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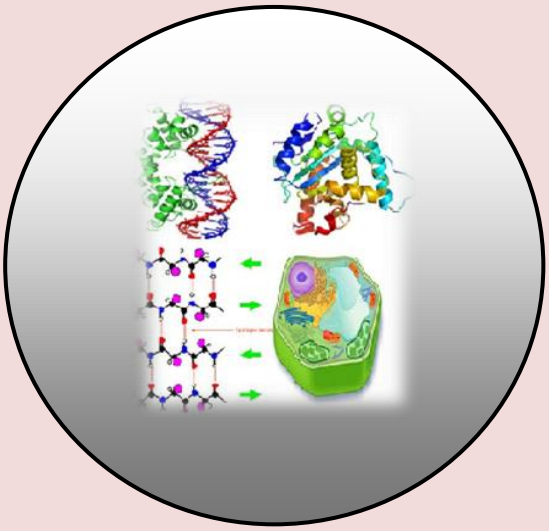

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RESEARCH PAPER

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Metformin as a Cause of Vitamin B12 Deficiency: Current Opinions and Mechanism

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ABSTRACT

Metformin is the most widely used agent to control type 2 diabetes mellitus (T2DM). Despite that about 60 years of clinic introduction of the drug, its action mechanism still not fully comprehended. Moreover, its probable interaction which causes vitamin B12 deficiency and its consequent serious complications are being of deep concern. In this review, the most recent advances in the mechanism of metformin action are presented. Different opinions regarding its responsibility in inducing B12 deficiency were reported and discussed. Literature suggested mechanisms by which this interaction proceeds were reviewed. Based on the structure-property relationship of biguanides, and the hypothesis that calcium is required for vitamin B12 absorption, a mechanism of the interaction was suggested, which depends on the formation of a complex between calcium and metformin.
Keywords: Metformin, Vitamin B12 deficiency, Biguanides and Structure-property relationship.

INTRODUCTION

Metformin is an oral diabetic drug that represent the most currently prescribed drug to control type 2 diabetes mellitus (T2DM) [Sally M. Marshall, 2017]. Its relative safety, high tolerance and cost effectivity are criteria that made it preferred over other hyperglycemic agents. Though long-period since it was introduced for the first time as T2DM agent, its mechanism of action is not fully understood. Despite its advantages over other T2DM drugs, it shows some undesired interactions or side effects. One of these interactions is its controversial interaction with Vitamin B12 and its association with vitamin B12deficiency.Overall, the effect still controversial and not yet completely resolved, since some reports have denied the effect. Moreover, the mechanism behind this interaction remains unclear. In this mini review we tried to include advances in metformin action mechanism and recent views about its interaction with vitamin B12. In particular we envisaged a new mechanism, in some of its details, to explain the mechanism by which metformin induces vitamin B12 level reduction, based on structure –property relationship of biguanides, the chemical group to which metformin belongs to. At the end of article we summarized some of literature updated recommendations for metformin treated T2DM patients and research community.

Metformin as T2DM drug

Metformin is currently the most widely used drug to control typ2 diabetic mellitus. It is an oral route drug and acts to suppress hepatic glucose production, decrease intestinal absorption and increase of insulin sensitivity by enhancing peripheral uptake and utilization [Elizabeth and Silvio, 2017, Lilian and Marilia, 2013]. In 1957, the published paper of Jeans stern was the date of initiation of metformin as an agent to control T2DM [Sterne, 1957]. Metformin origin is related to a particular structure of a compound called guanidine, which were found as constituent of a herbal plant called *Galliga Officinallis*.

It was being used as a traditional medicinal plant for treating worms and fever. In addition to metformin, phenformin and buformin are guanidines that were also used earlier as drugs for hyperglycemia but they were drown latter from markets of most countries as they were found to induce lactate acidolysis [Margaret et al., 1993, Clifford, 2017]. In contract, risk of lactate acidolysis is due to use of metformin is minimal [Robert, 2004, Sarah Vecchio and Alessandro Prott, 2011]. The structure of the three compounds contains fusion of two guanidine units and are called biguanides.

The use of metformin as T2DM medicine substantially grew after 1995, when food and drug administration in the USA approved the drug for treating T2DM patents. Nowadays, metformin is the first line drug for controlling T2DM [Clifford, 2017].

