Metformin and the risk of cancer in type 2 diabetes: methodological challenges and perspectives

Xilin Yang[1], Juliana CN Chan[2,3,4]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Tianjin Medical University, Tianjin 300070, China; [2]Department of Medicine and Therapeutics, [3]Hong Kong Institute of Diabetes and Obesity, [4]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China

Correspondence to: Dr. Xilin Yang. P.O. Box 154, Tianjin Medical University, Heping District, Tianjin 300070, China. Email: yxl@hotmail.com or yangxilin@tijmu.edu.cn.

Submitted Jun 10, 2014. Accepted for publication Jun 10, 2014.
doi: 10.3978/j.issn.2305-5839.2014.06.07
View this article at: http://10.3978/j.issn.2305-5839.2014.06.07

Metformin is a classical oral antidiabetes drug (OAD) that has been used for more than half a century for management of type 2 diabetes (T2D). Recently, there is growing interest in this drug because of its potential anticancer properties (1). A large number of clinical studies have studied the association of use of metformin and the risk of cancer for possible prevention and treatment; while many animal and cell-based studies have been conducted to explore the underlying mechanisms of the anticancer properties of metformin. On the other hand, clinical studies have been criticized to have a mysterious bias, immortal time bias, and the association between metformin use and the cancer risk was weak, just because these studies did not use methods proposed by some authors to control for immortal time bias (2).

Immortal time and other biases in epidemiological studies

Immortal time is a period in cohort study designs between the baseline of the study when risk factors for the clinical outcome were measured and the actual time of initiation of the drug treatment. During this period, the patient was not on the drug but classified as on the drug treatment. The misclassification leads to a false effectiveness of the drug for the clinical outcome (3). If so, can we use current users to test the effect of use of a drug for a clinical outcome? The answer is "no", for use of prevalent users may introduce prevalent user bias due to two reasons: (I) prevalent users were "survivors" of the use of the drug; (II) use of the drug had altered the level of risk factors and they were no longer a reflection of the risk of the clinical outcome users were to experience. The second question is whether use of the proposed methods such as use of time-varying exposure to the drug treatment during follow-up period is a solution to immortal time bias? Recent efforts to validate these proposed methods including time-varying exposure to drug in Cox proportional hazard analysis (4,5) to cope with immortal time bias shows the contrary: use of those proposed methods to control for immortal time bias themselves resulted in severely inflated relative risks and tended to lead to even more erroneous conclusions that use of OADs and other drugs in T2D increased the risk of clinical outcomes including cancers. Use of time-varying exposure Cox proportional hazard analysis is based on the assumption that exposure to the drug treatment occurs at random but this is rarely the case in real practice (5,6). OADs are used to control hyperglycemia in T2D and OAD users are more likely to have hyperglycemia. In this regard, several studies have demonstrated that high glucose was predictive of cancer among subjects without T2D (7) and among patients with T2D (8). In addition to hyperglycemia, many metabolic abnormalities, presumably stemming from insulin deficiency and abnormal cellular signalling, were found to be associated with increased risk of cancer in T2D (9-11). Users of OAD/s and other drugs in T2D are more likely at increased risk of cancer and indications to use these drugs can seriously bias the results in observational studies. Judgment regarding the reliability of the effectiveness of OADs and other drugs on cancer based entirely on whether those proposed methods for control of immortal

time bias have been used will do more harm than good. In this regard, at least three major biases, drug use indication bias, prevalent user bias and immortal time bias, must be considered simultaneously in evaluation of drug effects in T2D (4). The validation studies showed that exclusion of prevalent users to remove prevalent user bias, exclusion of immortal time from drug users to remove immortal time bias and adjustment for identified risk factors for cardiovascular diseases (CVD) resulted in the least inflated hazard ratio of renin-angiotensin system blockers for the risk of CVD (5). Use of this approach with adjustment for identified novel cancer subphenotypes (9-10) led to meaningful findings that renin-angiotensin system (RAS) blockers can reduce increased cancer risk related to low low-density lipoprotein cholesterol in T2D (11). However, this does not mean that this approach works for all the drugs. In fact, validation of statins on CVD risk using the same approach (12) generated a hazard ratio of 0.96 (95% CI: 0.72-1.27), or an inflation rate up to 52.38% against the reported effect of statins on CVD (hazard ratio: 0.63, 95% CI: 0.48-0.83) from the Collaborative Atorvastatin Diabetes Study (CARDS). It seems that unadjusted "time-varying" indications to use statins played an important role in our failure to validate the method. Further investigations are warranted to examine the feasibility for developing a time-dependent propensity score to consider the changing indication of use of drugs in T2D and its effect on removing the bias due to drug indications over time. Few studies, if any, which examined metformin's effects on cancer, have considered all these three major biases. Meta-analysis based on these observational studies did not add information but generated more confusion. In this regard, individual studies with detailed documentation of metabolic profile and drug use during long-term follow-up and good consideration of various biases including the three major biases may be more reliable than meta-analysis whose advantage is to overcome insufficient power in individual studies but not these biases.

