Chromium (III) Complexes of Metformin, Dapagliflozin, Vildagliptin and Glimepiride Potentiate Antidiabetic Activity in Animal Model

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INTRODUCTION

Diabetes is a chronic disease which increases morbidity, mortality along with social and economic costs. Chromium [Cr(III)] is a trace element which plays an important role in glucose and lipid metabolism i.e. in the treatment of diabetes.

OBJECTIVE: To synthesize some new chromium complexes with different classes of oral antidiabetic drugs and studied their antidiabetic potentials in mice.

METHODS: To increase the therapeutic efficacy of the drugs i.e, Metformin, Dapagliflozin, Vildagliptin and Glimepiride, Cr(III) complexes of these drugs were prepared and characterized by TLC, DSC, TGA and FTIR spectroscopy. The antidiabetic activity of the complexes was also evaluated in mice model.

RESULTS: At a dose of 150 mg/kg body weight of Cr(III)-drug complexes, the serum glucose levels reduced by 20.61% for Cr-metformin (Cr-Met), 13.07% for Cr-dapagliflozin (Cr-Dapa), 7.61% for Cr-vildagliptin (Cr-Vilda) and 4.07% for Cr-glimepiride (Cr-Glim) than the corresponding parent drugs metformin, dapagliflozin, vildagliptin and glimepiride, respectively after 14 days of treatment.

CONCLUSION: The complexes were found to be effective in lowering the serum glucose level in alloxan-induced diabetic mice.

Key Words: Metformin, Dapagliflozin, Vildagliptin, Glimepiride, Chromium (III), Antidiabetic
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Metformin, dapagliflozin, vildagliptin and glimepiride are common anti-diabetic drugs (Figure 1). Cr (III) is one of the important trace elements that the body can absorb. It is claimed that Cr (III) is the most stable and essential form, and accumulated in liver, spleen, soft tissues and bone. In the body, Cr (III) plays important roles in carbohydrate and lipid metabolism. It may increase the action of insulin by the interaction with the insulin receptor on the cell surface. Chromium also inhibits the liver hydroxymethylglutaryl-CoA reductase (rate-limiting enzyme of cholesterol synthesis). Cr deficiency in serum is reported in patients with glucose intolerance, diabetes mellitus, and hypercholesterolemia and also in aged people. But it is somehow difficult to intake the trace amount of Cr (III). There are chances of co-administration of Cr (III) as antidiabetic drug complexes to promote the therapeutic efficacy of antidiabetic drugs. Since it is reported that Cr (III)-metformin complex reduced the glucose level of diabetic rat. Therefore, we tried to synthesize some new chromium complexes with different classes of oral antidiabetic drugs and studied their antidiabetic potentials in mice. This paper describes the complex formation of Cr (III) with four popular antidiabetic drugs viz. metformin, dapagliflozin, vildagliptin and glimepiride, and in vivo study of their antidiabetic property in mice model (Figure 1).

**MATERIALS AND METHODS**

**Materials**

Analytical grade chemicals, solvents and chromium (III) chloride were used for all experimental purposes without further purification. The API of antidiabetic drugs metformin (purity 99%), vildagliptin (purity 99%), glimepiride (purity 99%), and dapagliflozin (purity 99%) were received as gift samples from ACI Pharmaceuticals Ltd., Dhaka, Bangladesh. Alloxan monohydrate (98%) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India.

**Synthesis of chromium (III) complexes with antidiabetic drugs**

The Cr(III)-metformin, Cr(III)-glimepiride, Cr(III)-dapagliflozin, Cr(III)-vildagliptin complexes were synthesized by dissolving each drug e. g. metformin hydrochloride (2 mmol, 0.388 g), glimepiride (0.5 mmol, 0.245 g), dapagliflozin (0.5 mmol, 0.2051 g), vildagliptin (0.5 mmol, 0.1517 g) in 25 mL of methanol and then mixed with 25 mL methanol solution of 1 mmol CrCl₃·6H₂O (0.202 g). The mixtures were heated at 70°C in a water bath (J.P.Selecta, Spain) with continuous stirring for 3.30 hours. Then the mixtures were left overnight for precipitation.

**Characterization of synthesized chromium (III) complexes with antidiabetic drugs**

**Differential scanning calorimetry (DSC)**

The phase change properties of the Cr(III) complexes were studied by differential scanning calorimetry (DSC) (DSC-60, Shimadzu, Japan). The range of temperature was up to 300°C and temperature rising rate was 10 °C/min at a flow rate of 20 mL/min in a nitrogen gas atmosphere.