In addition to glycemic control, it is useful in controlling, obesity [Hong-Hong Ning et al., 2018], endothelial dysfunction [Asma Nafisa et al., 2018], hemostasis [Lilian Beatriz Rojas and Marilia Brito Gomes, 2013], some types of cancer [Marie Daugana et al., 2016] and reduces vascular complications in T2DM patients [Libby, 2003].

The mechanism by which the drug performs its action still under controversial debate. However there is a common agreement that metformin doesn't increase insulin secretion yet increases its sensitivity. The earlier and currently the most accepted explanation is inhibition of complex I of the respiratory chain, suppressing ATP production needed for gluconeogenesis. Beside, inhibition of ATP lowers ATP to AMP ratio and thereby activates AMP kinase that results in inhibition of glucose synthesis and stimulation of glucose consumption [Bruno and Fabrizio, 2012]. An experimental study on rat liver mitochondria using phenformin inhibited of complexes II and IV as well [Drahota et al., 2014]. However, several researchers reported that this route of hepatic action will not, alone, lead to the observed acute lowering of blood glucose immediately following metformin administration [Gilligan, 2002]. Besides, the immediate lowering of glucose after metformin treatment occurs even mice that lack AMPK function, that indicates that hepatic mitochondria is not the only route for the action of metformin [Marc Foretz, 2010]. It was also assumed that short-term effect of metformin which is independent on the hepatic energy path may solely depend on inhibiting glucose transfer from intestine into blood stream by increasing glucose uptake by intestine tissues [Olga Horakova, 2019]. The method by which metformin performs its action in intestine has also been investigated in by Horakova et al. how reported the ability of intestine to uptake glucose and subsequent anaerobically metabolite it to produce lactate, which accumulates in gut and liver tissue. Introduction of Delayed release formulation of metformin (met DR) by Buse et al, results in lowering of plasma exposure and showed the non-change of efficacy in metformin – immediate lowering of glucose. A result that indicated that the central action of metformin is not on liver but residues in gut.

An interesting feature of metformin as hyperglycemic agent is that its variable response, i.e. its effect differs from an individual to another, which made some investigators to report it as heritably dependent behavior. The long-term response of metformin (AMPK activation) was found to be associated with the rs11212617 polymorphism in close proximity to the ataxia-telangiectasia mutated gene (*ATM*). In other hand, the short-terms action is connected to the organic cation transporters (OCl2 and OCl3) which are associated with three genetic variations rs3119309, rs2481030 and rs7757336. Anothergenetic related interesting features of metformin response, is that right handed type 2 patients were found to tolerate metformin more than left handed.

Vitamin B12, absorption mechanism

Vitamin b12 is a water soluble vitamin that is biologically essential and necessary for a variety of biological functions. It works a cofactor of transferring methyl malonic acid to succinic coenzyme A, and conversion of homocysteine to methionine Seeniann John and Carl Hoegerl, 2009].The only source of this vitamin to human is from the nutrient products come from animal as meat, milk and eggs. It is synthesized in the animal gastrointestinal tractsby microorganisms and transferred to the tissues of animal and then to human via food cycle [FAO Rome, 2001]. Natural vitamin B_{12} is being bound to proteins and, upon human feeding, is released in stomach and then bound to glucagon proteins (R-binders). R- binders are secreted by salivary glands and stomach and acts to protect vitamin B12 from denaturation in the high acidic medium of stomach. R-binders are digested upon entering the duodenum and free vitamin is released to bind immediately to another glycoprotein called intrinsic factor (IF) which passes the lower part of small bowel where it is absorbed by specific ileal receptors[26]. Binding of vitamin B_{12}-IF, to its binding receptors in the membrane of ileal cell was found to be strongly dependent of concentration of calcium ions in small bowe l. Chelation of calcium ions or decreasing pH reversibly were found to lower uptake of B12-IF, the result which was explained by presence of a type of calcium salt bridge or calcium dependent channel is necessary for vitamin B12-IF uptake. There is a common agreement that serum B_{12}level of less than 150±2 pmol/L (200 pg/mL) is indication of deficiency, and the border is in the range 150±2 to 220±2 pmol/L (200-300 pg/mL).

Metformin causes B12 deficiency

Though several advantages of metformin, it has some undesirable side effects. The most prominent and serious one is cause B12 deficiency. Most of the reports that were devoted to investigate the interaction of metformin and B12concluded that using metformin for treatment of DMT2 results in vitamin B12 deficiency in 10-30% of patients.

