Aspirin potentiates blood glucose lowering effect of glimepiride-pioglitazone combination in streptozotocin-induced diabetic rats

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Sir,

Patients of Type II Diabetes Mellitus (T2DM) need lifelong therapy with different oral hypoglycemic agents (OHAs). Many of them suffer from cardiac comorbidities and simultaneously receive lipid-lowering agents, antihypertensives, anti-platelet drugs like aspirin or clopidogrel. Though scanty, there are reports of aspirin altering blood glucose levels in euglycemic as well as hyperglycemic individuals.[1] Besides, aspirin's high (around 90%) affinity for binding plasma proteins[2] and its potential to displace concomitant medications from protein binding sites[3] are well-documented. Some oral hypoglycemic agents also are known to have high affinity for plasma proteins and hence, there is a likelihood that aspirin co-administration may favorably or adversely affect the blood glucose profile in patients receiving these OHAs, the impact of which, to the best of our knowledge, is not clearly known.

We attempted to explore whether aspirin had an intrinsic effect on blood glucose and if aspirin co-administration had an altering effect on the blood glucose control by oral hypoglycemic agents. Accordingly, we conducted a study in streptozotocin induced diabetic albino rats to observe the effect of subchronic aspirin administration on blood glucose levels and to see whether and to what extent, the glycemic control achieved by a combination therapy of two highly plasma protein binding OHAs, glimepiride and pioglitazone[4] was influenced by aspirin co-administration.

The study was performed with due approval from institutional animal ethics committee. Injection streptozotocin (Sisco Research Laboratories; batch T-835796), tablet aspirin (Ecosprin 75mg®; USV Ltd.; batch 04004359), tablet glimepiride (Glimestar 2mg®; Discovery Mankind; batch 11T-0332) and tablet pioglitazone (Pioglit 15mg®; Sun Pharmaceutical; batch ADL0095) were commercially procured. Daily doses of glimepiride 0.03 mg/kg (equivalent to human dose of 2 mg/day),[4] pioglitazone 0.21 mg/kg (equivalent to human dose of 15 mg/day),[4] low dose aspirin 4.64 mg/kg (equivalent to human doses of 325 mg/day which is an anti-platelet dose of aspirin)[2] and a higher, analgesic dose of aspirin 14.29 mg/kg (equivalent to human doses of 1000 mg/day)[2] were administered orally once daily after pulverizing and dissolving the tablets in carboxymethyl cellulose (CMC). Tail-vein blood samples were used to monitor fasting blood glucose (FBG) levels using glucometer and glucose strips (GlucoCheck Sensor, Roche Diagnostic Corporation).
After measuring FBG levels, 30 male Wistar Albino rats weighing 150-180 grams were injected with single intra-peritoneal injection of streptozotocin in 0.1 M citrate buffer (pH 4.5) at a dose of 55 mg/kg body-weight. Five days after streptozotocin administration, 24 rats having FBG > 200 mg/dl were selected and divided into four groups with 6 rats in each group. From next day, all groups started receiving drug treatment for 30 days as per following schedule:

- Group 1: Carboxymethyl cellulose for 30 days
- Group 2: Aspirin in low dose for first 15 days followed by higher dose of aspirin for next 15 days
- Group 3: Glimepiride and pioglitazone for 30 days
- Group 4: Glimepiride, pioglitazone and low-dose aspirin for initial 15 days followed by glimepiride, pioglitazone and high-dose aspirin for the next 15 days.

FBG levels were re-estimated at day 15 and day 30 of drug therapy.

Data were expressed as mean ± standard deviation (SD) and range. Between groups comparison was done using One way ANOVA followed by post-hoc Tukey's test and Independent t-test. Within group comparison at different time points was done using Repeated Measure ANOVA followed by post-hoc Tukey's test. A value of \( P < 0.05 \) was considered as significant in this study.

In Group 1, all the rats continued to remain hyperglycemic (FBG > 200 mg/dL) till day 30 of treatment.

In Group 2, there was a tendency to lowering of FBG levels in the diabetic rats with low-dose aspirin treatment for 15 days, though this reduction was not statistically significant. When aspirin was further continued for 15 more days with an escalated dose equivalent to standard analgesic dose, the FBG level was significantly reduced \( (P < 0.05) \) as compared to pretreatment value [Table 1]. However, the target FBG < 200 mg/dL was not achieved.

In Group 3, the 30-day treatment with glimepiride-pioglitazone combination caused significant reduction in FBG compared to pretreatment values \( (P < 0.05 \) by day _15 and \( P < 0.01 \) by day 30) [Table 1].

