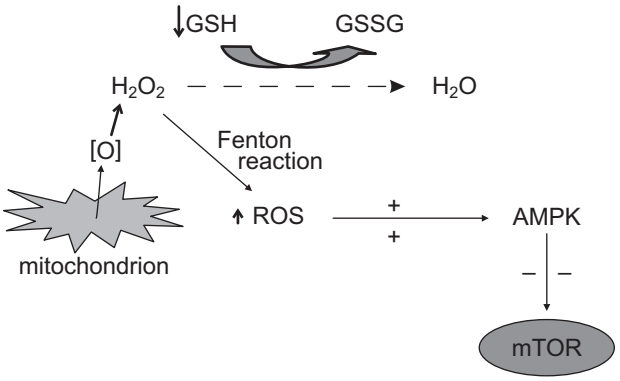

Figure 2. The reduction of glutathione (GSH) can compromise the conversion of hydrogen peroxide (H_2O_2) to water. Mitochondria produce superoxide, and the impaired clearance of H_2O_2 can cause the production of superhydroxide (OH) through the Fenton reaction. ROS: reactive oxygen species. (+) represents the activation of the pathway and (-) its inhibition.

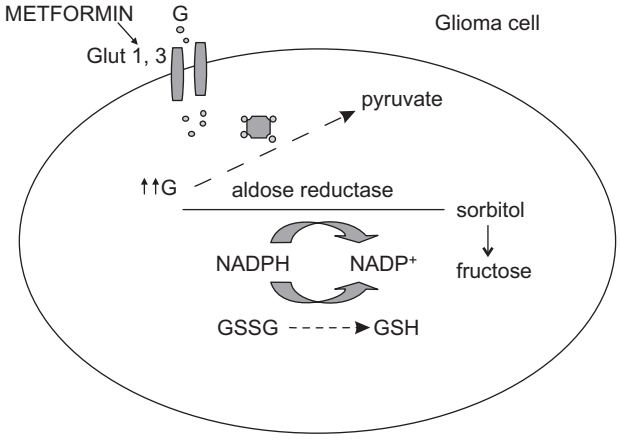

Figure 3. Metformin enhances the activity of glucose transporters (Glut1/Glut3), thus increasing the intracellular concentration of glucose. While glucose has a high affinity for hexokinase, the affinity for aldose reductase is low. As a result, in the presence of high glucose concentrations, hexokinase becomes saturated and the polyol pathway becomes activated. The excess glucose is metabolized by the enzyme aldose reductase to sorbitol, a polyol, and eventually to fructose, in a reaction that uses NADPH (the reduced form of nicotinamide dinucleotide phosphate) as a cofactor. NADPH is also required by the enzyme glutathione reductase in a reaction that regenerates reduced glutathione (GSH). This can compromise the recycling of glutathione disulphide (GSSG) to glutathione."G" stands for G-type protein.

mTOR signalling. Activation of AMPK by metformin results in phosphorylation and stabilization of tuberous sclerosis complex, which integrates regulatory inputs and transmits them to mTOR. These regulatory inputs include oxygen-dependent signals and growth factor-dependent signalling pathways such as the phosphatidylinositol 3-kinase (PI3K) and the AMPK signalling pathways [20]. It should be stated that mTOR phosphorylates down-stream mediators leading to the regulation of cell cycle progression, cell growth and angiogenesis, and the activation of mTOR-dependent protein translation correlates with malignant progression, adverse prognosis and resistance to both chemotherapy and targeted therapy such as trastuzumab [21].

Clinical trials using rapamycin analogues, such as temsirolimus and everolimus, currently registered for the treatment of advanced renal cell carcinoma, have validated the importance of mTOR inhibition as an anticancer treatment strategy. However, the antitumor activity as a single agent (e.g. everolimus) in renal cell carcinoma is modest. But interestingly, preclinical studies indicate that metformin lowers cell survival to a greater extent than the mTOR inhibitor rapamycin [22,23].

It has been shown that breast CSCs acquire less DNA damage following radiation compared to non-stem cells. Following a 10 Gy isodose, there is less DNA damage and decreased ROS in breast CSCs compared to non-stem tumor cells. There is also an increased expression of genes involved in GSH synthesis, suggesting that CSCs have an effective DNA repair mechanism through increased levels of ROS scavengers [24]. For example, exposure of CSCs to buthionine sulfoximine, a pharmacologic agent producing depletion of GSH, will eventually end up in CSCs radiotherapy resistance.