The effect is prevalent even with patients using combinations of metformin with other hyperglycemic drugs such as sulfonylureas [Jae-Seung et al., 2014]. The treatment period after which the effect manifests itself is of controversial. Some studies reported that 16 weeks (ca. 4 month) of metformin treatment is sufficient to cause reduction in serum B12, and even to make a significant increase in homocysteine. A systematic meta-analysis review included twenty six papers, from the period 1957 to 2013, showed that a period of six weeks to three months use of metformin by T2DMpatients may induce B12 reduction of 57 pmol/L. The deficiency become more prevalent as treating period get prolonged or with increasing the used dose of metformin [Tarek Wehbe et al., 2018]. Aroda et al. performed a systematic placebo control study and they concluded that risk of vitamin B_{12} deficiency occurs at 19.1% of subjects group after 5 years and at 20.3% after 13 years of metformin use compared to 9.5% and 15.6% after 5 and 10 years receptively with the placebo control group. Haeusler, in an extensive study in New Zealand, showed that in addition to correlation of dosage and duration, the reduction in serum B12 is also directly related an age of patient and dependent on and his ethnicity. The explanation that can be given for ethnicity dependent is based on variations the dietary cultures among different ethnic groups, where in some ethnic groups deficiency may result from insufficiency of B12 dietary sources.It was also found that male patients are liable to deficiency more than female sex. The deficiency of B_{12} imposes several adverse effect such as megaloblastic anemia and diabetic neuropathy.

Despite the large number of studies that confirmed correlation between metformin use and vitamin B12 deficiency, there is a controversial debate about the this interaction. The major number of the studies claimed metformin-cause of vitamin B12 deficiency were depending on serum vitamin B12 level. It was frequently found that serum vitamin B12 did not reflects cellular levels and, for a clear cut result, one must assure from the levels of active form of vitamin B_{12}, holocobalamin. Metabolic markers of vitamin B_{12} such as homocysteine and methyl malonic acid could also be more reliable. Biochemically, Metformin may interfere with vitamin B12 absorption, but this is of rare clinical significance. Greibe et. al. found that despite reduction of serum vitamin B12, its active form holocobalamin and methyl malonic acid were not affected by use of metformin up to six months. The same research team found that metformin induced vitamin B_{12} accumulation in liver of rats in account of its circulated quantity and they concluded no effect of metformin on absorption of vitamin B_{12} [Millerb et al., 2013]. Similar results were obtained by Obied et al. who studied status of vitamin B_{12} in diabetes mellitus patients and the effect of metformin treatment in extra and intracellular levels of vitamin B12. They concluded that no significant change in total vitamin B_{12} in diabetic patients, however, lower serumvitamin B_{12} were detected with no change in RBC-B_{12} due to metformin use. Recent and interesting study that was carried out by Elhadd et al. on vitamin B12 level in T2DM patients using metformin. They recruited 326 subjects with a mean age of around 54 years and mean diabetes duration of 7-8 years and concluded no vitamin B12 deficiency in type 2 diabetes patients treated with metformin. Further, they found that vitamin B12 reduction were more prevalence in non-metformin (19%) than metformin (8%) patients.

Due to the contradictions in definition of vitamin B12 deficiency, the debate is continuing. In one side, metformin induced vitamin B12 reduction recognized, but its functionality is not affected as there is increase in its liver stock that will continuing replenish any tissue deficiency. Beside, prevalence of serum vitamin B12 was correlated with type 2 diabetes mellitus itself. In a study of Bello et. al. patients not using metformin were found to be at risk of vitamin B12 deficiency which increase doubts about metformin intervention. Moreover, vitamin B_{12} deficiency may result from concomitant long-term use of other medication agents such as proton pump inhibitors. In other side, in addition to serum vitamin B12 levels, long-term metformin use was found to affect even its metabolic markers. In a systematic review and metal-analysis, Zhang et al, reported significant increase of homocysteine in subjects who do not take exogenous supplements of folate and B group vitamins, which in turn may increase risk of peripheral neuropathy. The study of Bello et al. has also indicated concomitant enhancement of B12 deficiency in T2DM patients using metformin compared to non-metforminT2DM. The above arguments, leads us to a conclusion that metformin will remain as a suspect of vitamin B12 deficiency cause until more systematic and comprehensive reports, that take control of all other variations such as age, treatment duration historical medications, heritability, make a decree.