**Thermogravimetric analysis (TGA)**

The thermogravimetric analysis (TGA) of the chromium (III) complexes was carried out by thermogravimetric analyzer (TGA-50, Shimadzu, Japan) at the temperature of up to

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**Figure 1:** Structures of metformin (A), vildagliptin (B), glimepiride (C), and dapagliflozin (D).
RESULTS AND DISCUSSION

After completing the reactions both crystalline and amorphous Cr-drug complexes were obtained. The formation of complexes with drugs was confirmed by TLC, DSC, TGA and FTIR spectroscopy.

At first, TLC of the Cr-drug complexes was carried in methanol-dichloromethane in the different ratio for different complexes. Single spot from the complexes which were varied from their precursor drugs was found (Table 1). Each spot indicated the presence of a new complex.

Table 1: Rf values of drugs and their complexes on TLC

<table>
<thead>
<tr>
<th>Item</th>
<th>Mobile phase</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin HCl</td>
<td>Methanol/dichloromethane (3:7)</td>
<td>0.6</td>
</tr>
<tr>
<td>Cr-metformin complex</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Dapagliflozin</td>
<td>Methanol/dichloromethane (8:2)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cr-dapagliflozin complex</td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>Vildagliptin</td>
<td>Methanol/dichloromethane (8:2)</td>
<td>0.4</td>
</tr>
<tr>
<td>Cr-vildagliptin complex</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Glimepiride</td>
<td>Methanol/dichloromethane (2:8)</td>
<td>0.65</td>
</tr>
<tr>
<td>Cr-glimepiride complex</td>
<td></td>
<td>0.5</td>
</tr>
</tbody>
</table>

The phase changes of the pure anti-diabetic drugs and their Cr-complexes were investigated by DSC. Pure metformin displayed melting endotherm at 231°C and its Cr-complex exhibited at 224°C (Figure 2). Dapagliflozin showed melting endotherm at the point of 77.98°C and its Cr-complex showed different peaks in the thermogram (Figure 2). Vildagliptin and glimepiride indicated melting points at 152°C and 213°C, respectively whereas their Cr-complexes showed different melting endotherms, which revealed the formation of new complexes (Figure 2).

The percentages of weight loss against the increase in temperature for pure antidiabetic drugs and their complexes were investigated by thermogravimetric analysis. For pure metformin, 4.29% was found to be degraded at 205°C. It also degraded by 88% at 356°C probably by the removal of methyl groups.22 It was also found to be degraded by 97% at 599°C due to the loss of amino groups. However, Cr-metformin complex showed completely different degradation pattern (Figure 3).
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Figure 2: Overlaid DSC thermograms: metformin and Cr-metformin complex (A), dapagliflozin and Cr-dapagliflozin complex (B), vildagliptin and Cr-vildagliptin complex (C), glimepiride and Cr-glimepiride complex (D).

Figure 3: Overlaid TGA thermograms: metformin and Cr-metformin complex (A), dapagliflozin and Cr-dapagliflozin complex (B), vildagliptin and Cr-vildagliptin complex (C), glimepiride and Cr-glimepiride complex (D).

Figure 4: Overlaid IR spectra of metformin and Cr-metformin complex (A), dapagliflozin and Cr-dapagliflozin complex (B), vildagliptin and Cr-vildagliptin complex (C), glimepiride and Cr-glimepiride complex (D).

In the case of pure dapagliflozin, the degradation pattern showed 17% degradation at 189°C, 86% at 389°C and 94% at 516°C, which are due to release of hydroxyl molecules and methyl groups. Similarly, vildagliptin and glimepiride and their Cr-complexes, were showed different degradation pattern from pure drugs. The FTIR spectra were studied to measure the wavelength and intensity of transmission/absorption which have characteristics of specific types of molecular vibration and stretching that help to identify functional groups of new complexes. The FTIR spectra of pure drugs metformin, dapagliflozin, vildagliptin, glimepiride and their Cr-complexes are shown in figure 4. If the pure drugs and complexes displayed same IR spectrum it can be claimed that they are the same compounds. Therefore, any disappearance or shifts of peaks will indicate the presence of new compounds.

The characteristic stretching peak of -NH₂ of metformin seen at 3742.93 cm⁻¹ was obtained in the downfield at 3706 cm⁻¹ for Cr-metformin complexes (Figure 4). Likewise, the characteristic peaks of OH stretching of dapagliflozin, vildagliptin and glimepiride observed at 3352.28 cm⁻¹, 3741 cm⁻¹, 3772 cm⁻¹, respectively shifted in the IR spectra of the respective Cr-drug complexes to 3390 cm⁻¹ for Cr-dapagliflozin and 3402.00 cm⁻¹ for Cr-vildagliptin and 3569.54cm⁻¹ for Cr-glimepiride. These changes in the absorption in the IR spectra indicated the formation of the complex between the drug and chromium.

