High-fat Diet Aggravates Islet Beta-cell Toxicity in Mice Treated with Clozapine

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Background: Clozapine, an atypical antipsychotic drug, induces derangements in glucose homeostasis in certain patients. This study investigated the mechanisms of clozapine-induced beta-cell toxicity.

Methods: Fifty-two healthy C57BL/6 male mice were randomized into 4 groups to study the effects of clozapine (group C, D) and a high-fat diet (group B, D). Three mice from each group were randomly selected to determine the amount of food intake on days 8–10, and their pancreases were removed for histological examination on day 11. The remaining 10 mice in each group were sacrificed at the 8th week to measure pancreatic insulin content (PIC).

Results: Mice given clozapine for 8 weeks demonstrated trends of lower PIC. The histological examination of the pancreases retrieved on day 11 already revealed apoptotic changes and suppression of cell proliferation. Although mice fed high-fat chow gained weight, mice given both clozapine and a high-fat diet showed less weight gain and more severe histological deterioration, and had the lowest PIC levels of the 4 groups.

Conclusion: Pancreatic beta-cell apoptosis, suppression of cell proliferation, and trends of reduction in pancreatic insulin content were observed in mice taking clozapine. The findings of clozapine induced beta-cell toxicity were further aggravated when mice were concomitantly fed a high-fat diet.

Key words: beta-cell toxicity, clozapine, high-fat diet, apoptosis, Ki67, pancreatic insulin content
reversible after the mice recovered from the acute
dose of medication.22
Not all schizophrenic patients taking clozapine
develop overt diabetes.8,9 Weight gain and metabolic
syndrome are commonly observed in patients treated
for schizophrenia.10,11 We were interested in studying
the beta-cell damage since acute decreased insulin
secretion was reported by Chintoh et al.22 Therefore
we designed this study to examine beta-cell histol-
ogy and insulin content in mice treated with clozap-
ine for 8 weeks. Also, we introduced a high-fat diet
model in addition to clozapine treatment to test fur-
ther beta-cell changes while adaptive compensation
of beta-cells takes place.10,11

METHODS
Male C57BL/6 mice (8–12-week old) were
obtained from a local breeder and 3 to 5 mice were
housed in each cage and fed pelleted food and tap
water ad libitum. Mice were fed either regular chow
(5001 LabDiet, 3.17 Kcal/g, 12% kcal from fat) or a
high-fat diet (TestDiet, 5.17 Kcal/g, 60% kcal from
fat), both obtained from Purina (Richmond, IN,
U.S.A.), for 8 weeks. The animals were maintained
on a lighting cycle of 12 h light and 12 h darkness.
Fifty-two healthy C57BL/6 male mice were ran-
domly separated into 4 groups. Mice in groups C and
D were orally administrated 13.5 mg/kg body weight
of clozapine six days a week between 8 AM to 9
AM. Distilled water was given to mice in groups A
and B as control groups. Mice in groups A and C
were fed a regular diet while mice in groups B and D
were fed a high-fat diet.
The non-fasting blood glucose was measured at
8AM daily and body weight was measured twice a
week. The change of body weight (ΔBW) was calcu-
lated as the difference in the average body weight
between the first and eighth weeks.
Three mice from each group were randomly
selected to be housed in metabolic cages between the
7th to 11th days to measure diet intake. Then, the mice
were sacrificed for histological examination of the
pancreases on day 11. The apoptotic and proliferative
nuclei in the islets of paraffin-embedded pancreatic
sections were detected using the TUNEL Apoptosis
Detection Kit bought from GenScrip (Piscataway,
NJ, U.S.A.) and an immunohistochemical examina-
tion was done using anti-Ki67 antibody (ab15580)
obtained from Abcam Inc. (Cambridge, MA,
U.S.A.).
The remaining 10 mice in each group were sac-
cificed at the end of 8 weeks and the pancreases were
removed to measure pancreatic insulin content (PIC)
using an acid-ethanol extraction and insulin kit (#
RI-13k) bought from LINCO Research, Inc. (St.
Charles, MO, U.S.A.). The animals were treated
humanely in accordance with the laboratory animal
guidelines of Chang Gung Memorial Hospital. Since
normality was not achieved, the results were given as
median values [minimum; maximum], and the non-
parametric Wilcoxon signed-rank test, Mann-
Whitney test, and Kruskal-Wallis test were per-
formed for comparisons as appropriate. A value of p
< 0.05 was considered significant.