Mechanism of metformin induced vitamin B12 deficiency

In spite tothe intensive publications regarding association of metformin treated T2DM with vitamin B12 deficiency, little interest is observed in the mechanism by which this effect may proceed. The attention was not drown to the mechanism may be due to continuous ongoing controversial debate about the effect itself. Though insufficiency of literature, we found several mechanisms that were suggested on some spaced time periods. The simplest one assumes alterations of small bowel motility leads to bacterial overgrowth and subsequent consumption of vitamin B12. It is reported that diabetic patients may exhibit slow small bowel transit and bacteria overgrowth resulting in reduction of absorbed vitamin B12. However, Liu et al., through their experiments reported no significant change in intestine motility as a result of metformin administration [Scarpello et al., 1998]. They revealed that, with exception of inhibiting bile slat absorption and its accumulation in colon, Metformin does not cause motility or microbiome change of gut.

There is also other explanation of metformin induced B12 deficiency that based on inhibition of intrinsic factor by in metformin T2DM patients. Adams et al. carried out experimental investigation on effect of metformin on intrinsic factor secretion by saliva and stomach and found a remarkable lowering in serum vitamin B_{12}level associated decreased secretion intrinsic factor in gastrointestinal tract of human subjects. They observed vitamin B12 malabsorption in 30% of subjects treated with metformin in accompanying with decreased secretion of intrinsic factor that persists even after withdrawal of metformin.

The mechanisms that receive attention are those based on metformin-inducing biochemical alterations of calcium status that is required for the uptake of vitamin B12-intrisic factor by receptors of the ileal cells. One of the hypothesis by which this effect occurs was formulated by Haeusler, states that vitamin binds to tubulin receptors and transferred through channels that are calcium-dependent membrane potential. Metformin arrived in the ilium binds competitively to cubulin-vitamin B12, leads to removal of calcium ions and subsequent potential alterations and ultimately, absorption is inhibited. This mechanism can't explain the results indicated that that the effect can be reversed or decreased significantly by giving metformin-treated patients supplementary of calcium.

Here, we present our thoughts in a mechanism that based on complexation interaction between metformin and calcium which lowers bioavailability of calcium ions required for vitamin B12 absorption. The binding of calcium ions to ligand such as EDTA in Biosystems is well known [Mackenzie and Donaldson, 1972]. Moreover, the binding properties of metformin to divalent metals was suggested to be the route of actions of metformin in most of cellular effects. Indeed, stable square planar metformin complexes were prepared with some divalent and trivalent cations of Cu, Ni, Mn and Zn, Au, Cr and V. Based on the aforementioned side literature, ability of metformin to form complexes with divalent cations could be a strong indication that metformin may chelate calcium cations thereby decreasing bioavailability of these ions for vitamin B_{12} absorption. It was well-known that stabilities of these type of complexes are dependenton type of divalent ion and pH value. Though no experimental investigations were reported chelation of calcium ions by metformin and pH conditions for this type of process, we believe that metformin inhibits vitamin B_{12} absorption by complexing with calcium ions required for uptake of B12-IF. We assume that Ca-metformin complex has its own pH stability range. So there will be a competence between metformin and vitamin B_{12}-IF toward calcium ions. If the pH of small bowel ileum appropriate for chelation of calcium ions with metformin, vitamin B_{12} absorption will be inhibited which may lead to vitamin B_{12}deficiency with prolonged period of metformin use. Even when the hypothesized complex is somewhat of weak stability at physiological conditions, relatively high concentration of metformin in gut compared to other interventions (calcium and IF-B12 levels and pH) will make conditions in favour of its formation. This mechanism can give a plausible explanation for the interaction is being reversible, pH dependence and a direct relation with metformin dosage. Further evidence for the role of calcium-metformin complex in vitamin B12 malabsorption would result when we take a look in structure-relationship of two types of biguanide (metformin and phenformin) which have been used as an agents forT2DM. In contrast to the classical representation, it was found that the correct structure is the tautomeric form, Figure 1, rather than classical one which used be presented in most literature reports. The structures of the two biguanides shows the difference in the alkyl structure connected to the terminal nitrogen atoms, which is sp3 hybridized in metformin and sp2 in phenformin. It is expected this structure will make metformin more hydrophilic and more active as bidentate legend.In contrast to metformin, literature shows no short-term effect of phenformin treatmenton vitamin B_{12}absorption. However, in a study used Schilling test for measuring urine B12 as ^{58}Co to assess the influence of long-term phenformin treatment on B12 vitamin absorption and compare results to those of metformin, it was found that the occurring incidence of malabsorption was more incidents with phenformin (46%) compared to that with metformin (30%). However, magnitude of serum B12 depression was larger with metformin users (23%) compared to phenformin (2%), which indicates that phenformin induced B_{12} malabsorption is less severe [Gerald, 1973]. As Phenformin is being of lower potentiality to cause vitamin B12 deficiency compared to metformin, this may present and evidence of that calcium ions chelating biguanides ligand. It is well known that phenformin has higher potency as hyperglycemic agent than metformin, yet it has a milder effect regarding vitamin B12 malabsorption. It is expected that the ability of biguanides molecule as a ligand to chelate metal ions is dependent on the tendency of two nitrogen atoms to donate their lone pairs to the metal. In other words, stronger basic molecule is expected to be more active chelating ligand. Literature reports that phenformin is expected to be slightly lower in basicity [Ying Woo, 1998] and more hydrophilic [Graham et al., 2013] than phenformin which make it more active toward chelation of Caions. Revealing literature, we found several reports concerningmetal complexes of metformin while very few described those with phenformin and other biguanides. This argument, has presented an evidence of calcium-metformin complex formation as a potential mechanism for vitamin B12 malabsorption and an appropriate explanation for the relatively non-significant effect of phenformin on it.