Mechanisms of anticancer properties of metformin

Metformin acutely decreases hepatic glucose production via inhibition of the mitochondrial respiratory chain complex I and the resulting decrease in hepatic energy status activates AMP-activated protein kinase (AMPK), a main sensor and regulator in energy metabolism (13). In addition, metformin may downregulate sterol-element binding protein-1c and activates phosphorylation and inactivates acetyl-CoA carboxylase (ACC), a critical precursor for fatty acid biosynthesis to increase fatty acid oxidation and inhibition of lipogenesis (13). A large number of studies explored whether metformin has anticancer properties through an AMPK-dependent mechanism. For example, using a human pancreatic cancer cell based study, Sinnett-Smith *et al*. (14) found that metformin at low concentrations inhibited DNA synthesis through an AMPK-dependent mechanism. Qu *et al*. (15) reported that metformin can re-sensitize multidrug-resistant breast cancer cells due to activating AMPK signal pathway. Many studies also found that metformin may have anticancer properties through an AMPK-independent mechanism. For example, Liu *et al*. (16) found that metformin also has a direct inhibitory effect on mTOR, independent of AMPK. Zhang *et al*. (17) reported that metformin sensitizes human bladder cancer cells to tumor necrosis factor-related apoptosis mediated by the mTOR pathway, but independent of AMPK.

Additive interaction: a method to address subgroup effects of metformin on cancer

Many cell-based and animal-based studies used concentrations much higher than those used by patients with T2D. Indeed, these experimental findings need to be supported by evidence of epidemiological investigations before large expensive trials can be conducted. Currently, available data that can be used to address the association of metformin usage and the risk of cancer are mainly limited to patients with T2D. These patients are at increased risk of a spectrum of site-specific cancers. Thus, the critical questions that should be asked are who would benefit from use of metformin for prevention of cancer, all the patients with T2D or subgroups of patients who have increased cancer risk due to manifestation of particular subphenotypes? In other words, do we have a way to address whether metformin can reduce cancer risk through the AMPK-dependent mechanism or AMPK-independent mechanisms? Testing additive interaction may serve the purpose to address the subgroup effect of metformin on cancer. An additive interaction between two risk factors means that co-exposure to two risk factors confers a risk larger than the summation of exposure to the two risk factors individually (18). If we assign nonuse of metformin as a risk factor and an identified risk for cancer in T2D as another risk factor, the additive interaction between the identified subphenotypes and nonuse of metformin therefore indicates an effect of metformin among the subgroup with the identified

subphenotypes, i.e., metformin has a specific effect to reduce the increased cancer risk related to the identified subphenotypes, which also suggest that the mechanism related with the identified subphenotypes and metformin is a possible mechanism to link diabetes and cancer. We previously identified that low high-density lipoprotein cholesterol (HDL-C) was a subphenotypes for cancer in T2D (19). As low HDL-C is associated with an inhibited AMPK pathway (20), a significant additive interaction between low HDL-C and nonuse of metformin suggest that an abnormal AMPK signaling pathway is one possible mechanism linking T2D and cancer and use of metformin may have potential to prevent cancer in this high risk group. Along the way, we demonstrated that there was a significant additive interaction between HDL-C <1.0 mmol/L and nonuse of metformin for cancer among Chinese with T2D after considering prevalent user bias and drug use indication bias (20). Using the validated method (5) and further exclusion of immortal time among metformin users, the additive interaction between nonuse of metformin and HDL cholesterol <1.0 mmol/L remained significant: use of metformin reduced the hazard ratio of HDL-C <1.0 mmol/L for cancer from 3.90 (95% CI: 2.07-7.35) to 1.56 (0.71 to 3.42) with significant attributable proportion due to interaction (AP: 0.48, 95% CI: 0.11-0.84), suggesting that abnormal AMPK pathway is one possible mechanism linking T2D and cancer, and patients with abnormal AMPK pathway may benefit from use of metformin for prevention of cancer.

Future research in subgroup effects of metformin on cancer

Metformin may have anticancer properties through AMPK-dependent and AMPK-independent mechanisms in cell or animal-based studies. Our data support that abnormal AMPK pathway is a possible biological link between T2D and cancer and metformin can attenuate the increased cancer risk related to abnormal AMPK pathway. Nevertheless, we have failed to provide evidence that metformin can specifically attenuate increased cancer risks related to other cancer subphenotypes in T2D (9). Addressing effects of drug usage on cancer in T2D including metformin and other drugs poses a methodological challenge. In observational studies, we must consider biases from prevalent users, drug indications and immortal time simultaneously. Further validations of methods to control for these biases and development of time-dependent propensity scores for OADs and other drugs in T2D are needed, given the finding that diabetes increases the risk of many cancers. We have identified a group of novel subphenotypes for cancer in T2D (9): (I) HDL-C <1.0 mmol/L; (II) low-density lipoprotein cholesterol <2.8 mmol/L + albuminuria; (III) LDL-C <2.8 mmol/L + triglyceride <1.70 mmol/L; and (IV) LDL-C ≥3.80 mmol/L. Further explorations of subphenotypes for cancer in T2D, especially those related to the AMPK and suspected non-AMPK pathways, and to test whether metformin can reduce the increased cancer risk related with these subphenotypes will have strong implications for designing large randomized clinical trials for prevention of cancer in T2D and non-T2D.

