Data mining approach for the treatment of drug adverse effects: olanzapine-induced hyperglycemia can be inhibited by vitamin D

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Abstract

Every pharmaceutical agent has both directions of effects; that is, therapeutic main effects and undesirable adverse effects. Surveillance of adverse drug reactions has been performed in the post-marketing period by collecting self-reports of clinical cases in which some of adverse events are observed. FDA Adverse Event Reporting System (FAERS) is one of the publicly available databases of adverse event cases. To find a practical solution for reducing adverse events, we have shown that FAERS can be used for finding a drug combination in which the odds ratio of adverse events is statistically lowered with a concomitant drug. In the present study, we focused on the atypical antipsychotic olanzapine, which frequently causes hyperglycemia in patients. Our analysis of FAERS lead to a hypothesis that olanzapine-induced hyperglycemia is mitigated by concomitant use of vitamin D. Subsequent in vitro experiment using cultured myocytes demonstrated that olanzapine induces insulin resistance that can be prevented by vitamin D pretreatment.

Key words: Adverse effects, atypical antipsychotics, olanzapine, hyperglycemia, vitamin D

Introduction

Drugs not only have beneficial, main effects but also exert harmful effects on humans. Since most of the mechanisms of adverse events are still unknown, it is difficult to prevent them, leading to the accumulation of self-reports of such events. The FDA Adverse Event Reporting system (FAERS) is the largest worldwide database of self-reports of adverse reactions. Until now, more than eight million case reports have been accumulated and become freely accessible to the public.

Traditionally, FAERS has been used to clarify a "positive" correlation between a drug (drug A) and its adverse effect by calculating odds ratio. Previously, we came up with an idea that it is possible to identify a concomitant drug B that could mitigate the risk of adverse events associated with the use of drug A by finding a "negative" correlation of drug B and the drug A-induced events. By challenging this approach, we suggested a new therapy where quetiapine-induced hyperglycemia will be prevented by the concomitant use of vitamin D. [(1)]

Atypical antipsychotics, such as quetiapine and olanzapine, are clinically used to treat a wide variety of mental disorders, including schizophrenia, bipolar disorder and depression. However, these drugs are often associated with the occurrence of acute hyperglycemia, which frequently leads to new-onset diabetes mellitus (DM), thus limiting their clinical use. [(2,3)] Our previous study demonstrated that this problem can be solved by the concomitant use of vitamin D, which is a simple, secure and inexpensive way. However, olanzapine, which is said to cause hyperglycemia more frequently than quetiapine, has not been studied yet. Therefore, we investigated whether the olanzapine-induced hyperglycemia can also be inhibited by vitamin D with a combination of datamining of FAERS and in vitro experiments.

Materials and Methods

1. Analysis of the FAERS database.

FAERS adverse event reports were obtained from the FDA website (http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Surveillance/AdverseDrugEffects/). Duplicated reports (among a total of 8,238,511 reports) from the first quarter of 2004 through the second quarter of 2016 were filtered by applying the FDA's recommendation of adopting the most recent case number. Consequently, 6,769,743 remaining reports were analyzed in this study. Arbitrary drug names, including trade names and abbreviations, were mapped into unified generic names via text mining. Adverse event risk was evaluated by calculating the reporting odds ratio (OR) with 95% confidence interval (CI).

Briefly, individuals in the FAERS database were divided into the following four groups: (a) individuals who received the drug of interest (i.e., olanzapine or vitamin D) and exhibited DM-related adverse events; (b) individuals who received

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the drug of interest, but did not exhibit DM-related adverse events; (c) individuals who did not receive the drug of interest and exhibited DM-related adverse events; and (d) individuals who did not receive the drug of interest and did not exhibit DM-related adverse events. The OR with 95% CI was calculated as follows:

$$OR = \frac{a/b}{c/d},$$

$$95\%\ \mathrm{CI} = \exp\left\{\log(\mathrm{OR}) \pm 1.96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}\right\}$$

where a, b, c, and d refer to the number of individuals in each group, and log refers to the natural logarithm. If the lower limit of the 95% CI was > 1, a significant association was assumed between use of the drug of interest (i.e., olanzapine) and the increased occurrence of DM-related adverse events. Oppositely, if the upper limit of the 95% CI was < 1, a significant association was assumed between use of the drug of interest (i.e., vitamin D) and the decreased occurrence of DM-related adverse events. The search terms for "DM" and "vitamin D" are described in Tables 1 and 2, respectively.

2. Drugs and reagents.

Olanzapine was purchased from Tokyo Chemical Industry (Tokyo, Japan). Insulin was from Biological Industries (Cromwell, CT, USA). 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-d-glucose (2-NBDG) was from Cayman Chemical (Ann Arbor, MI, USA). Calcitriol was from Toronto Research Chemicals (Ontario, Canada). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were from Sigma-Aldrich (Saint-Louis, MO, USA). Horse serum (HS) was from Invitrogen Japan (Tokyo, Japan).