In Group 4, when aspirin in low dose was added to glimepiride-pioglitazone combination for 15 days and then the dose of aspirin was increased to the analgesic equivalent dose for another 15 days, either regime synergized the blood sugar lowering effect when compared to respective group 3 values, though not being statistically significant. However, when the mean reduction of FBG levels at fifteenth and thirtieth day of treatment relative to respective pretreatment values were compared between Group 3 and Group 4, both low-dose and high-dose add-on aspirin regimens demonstrated a statistically significant mean reduction in FBG \( (P < 0.01 \) at day _15 and \( P < 0.05 \) at day 30) [Table 2].

In this study, aspirin monotherapy in low dose for 15 days was seen to cause slight reduction in FBG levels which was not significant statistically. However, continuing aspirin in higher dose for 15 more days demonstrated statistically significant lowering in FBG. Furthermore, sub-chronic aspirin treatment in both low-dose and in low-followed-by-higher dose significantly potentiated the glucose lowering effect of glimepiride-pioglitazone combination.

A diligent literature search to find documented clinical corollary to such an observation could only reveal a few case reports depicting aspirin-induced hypoglycemia among inpatients.[1] These evidences strengthen our finding and highlight their possible clinical relevance.

The mechanisms by which aspirin alters glucose metabolism are unclear, and though few studies have described moderate and high doses of aspirin inhibiting hepatic glucose production as well as improving insulin action from inhibition of kinases IKKβ (a key downstream mediator of insulin resistance),[1,6] no conclusive study on lower doses of aspirin were found. Plasma protein binding of both glimepiride and pioglitazone are reportedly >99%[4] and aspirin is known to displace highly plasma protein binding drugs
from protein binding sites. Though the exact mechanism is unknown, our findings of aspirin synergising the blood glucose lowering action of glimepiride and pioglitazone could possibly be attributed to two properties of aspirin – intrinsic glucose lowering action and plasma protein binding drug interaction. The findings of this study tend to suggest that diabetic patients on chronic aspirin treatment may require tailoring of OHAs doses, particularly for the ones with protein-binding property.

We contemplate to commission a correlation study in clinical setting to test this hypothesis.

**Acknowledgement**

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**References**


**Figures and Tables**

**Table 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline (log_b)</th>
<th>5 days after STZ (log_STZ)</th>
<th>After 15 days of treatment (log_15)</th>
<th>After 30 days of treatment (log_30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=6)</td>
<td>73.3±4.68 (68.8-80)</td>
<td>207.8±3.48 (203-212)</td>
<td>212.6±3.39 (207-222)</td>
<td>216.5±2.59 (213-220)</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>76.8±5.05 (69-82)</td>
<td>222.1±5.12 (217-230)</td>
<td>219.8±5.08 (208-228)</td>
<td>161.5±7.92 (152-172)</td>
</tr>
<tr>
<td>3 (n=6)</td>
<td>72.8±8.14 (68.8-89)</td>
<td>219.8±2.86 (216-224)</td>
<td>139.1±7.49 <strong>(p&lt;0.05)</strong> (125-146)</td>
<td>110.3±5.48 <strong>(p&lt;0.01)</strong> (94.117)</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>79.5±7.23 (72-93)</td>
<td>230.3±4.55 <strong>(p&lt;0.05)</strong> (225-238)</td>
<td>134.6±4.46 <strong>(p&lt;0.01)</strong> (128-139)</td>
<td>101.8±3.65 <strong>(p&lt;0.01)</strong> (89-123)</td>
</tr>
</tbody>
</table>

*P<0.001 in comparison to group 1 value (One way ANOVA followed by post hoc Tukey’s test), **P<0.05 in comparison to group 3 value (One way ANOVA followed by post hoc Tukey’s test), ***P<0.01 in comparison to group 2 value (One way ANOVA followed by post hoc Tukey’s test), **P<0.05 in comparison to log_b value (Repeated measure ANOVA followed by post hoc Tukey’s test), ***P<0.01 in comparison to log_STZ value (Repeated measure ANOVA followed by post hoc Tukey’s test), ****P<0.05 in comparison to log_15 value (Repeated measure ANOVA followed by post hoc Tukey’s test)***

Comparison of fasting blood glucose levels (mg/dL) between all groups, at different time points.
### Table 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean reduction of FBG level at 15th day of treatment (FBG_15)</th>
<th>Mean reduction of FBG level at 30th day of treatment (FBG_30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (n=6)</td>
<td>80.67±6.31 (73-91)</td>
<td>109.50±9.4 (102-128)</td>
</tr>
<tr>
<td>4 (n=6)</td>
<td>95.67±6.22 * (86-103)</td>
<td>128.50±13.87 *(106-142)</td>
</tr>
</tbody>
</table>

*P<0.01 in comparison to group 3 (independent t test) *P<0.05 in comparison to group 3 (independent t test)

Comparison of mean reduction of fasting blood glucose levels (mg/dL) between groups 3 and 4, at day 15 and day 30 of treatment