Acknowledgements

Disclosure: The authors declare no conflict of interest.

References

1. Franciosi M, Lucisano G, Lapice E, et al. Metformin therapy and risk of cancer in patients with type 2 diabetes: systematic review. PLoS One 2013;8:e71583.
2. Suissa S, Azoulay L. Metformin and the risk of cancer: time-related biases in observational studies. Diabetes Care 2012;35:2665-73.
3. Lévesque LE, Hanley JA, Kezouh A, et al. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. BMJ 2010;340:b5087.
4. Yang XL, Ma RC, So WY, et al. Addressing different biases in analysing drug use on cancer risk in diabetes in non-clinical trial settings--what, why and how? Diabetes Obes Metab 2012;14:579-85.
5. Yang X, Kong AP, Luk AO, et al. Validation of Methods to Control for Immortal Time Bias in a Pharmacoepidemiologic Analysis of Renin-Angiotensin System Inhibitors in Type 2 Diabetes. J Epidemiol 2014. [Epub ahead of print].
6. Zhou Z, Rahme E, Abrahamowicz M, et al. Survival bias associated with time-to-treatment initiation in drug effectiveness evaluation: a comparison of methods. Am J Epidemiol 2005;162:1016-23.
7. Jee SH, Ohrr H, Sull JW, et al. Fasting serum glucose level and cancer risk in Korean men and women. JAMA 2005;293:194-202.
8. Yang X, Ko GT, So WY, et al. Associations of

hyperglycemia and insulin usage with the risk of cancer in type 2 diabetes: the Hong Kong diabetes registry. Diabetes 2010;59:1254-60.
9. Yang X, So WY, Ma RC, et al. Diabetes and cancer: the mechanistic implications of epidemiological analyses from the Hong Kong Diabetes Registry. Diabetes Metab Res Rev 2012;28:379-87.
10. Yang X, So WY, Ma RC, et al. Synergistic effects of low LDL cholesterol with other factors for the risk of cancer in type 2 diabetes: the Hong Kong Diabetes Registry. Acta Diabetol 2012;49 Suppl 1:S185-93.
11. Yang X, Ma RC, So WY, et al. Renin angiotensin system inhibitors may attenuate low LDL cholesterol-related cancer risk in type 2 diabetes. Diabetes Metab Res Rev 2013. [Epub ahead of print].
12. Kong AP, Yang X, So WY, et al. Additive effects of blood glucose lowering drugs, statins and renin-angiotensin system blockers on all-site cancer risk in patients with type 2 diabetes. BMC Med 2014;12:76.
13. Viollet B, Guigas B, Sanz Garcia N, et al. Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond) 2012;122:253-70.
14. Sinnett-Smith J, Kisfalvi K, Kui R, et al. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. Biochem Biophys Res Commun 2013;430:352-7.
15. Qu C, Zhang W, Zheng G, et al. Metformin reverses multidrug resistance and epithelial-mesenchymal transition (EMT) via activating AMP-activated protein kinase (AMPK) in human breast cancer cells. Mol Cell Biochem 2014;386:63-71.
16. Liu X, Chhipa RR, Pooya S, et al. Discrete mechanisms of mTOR and cell cycle regulation by AMPK agonists independent of AMPK. Proc Natl Acad Sci U S A 2014;111:E435-44.
17. Zhang T, Wang X, He D, et al. Metformin sensitizes human bladder cancer cells to TRAIL-induced apoptosis through mTOR/S6K1-mediated downregulation of c-FLIP. Anticancer Drugs 2014. [Epub ahead of print].
18. Andersson T, Alfredsson L, Källberg H, et al. Calculating measures of biological interaction. Eur J Epidemiol 2005;20:575-9.
19. Yang X, So WY, Ma RC, et al. Predicting values of lipids and white blood cell count for all-site cancer in type 2 diabetes. Endocr Relat Cancer 2008;15:597-607.
20. Yang X, So WY, Ma RC, et al. Low HDL cholesterol, metformin use, and cancer risk in type 2 diabetes: the Hong Kong Diabetes Registry. Diabetes Care 2011;34:375-80.

Cite this article as: Yang X, Chan JC. Metformin and the risk of cancer in type 2 diabetes: methodological challenges and perspectives. Ann Transl Med 2014;2(6):52. doi: 10.3978/j.issn.2305-5839.2014.06.07