Metformin use in medical oncology. A bridge towards brain cancer research

Metformin is a biguanide, a widely prescribed oral medication used as front-line therapy for type 2 diabetes and polycystic ovary syndrome [25,26]. Population studies suggest that metformin decreases the incidence of cancer and cancer-related mortality in diabetic patients. Recently, exciting preclinical studies have shown that metformin can inhibit the growth of cancer cells, including breast cancer *in vitro* and of tumors *in vivo*. More recently, a retrospective study of patients who received neoadjuvant chemotherapy for breast cancer showed that diabetic cancer patients receiving metformin during their neoadjuvant chemotherapy had a higher pathological complete response rate than diabetic patients not receiving metformin [27].

Initial experiments showed that metformin was capable of reducing proliferation in prostate, colon, and breast cancer cell lines through cell cycle inhibition shown by an important decrease of cyclin D1 protein level [28]. Subsequently in vivo experiments using intraperitoneal or oral metformin in nude mice resulted in tumor growth inhibition. To evaluate the effect of metformin on cell proliferation, investigators looked at the effect of this drug *in vitro* in a group of breast, ovarian, and prostate cancer cells lines. In MCF-7 human breast

cancer cells, metformin acted as a growth inhibitor rather than an insulin sensitizer [29]. In addition, they found that exposure to a growth inhibitory concentration of drug by means of the AMPK pathway activation and mTOR inhibition can lead to decreased protein synthesis, blocking both growth and proliferation.

In nude mice metformin modestly inhibits tumor growth of xenografts of a triple-negative breast cancer cell line that lacks the estrogen, progesterone, and HER2 receptors [30]. These observations finally suggest the possibility that metformin might be useful as an anticancer drug in nondiabetic contexts. Other data show that metformin selectively kills breast cancer stem cells. Investigators took four genetically different types of breast cancer cells and added metformin to doxorubicin. The combination was able to kill both non-stem and CSCs in culture, and showed reduced tumor mass and prolonged remission more than with either drug alone in a xenograft mouse model. All of these preclinical oncology models demonstrated the anticancer effects of metformin in all breast cancer subtypes as well as in cytotoxic therapy-resistant models and provided a rationale to study this drug in the clinic.

Glucose transport in the brain is achieved by glucose transporters, including GLUT or SLC2A1, with at least 12 GLUT subtypes known so far. In humans very little data have been published on this topic, but, nevertheless, a slight interest on the expression of GLUT-1 and GLUT-3 has been reported. A wide variety of researches have shown a close relationship between GLUT-3 and carcinogenesis, tumor development or the unfavorable prognosis of various malignancies [31,32]. Expression of GLUT-3 was detected in glioma cell membrane and cytoplasm, which was increasing gradually with the increase of the World Health Organisation (WHO) grade [33]. Boado et al. have proven that in malignant glial cells, the expression level of GLUT-3 correlated with the biologic aggressiveness of the tumor. Similar studies also testify that GLUT-3 is more detectable in high-grade than in low-grade gliomas, indicating that GLUT-3 may be the predominant glucose transporter in the GLUT family of highly malignant human glioma [34].

Concluding remarks

The association between metabolic syndrome and cancer has been a subject of debate in internal medicine for the last decades, but only recently a clear link has been proven in the case of breast cancer patients previously diagnosed with type 2 diabetes mellitus. The overall agreement is that current evidence is just not enough to dramatically change the clinical practice of oncology, and in our case brain cancer treatment, and that further research is needed to address the relationship between diabetes, metabolism, insulin analogues and neoplasia. Still, it is very interesting to explore the potentially beneficial effects of metformin in glioma chemo/immunotherapy and wait for results in the clinic.

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References

1. Jordan CT, Guzman ML, Noble N. Cancer stem cells. N Engl J Med 2006; 355: 1253-1261.
2. Tomuleasa C, Soritau O, Rus-Ciuca D et al. Isolation and characterization of hepatic cancer cells with stem-like properties from hepatocellular carcinoma. J Gastrointestin Liver Dis 2010; 19: 61-67.
3. Behin A, Hoang-Xuan K, Carpentier AF, Delattre JY. Primary brain tumours in adults. Lancet 2003; 361(9353): 323-331.
4. Wen PY, Kesari S. Malignant gliomas in adults. N Engl J Med 2008; 359: 492-507.
5. Stupp R, Tonn JC, Brada M, Pantheroudakis G. High-grade malignant gliomas: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21(Suppl 5): v190-v193.
6. Albesiano E, Han JE, Lin M. Mechanisms of local immunoresistance in glioma. Neurosurg Clin N Am 2010; 21: 17-29.
7. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care 2010; 33: 1304-1308.
8. Jalving M, Gietema JA, Lefrandt JD et al. Metformin: taking away the candy for cancer? Eur J Cancer 2010; 46: 2369-2380.
9. Jansen M, Ten Klooster JP, Offerhaus GJ, Clevers H. LKB1 and AMPK family signaling: the intimate link between cell polarity and energy metabolism. Physiol Rev 2009; 89: 777-798.
10. Lineham WM, Srinivasam R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol 2010; 7: 277-285.
11. Emerling BM, Weinberg F, Snyder C et al. Hypoxic activation of AMPK is dependent on mitochondrial ROS but independent of an increase in AMP/ATP ratio. Free Radic Biol Med 2009; 46: 1386-1391.
12. Grisouard J, Timper K, Radimerski TM et al. Mechanisms of metformin action on glucose transport and metabolism in human adipocytes. Biochem Pharmacol 2010; 80: 1736-1745.
13. Stockhammer F, von Deimling A, Synowitz M, Blechmidt C, van Landeghem FK. Expression of glucose transporter 1 is associated with loss of heterozygosity of chromosome 1p