3. Cell culture and differentiation.

Mouse C2C12 myoblast cell line was used for the experiments. The cells were cultured in 100-mm dishes in DMEM containing 10% heat-inactivated FBS at 37°C with 5% CO_2. One day after seeding cells in black 96-well plates (> 70% confluence), the medium was switched to DMEM with 2% HS to differentiate cells into myotubes. The myotubes were used for experiments 3–5 days following differentiation.

4. Glucose uptake assay.

Glucose uptake assay was performed by measuring the uptake of 2-NBDG, a fluorescent derivative of glucose. Cells were stimulated with or without insulin (1 μM) dissolved in KRPH buffer (136 mM NaCl, 4.7 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 5 mM KH_2PO_4, 20 mM HEPES) for 15 min followed by the addition of 2-NBDG (50 μM) for 20 min. After incubation, free 2-NBDG was washed out 3 times with Krebs-Ringer-Phosphate-HEPES (KRPH) buffer. The fluorescence retained in the cells was measured with a Wallac 1420 Arvo SXFL multilabel counter (PerkinElmer, Waltham, MA, USA) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

5. Statistical analysis.

Data obtained from the cellular experiments were analyzed with GraphPad Prism 5 Software (GraphPad, San Diego, CA, USA). Differences between two groups were compared via a Mann-Whitney *U* test or an unpaired two-tailed Welch's *t*-test. Holm's correction was applied to adjust for multiple comparisons. Differences were considered significant at $p < 0.05$.

Table 2. Search terms for vitamin D analogs in the analysis of FAERS

Resolved drug names	
Vitamin D	Dihydrocholecalciferol
Vitamin D-calcium combination	Calcitriol
Cholecalciferol	Falecalcitriol
Paricalcitol	Eldecalcitol
Ergocalciferol	Maxacalcitol
Doxercalciferol	Tacalcitol
Alendronate-cholecalciferol combination	Calcifediol
	Alphacalcidol

Table 1. Search terms for DM-related adverse events in the analysis of FAERS

Adverse event name	
diabetes mellitus inadequate control	type 2 diabetes mellitus
diabetes mellitus insulin-dependent	diabetes mellitus
diabetes mellitus non-insulin-dependent	type 1 diabetes mellitus
insulin-requiring type II diabetes mellitus	diabetic coma
latent autoimmune diabetes in adults	insulin resistant diabetes
insulin-requiring type 2 diabetes mellitus	diabetic ketoacidosis
fulminant type 1 diabetes mellitus	diabetic hyperosmolar coma
diabetic hyperglycaemic coma	diabetes with hyperosmolarity
diabetic ketoacidotic hyperglycaemic coma	

Results

1. Olanzapine increases the risk of DM-related adverse events in the FAERS database.

We first investigated the association between olanzapine usage and DM-related adverse events in the FAERS database (Table 3). The search terms for DM-related adverse events are described in Table 1. A disproportionality analysis of the database revealed a significant association between olanzapine use and the increased occurrence of DM-related events. A strong association (OR > 20) was observed, which confirmed the hypothesis that the risk of olanzapine-induced hyperglycemia is properly reflected in the clinical database.

2. Vitamin D decreases the occurrence of olanzapine-induced, DM-related adverse events.

We next investigated whether vitamin D (see Table 2 for the search terms for vitamin D analogs) has the capacity to decrease the risk of olanzapine-induced DM (Table 4). The analysis of the database indicated that vitamin D was associated with a decreased occurrence of olanzapine-induced, DM-related adverse events.

3. Calcitriol improved olanzapine-induced insulin resistance in C2C12 myotubes.

To verify the hypothesis that vitamin D mitigates the risk of olanzapine-induced hyperglycemia, we conducted an in vitro glucose uptake assay using C2C12 mouse myotubes (Fig. 1). Treatment with olanzapine (10–1000 nM) for 1 h inhibited insulin-stimulated glucose uptake in a concentration-dependent manner. Significant inhibition was observed at 1,000 nM olanzapine (Fig. 1a), suggesting the induction of insulin resistance by olanzapine. The olanzapine-induced decrease in glucose uptake was improved by pretreatment of the cells with calcitriol (1,25-dihydroxycholecalciferol, the biologically active form of cholecalciferol/vitamin D_3) in a concentration-dependent manner at 0.1–10 nM for 24 h, and significant improvement was achieved at 10 nM (Fig. 1b). These data demonstrate that vitamin D can improve olanzapine-induced insulin resistance.