The Cr-drug complexes and standard antidiabetic drugs when administered to alloxan-induced diabetic mice significantly reduced the blood glucose level as compared to alloxan control mice which received distilled water and normal food (Table2). Cr-metformin complex reduced blood glucose level significantly and it was found to be 15.10 mmol/L whereas pure metformin reduced blood glucose level to 19.02 mmol/L after 14 days of treatment.
Table 2: In vivo antidiabetic property of drugs and their Cr-complexes in mice model

<table>
<thead>
<tr>
<th>Drug and their complexes</th>
<th>Before Alloxan treatment (mmol/L)</th>
<th>±SDV</th>
<th>After Alloxan treatment (mmol/L)</th>
<th>±SDV</th>
<th>After 14 days of drug treatment (mmol/L)</th>
<th>±SDV</th>
<th>% of reduction of blood sugar level from corresponding drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>5.24</td>
<td>0.64</td>
<td>31.54</td>
<td>0.68</td>
<td>19.02</td>
<td>0.64</td>
<td>20.61</td>
</tr>
<tr>
<td>Cr-Met</td>
<td>5.40</td>
<td>1.12</td>
<td>31.00</td>
<td>1.87</td>
<td>15.10</td>
<td>2.12</td>
<td></td>
</tr>
<tr>
<td>Dapa</td>
<td>5.44</td>
<td>0.27</td>
<td>30.37</td>
<td>0.80</td>
<td>17.60</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>Cr-Dapa</td>
<td>5.50</td>
<td>1.25</td>
<td>29.40</td>
<td>0.98</td>
<td>15.30</td>
<td>0.88</td>
<td>13.07</td>
</tr>
<tr>
<td>Vilda</td>
<td>5.20</td>
<td>0.71</td>
<td>31.50</td>
<td>0.85</td>
<td>19.70</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Cr-Vilda</td>
<td>5.10</td>
<td>0.90</td>
<td>31.60</td>
<td>0.97</td>
<td>18.20</td>
<td>1.21</td>
<td></td>
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<tr>
<td>Glim</td>
<td>5.40</td>
<td>0.28</td>
<td>30.24</td>
<td>1.29</td>
<td>17.20</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>Cr-Glim</td>
<td>5.50</td>
<td>0.98</td>
<td>21.70</td>
<td>0.88</td>
<td>16.50</td>
<td>1.02</td>
<td>4.07</td>
</tr>
</tbody>
</table>

CONCLUSION

Chromium (Cr) (III) reacted with four anti-diabetic drugs namely metformin, dapagliflozin, vildagliptin, glimepiride at high temperature and produced complexes which were justified by studying their thermochemical properties e.g. DSC, TGA, TLC and IR spectroscopy. The Cr-drug complexes and standard anti-diabetic drugs when administered to alloxan-induced diabetic mice significantly reduced the blood glucose level as compared to alloxan control mice which received distilled water and normal food. It was found that after 14 days of treatment with metformin, dapagliflozin, vildagliptin, glimepiride, the average glucose levels of mice reduced from 31.54 to 19.02, 30.37 to 17.60, 31.50 to 19.70 and 30.24 to 17.20 mmol/L, respectively; whereas chromium complexes of these drugs i.e. Cr-metformin, Cr-dapagliflozin, Cr-vildagliptin and Cr-glimepiride were found to be reduced blood glucose levels in mice from 31.0 to 15.10, 29.40 to 15.30, 31.60 to 18.20 and 21.70 to 16.50 mmol/L, respectively. The Cr-complexes were found to be more potent than the corresponding pure drugs viz. Cr-metformin, Cr-dapagliflozin, Cr-vildagliptin and Cr-glimepiride showed 20.61%, 13.07%, 7.61%, 4.07% more effective than metformin, dapagliflozin, vildagliptin, glimepiride drugs, respectively. Among them, Cr-metformin complex reduced blood glucose level significantly and it was found to exhibit 20.61% higher antidiabetic activity than the pure metformin. However, the untoward effects of these complexes could not be established at this moment. Whether the Cr-complexes have long-term health benefits or untoward effects are not yet known, although the mice model experimental data indicated that Cr-complexes can improve glucose metabolism. Therefore, comprehensive studies, including in vivo model will be required to establish other beneficial and/or untoward effects of these Cr-drug complexes. Conducted study reveals that the chromium complexes of traditionally used antidiabetic drugs increase the efficacy of the drugs. The finding opens the possibility to promote the production and utilization of chromium complexes of metformin, dapagliflozin, vildagliptin and glimepiride for treating diabetes mellitus.

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