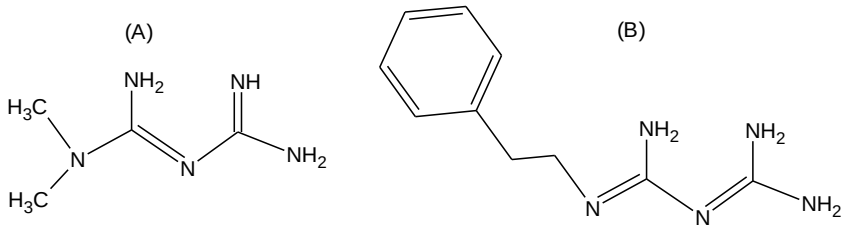

Figure 1. Structures of metformin (A) and phenformin (B).

Another indirect role of metformin in reducing calcium in gut may come from its effect in inhibiting bile salts absorption and causing their accumulation in colon [Liu et al., 2011]. Bile slats were reported to chelate calcium [Verónica Urdaneta and Josep Casadesús, 2017], and hence participate in its level diminishing.

Recommendations

It is useful to introduce useful recommendations that may be useful for patients of T2DM patients treated with metformin and to the scientific community concerns with improving agents of hyperglycemia. Structure-property relation-ship comparing between biguanides would be very useful in developing safe and efficient agents for T2DM. Metformin, despite its lower efficacy as diabetes mellitus agent, is safer and does not induce significant lactic acidosis but is of high potential cause vitamin B12 deficiency. In the other side, phenformin was found to be higher potent as type 2 diabetes mellitus, and does not cause significant vitamin B12 deficiency, but itis toxic and induce higher lactate acidosis. So, investigating structure- property relationships in both compounds could lead to formulating safer and higher potent biguanide T2DM drug.

Patients of type 2 diabetes mellitus treated with metformin are advised to take a vitamin B12 (2.4 µg/day) and undergo annual serum B12 screening. The patients with vitamin B12 in the border are advised to screen for homocysteine and methyl malonic acid [Anuvarsha et al., 2018]. It was also recommended that an annual injection of 1000 mcg (1 mg)of vitamin B12 would be sufficient to prevent deficiency in patients using metformin and screening for serum vitamin B12 is also necessary before initiating administration [Wendimere Reilly and Jasminka Z. Ilich, 2016, Mahajan and Gupta, 2010]. Patients with acute or severe neuropathic complications, are advised to take daily supplement of1 mg for a week, followed by 1 mg weekly for four weeks via parenteral route. Recent study of Silverstein et al. showed that oral vitamin B12 supplementation is costly more effective and enhances serum B12 level substantially compared to parenteral [William et al., 2019], but this may work for non-metformin induced deficiency. Since metformin exerts the major of its action in gut, parenteral treatment is more effective. This also may explain the results of National Health and Nutrition examination survey that indicated non successful of oral vitamin B12 supplements with T2DM patients treated with metformin. In all cases, caution has to be taken, since high plasma vitamin B12 concentration might be associated with risk of all-cause mortality [Isidor Minović Jose et al., 2020].

Finally, Spreading awareness of screening and controlling vitamin B12 among metformin treated patients is necessary. Unfortunately, despite the recommendation for serum B12 screening was suggested as early as 40 years, patients and health community do not take this action in serious and therefore high percentage of patients do not undergo serum B12 screening.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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