in oligodendroglial tumors WHO grade II. J Mol Histol 2008; 39: 553-560.
14. Brooks GA. Cell-cell and intracellular lactate shuttles. J Physiol 2009; 587: 5591-5600.
15. Jezek P, Plecita-Hlavata L, Smolkova K, Rossignol R. Distinctions and similarities of cell bioenergetics and the role of mitochondria in hypoxia, cancer, and embryonic development. Int J Biochem Cell Biol 2010; 42: 604-622.
16. Peng TI, Jou MJ. Oxidative stress caused by mitochondrial calcium overload. Ann N Y Acad Sci 2010; 1201: 183-188.
17. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unraveling the mechanism. Lancet 2010; 375(9733): 2267-2277.
18. Corti A, Casini AF, Pompella A. Cellular pathways for transport and efflux of ascorbate and dehydroascorbate. Arch Biochem Biophys 2010; 500: 107-115.
19. Chung SS, Ho EC, Lam KS, Chung SK. Contribution of polyol pathway to diabetes-induced oxidative stress. J Am Soc Nephrol 2003; 14(8 Suppl 3): S233-236.
20. Zadra G, Priolo C, Patnaik A, Loda M. New strategies in prostate cancer: targeting lipogenic pathways and the energy sensor AMPK. Clin Cancer Res 2010; 16: 3322-3328.
21. Jones KL. Buzdar AU. Evolving novel anti-HER2 strategies. Lancet Oncol 2009; 10: 1179-1187.
22. Motzer RJ, Escudier B, Oudard S et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomized, placebo-controlled phase III trial. Lancet 2008; 372(9637): 449-456.
23. Amato RJ. Current immunotherapeutic strategies in renal cell carcinoma. Surg Oncol Clin N Am 2007; 16: 975-986.
24. Cook JA, Gius D, Wink DA, Krishna MC, Russo A, Mitchell JB. Oxidative stress, redox, and the tumor microenvironment. Semin Radiat Oncol 2004; 14: 259-266.
25. Diamanti-Kandarakis E, Economou F, Palimeri S, Christakou C. Metformin in polycystic ovary syndrome. Ann N Y Acad Sci 2010; 1205: 192-198.
26. BARI 2D Study Group, Frye RL, August P et al. A randomized trial of therapies for type 2 diabetes and coronary artery disease. N Engl J Med 2009; 360: 2503-2515.
27. Martin-Castillo B, Dorca J, Vazquez-Martin A et al. Incorporating the antidiabetic drug metformin in HER2-positive breast cancer treated with neo-adjuvant chemotherapy and trastuzumab: an outgoing clinical-translational research experience at the Catalan Institute of Oncology. Ann Oncol 2010; 21: 187-189.
28. Chong CR, Chamber BA. Mysterious metformin. Oncologist 2009; 14: 1178-1181.
29. Berstein LM, Yue W, Wang JP, Santen RJ. Isolated and combined action of tamoxifen and metformin in wild-type, tamoxifen-resistant, and estrogen-deprived MCF-7 cells. Breast Cancer Res Treat 2010 (in press).
30. Liu B, Fan Z, Edgerton SM et al. Metformin induces unique biological and molecular responses in triple negative breast cancer cells. Cell Cycle 2009; 8: 2031-2040.
31. Kaida H, Ishibashi M, Yuzuriha M et al. Glucose transporter expression of an esophageal gastrointestinal tumor detected by F-18 FDG PET/CT. Clin Nucl Med 2010; 35: 5050-5059.
32. Ayala FR, Rocha RM, Carvalho KC et al. GLUT1 and GLUT3 as prognostic markers for oral squamous cell carcinoma. Molecules 2010; 15: 2374-2387.
33. Liu Y, Li YM, Tian RF et al. The expression and significance of HIF-1alpha and GLUT-3 in glioma. Brain Res 2009; 1304: 149-154.
34. Boado RJ, Black KL, Partridge WM. Gene expression of GLUT3 and GLUT1 glucose transporters in human brain tumors. Brain Res Mol Brain Res 1994; 27: 51-57.