RESULTS
The dietary intake measured between day 8 to
day 10 was significantly decreased in mice fed a
high-fat diet. Nevertheless, the daily caloric intake
did not differ among 4 groups (Table 1). Mildly ele-
vated blood glucose levels were observed only in
mice fed high-fat chow (group B) on day 56 (Table
2). Among the 4 groups, group B (high-fat chow)
had a significant weight gain while group C (clozap-
ine) had a weight loss at the 8th week. The mice fed
high-fat chow and treated by clozapine simultane-
ously (Group D) had a significantly lower weight
than those fed only a high-fat diet (Group B), indi-
cating clozapine attenuated the weight gain effect
that was induced by the high-fat diet.
Pancreatic histology showed higher TUNEL
apoptosis and less anti-Ki67 detection on day 11 in
the beta-cells of mice given clozapine (group C)
(Table 1), compared with the control (group A) and
mice fed high-fat chow (group B). The results indi-
cated more apoptosis and less pancreatic prolifera-
tion developed in mice treated with clozapine. These
findings of beta-cell toxicity caused by clozapine
administration were further aggravated when mice
were simultaneously fed a high-fat diet (group D).
At the 8th week, trends of a reduced PIC were
observed in group C while an increased PIC was
observed in group B, although this did not reach sig-
nificance in our study (Table 2). The PIC in mice
simultaneously treated with clozapine and a high-fat
diet (group D) showed the lowest level of all 4
High-fat and clozapine beta-cell injury

Chung-Huei Huang, et al

In this study, we found that clozapine-induced apoptosis of mouse pancreatic islet cells occurred as early as 10 days after drug administration. Moreover, a high-fat diet aggravated apoptosis and inhibited proliferation of islet cells in mice taking clozapine. The long-term toxic effects of the combination of clozapine and a high-fat diet were reflected in the significant reduction in the PIC in mice given a high-fat diet and clozapine for 8 weeks.

The mechanism of action of clozapine-induced pancreatic toxicity is not yet clear. Chintoh et al. reported that deterioration of plasma insulin secretion can be observed immediately following a single dose of clozapine injection. Subsequently, they found beta-cell function can be restored. Our study observed significant damage to beta-cells when mice were treated with clozapine for 10 days. Although no statistical difference was reached, a trend of PIC reduction was demonstrated in group C following 8 weeks of treatment, implicating beta-cell toxicity from clozapine. It has been reported that a longer duration of atypical antipsychotics is associated with a higher risk of diabetes. The trend of PIC loss could be anticipated after further chronic treatment with clozapine. The beta-cell toxicity we observed may correlate with clinical reports of diabetic ketoacidosis in patients taking atypical antipsychotics.

Feeding high-fat chow resulted in weight gain and a trend of PIC increment in mice, suggesting beta-cell hyperplasia in mice given a high-fat diet. When mice received both clozapine treatment and a high-fat diet, the PIC was much lower when compared with control mice (group A) or mice fed a high-fat diet alone. The results are given as median values [minimum; maximum]. No statistical differences were observed in daily intake and calories among the four groups (Kruskal-Wallis test).