Table 3. Association between olanzapine and the occurrence of DM in FAERS

	DM	No DM	Report rate (%)	Odds Ratio (95% CI)	*p* value
Usage of olanzapine	7,256	39,168	15.63	20.81 (20.27–21.37)	< 0.001
No usage of olanzapine	59,311	6,664,008	0.88		

Table 4. Decrease of the occurrence of olanzapine-induced DM by vitamin D in FAERS

	DM	No DM	Report rate (%)	Odds Ratio (95% CI)	*p* value
Usage of vitamin D	34	929	3.53	0.19 (0.14–0.27)	< 0.001
No usage of vitamin D	7,222	38,239	15.89		

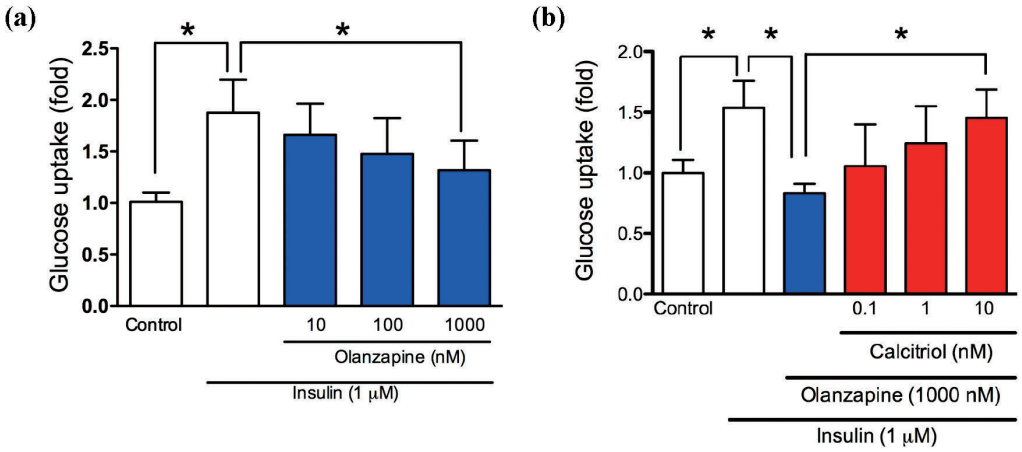

Fig. 1. Calcitriol improved olanzapine-induced insulin resistance in C2C12 myotubes. Glucose uptake was evaluated using 2-NBDG as described in Materials and Methods. The data are normalized to the control group and presented as mean ± S.E.M. **(a)** Concentration-dependent effect of olanzapine on the insulin-stimulated glucose uptake. Differentiated C2C12 cells were treated with olanzapine (10–1,000 nM) for 1 h. The cells were then stimulated with or without insulin (1 μM) for 15 min. n = 17–21. *$p < 0.05$. **(b)** Concentration-dependent effect of calcitriol on the olanzapine-induced insulin resistance. Differentiated C2C12 cells were treated with calcitriol (0.1–10 nM) for 24 h, followed by olanzapine (1,000 nM) for 1 h. The cells were then stimulated with or without insulin (1 μM) for 15 min. n = 15. *$p < 0.05$.

Discussion

To the best of our knowledge, this is the first study demonstrating that olanzapine-induced hyperglycemia can be prevented by pretreatment with vitamin D.

We have already shown that vitamin D also prevents quetiapine-induced hyperglycemia. Although both olanzapine and quetiapine are classified as atypical antipsychotics sharing some of the target receptors, the chemical structures of these drugs do not closely match each other. Considering the fact that hyperglycemia induced by different drugs (quetiapine and olanzapine) can be prevented by a single drug (vitamin D), the hyperglycemia caused by both drugs may have the same molecular mechanism. According to our previous report on quetiapine, [(1)] the olanzapine-induced hyperglycemia may also be mediated via *Pik3r1*, though further investigations with molecular docking analysis and experimental validation study are needed.

Since olanzapine is more frequently used in Japan, and has more severe risk of DM than quetiapine, [(2)] the present finding is worthwhile to try the concomitant use of vitamin D in preventing hyperglycemia acutely induced by dosing of atypical antipsychotics. Future clinical trials are awaited.

結論／ Conclusion

In order to search for the solution for olanzapine-induced hyperglycemia, we combined analysis of FAERS with pharmacological experiments. As a result, analysis of FAERS lead to a hypothesis that olanzapine-induced hyperglycemia might be prevented by vitamin D. Following pharmacological experiments verified that the olanzapine-induced insulin resistance could be prevented by vitamin D

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副作用の軽減策を臨床ビッグデータから探す：オランザピン誘発高血糖のビタミンDによる予防

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要旨

薬には本来の効能である主作用と望ましくない有害作用（副作用）がある．この副作用は市販後にモニターされており，有害事象のセルフレポートとして蓄積されている．米国食品医薬品局FDAが公開している副作用報告データFAERSでは世界中で起こった有害事象例が検証できる．我々はこれまで有害事象を減らす実際的な方策として，FAERSを用いて特定の副作用を軽減する併用薬を探索することが可能であることを示してきた．そこで本研究では，非定型統合失調治療薬であるオランザピンの副作用として知られる高血糖に着目した．我々が行ったFAERSの解析から，オランザピンが誘発する高血糖はビタミンDの併用によって改善するという仮説が導出された．さらに培養骨格筋細胞を用いた実験により，オランザピンが誘発するインスリン抵抗性がビタミンD前処置で改善されることが実証された．

重要語句：有害事象，非定型統合失調症治療薬，オランザピン，高血糖，ビタミンD

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