Table 2. Effects of Clozapine and High-fat Diet on Mouse Appetite and Apoptosis and Proliferation of Pancreatic Beta Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Clozapine</th>
<th>HF diet</th>
<th>Daily intake (g/100 g BW)</th>
<th>Daily calories (Kcal/100 g BW)</th>
<th>Apoptosis</th>
<th>Ki67 positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>-</td>
<td>14.8 [13.6; 15.0]</td>
<td>46.9 [43.2; 47.6]</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
<td>-</td>
<td>6.6 [6.6; 8.5]</td>
<td>33.9 [33.8; 45.8]</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>+</td>
<td>14.5 [12.4; 15.0]</td>
<td>46.0 [39.2; 47.7]</td>
<td>+ +</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>+</td>
<td>6.9 [6.5; 7.2]</td>
<td>37.0 [33.3; 37.3]</td>
<td>+ + +</td>
<td>+ / -</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study, we found that clozapine-induced apoptosis of mouse pancreatic islet cells occurred as early as 10 days after drug administration. Moreover, a high-fat diet aggravated apoptosis and inhibited proliferation of islet cells in mice taking clozapine. The long-term toxic effects of the combination of clozapine and a high-fat diet were reflected in the significant reduction in the PIC in mice given a high-fat diet and clozapine for 8 weeks.

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high-fat diet without clozapine (group B). It is possible that the compensating islet beta-cells are more vulnerable to clozapine administration. However, further research is required to elucidate the underlying mechanism. Our findings in mice might relate to the clinical study recently reported by Argo et al. Although we reported beta-cell damage in our experiment, the PIC in group D was 55.8% of that in group A. A greater than 80-90% reduction of beta-cell mass is required for development of overt diabetes. Although a decreased daily intake was observed, body weight gain was still noted in our study in the high-fat chow groups. A further explanation of metabolic efficiency may be needed. A large study of mice with a high-fat diet model indicated that weight gain in mice given a high-fat diet is not fully explained by energy intake but is also correlated with a reduction in the metabolic rate. In addition, since the administration of clozapine did not change the amount of chow and the daily calorie intake, the explanation for the lower increment of body weight in mice taking clozapine is not clear.

In conclusion, we found that feeding a high-fat diet aggravates clozapine-induced toxicity in islet beta-cells of healthy adult mice. High-fat intake should be carefully controlled in patients taking clozapine for treatment of schizophrenia.

Acknowledgements
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REFERENCES
高脂肪食物加重 clozapine 對胰島β細胞的毒害性

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背 景：clozapine 是一種非典型抗精神病藥物，臨床上使用此類藥物易造成葡萄糖代謝不平衡失調。本研究探討 clozapine 對胰島β細胞的毒害及與高脂肪飲食的相關性。

方 法：隨機將 52 隻 C57BL/6 雄性小鼠分成四組。A、B 組飼食高鈣水，C、D 組飼食 clozapine。此外 A、C 組飼食一般飼料，B、D 組則給予高脂肪飼料。分別記錄小鼠的體重、體重及非空腹血糖。第 11 天每組隨機取三隻小鼠對其胰臟作組織檢查，研究胰島細胞凋亡 (apoptosis) 及進行 Ki67 免疫染色。其餘實驗鼠則在第 8 週檢驗其胰臟中胰島素含量。

結 果：本實驗顯示 clozapine 飼食會造成小鼠體重降低、胰島細胞凋亡及胰島細胞增生減少，長期使用造成胰臟胰島素含量降低。高脂肪飼食會使實驗鼠體重增加、血糖上升及胰臟中胰島素含量增加。然而，在 clozapine 治療組時飼食高脂肪飼料會加重胰島細胞凋亡，顯著減少胰臟中胰島素含量。

結 論：我們發現 clozapine 對小鼠胰島β細胞具明顯毒害性。而高脂肪食物會惡化 clozapine 的胰島破壞。建議臨床上病人使用此類藥物治療精神疾病須避免高脂肪飲食。

(長庚醫訊 2012;35:318-22)

關 鍵 詞：胰島細胞毒性，clozapine，高脂肪飲食，自體凋亡，Ki67，胰臟胰島素含量

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