Metabolic Effect of Co-Administered Lopinavir/Ritonavir and Sulfamethoxazole/Trimethoprim in Albino Rats

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Abstract: Antiretroviral drugs containing lopinavir/ritonavir (LPV/r) are used concurrently with sulfamethoxazole/trimethoprim (SMX/TMP) in the management of HIV associated co-infections. These drugs have been individually implicated in metabolic disorders; therefore this research evaluates the effects of the co administration of these agents on lipid profile, glucose level, pancreatic enzymatic antioxidants malondialdehyde and pancreas architecture in rats. Eighty (80) adult albino rats which were divided into 5 groups (A-E) were used in this study. Animals were treated as follows: A (control) (1% ethanol), B (22.4/4.6 mg/kg SMX/TMP), C (22.8/5.8mg/kg LPV/r) and D (SMX/TMP+ LPV/r) for 2-8 weeks. SMX/TMP exhibited no significant increase in serum triglyceride (TG), cholesterol (CH), low density lipoprotein (LDL) and glucose (GL) levels while LPV/r treatment showed significant increase in these parameters. Treatment with LPV/r produced significant increase in MDA with decrease in SOD and GSH while SMX/TMP did not produce any significant change in these parameters. Synergistic effects were not observed in any of these parameters when LPV/r and SMX/TMP were co administered. Pancreatic architecture of animals treated with single and combined doses of these agents showed normal exocrine and endocrine gland infiltrated by mononuclear inflammatory cells. Concurrent use of these agents in HIV associated co-infection may not be associated with metabolic disorder with respect to dose used in this study.

Key words: Metabolic Disorder Lopinavir - Ritonavir Sulphamethoxazole - Trimethoprim Rats

INTRODUCTION

Lopinavir/ritonavir is a component of highly active antiretroviral therapy that belongs to the protease inhibitors family. It is the first protease inhibitors fixed dose combination employed in the management of HIV/AIDS. This combination is of clinical importance because lopinavir is extensively metabolized by Cytochrome 3A4 and 3A5 [1]. Ritonavir is a potent inhibitor of hepatic enzymes (Cytochrome 3A4 and 3A5). The use of ritonavir with lopinavir inhibits the metabolism of lopinavir by these hepatic enzymes thereby increasing its concentration [2]. Lopinavir /ritonavir combination is reported to be well tolerated after oral administration but reports have associated it with some toxicological effects. It has been implicated in metabolic disorder characterized by elevations in total cholesterol, low density lipoprotein, triglyceride, insulin resistance and hyperglycemia [3, 4].

Sulfamethoxazole/ trimethoprim is a fixed dose combination drug with a broad spectrum of antimicrobial activity against aerobicGram positive and negative organisms, fungi and protozoan. It has excellent chemoprophylactic activity against P. jiroveci Pneumonia, T. gondii, it also reduces the risk of malaria, isosporiasis and KaposisSarcoma[5]. Probably due to its broad spectrum of activity, WHO recommends the use of this agent as prophylaxis for adult HIV patients with clinical stages 2, 3 or 4 HIV infection[6]. Sulfamethoxazole/trimethoprim is well tolerated with few adverse effects but some scholars have associated it with some possible metabolic effects [7, 8].

Reports have associated HIV/AIDS with co-morbidity and co-infection which is of clinical concern [9]. This may necessitate the concurrent use of antiretroviral agents with broad spectrum antimicrobial agents. The concurrent use of these agents may add to pill burden and
possibly adverse effects on body tissue and organs which calls for evaluation. In this study we evaluated the effects of co-administered lopinavir/ritonavir and sulfamethoxazole/trimethoprim which can be used concurrently in the management of HIV/AIDS associated co-morbidity and co-infection in rats. Effects were evaluated on lipid profile, glucose level, pancreatic enzymatic antioxidants, malondialdehyde and pancreas architecture.

**MATERIALS AND METHODS**

**Drugs:** Lopinavir/ritonavir used was manufactured by Myland Laboratories Limited India while sulfamethoxazole/trimethoprim was manufactured by CSPC Ouyi Pharmaceuticals China. Both drugs were of analytical grade.

**Animals:** The animals used in this research work were obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The animals were allowed free access to food and water ad libitum and were allowed to acclimatize for 14 days. Animals were handled according to Helsinki declaration on the handling and use of animals.

**Dose Selection:** 22.4/4.6 mg/kg of sulfamethoxazole/trimethoprim and 22.8/5.8 mg/kg of Lopinavir/ritonavir were used in this study [10, 11].

**Preparation of Drug:** Lopinavir/ritonavir tablets were crushed and dissolved in 1% ethanol while sulfamethoxazole/trimethoprim tablets were also crushed and suspended in water [12].

**Grouping of Animals:** Eighty healthy male adult rats of average weight 300±5 g were used in this study. The rats were divided into five groups A B C D and E.

**Drug Administration:**

**Group A:** This served as the control and contained twenty animals which were treated with water 1% ethanol orally.

**Group B:** This group contained 15 animals which were further divided into three subgroups (B1-B3). Animals in sub group B1 were treated with 22.4/4.6 mg/kg of SMX/TMP. Animals in subgroup B2 were treated with 22.8/5.8 mg/kg of LPV/r. Animals in subgroup B3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 2 weeks.

**Group C:** This group contained 15 animals which were further divided into three subgroups (C1-C3). Animals in sub group C1 were treated with 22.4/4.6 mg/kg SMX/TMP. Animals in subgroup C2 were treated with 22.8/5.8 mg/kg of LPV/r. Animals in subgroup C3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 4 weeks.

**Group D:** This group contained 15 rats which were further divided into three subgroups (D1-D3). Animals in sub group D1 were treated with 22.4/4.6 mg/kg of SMX/TMP. Animals in subgroup D2 were treated with 22.8/5.8 mg/kg of LPV/r. Animals in subgroup D3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 6 weeks.

**Group E:** This group contained 15 animals which were further divided into three subgroups (E1-E3). Animals in sub group E1 were treated with 22.4/4.6 mg/kg of SMX/TMP. Animals in subgroup E2 were treated with 22.8/5.8 mg/kg of LPV/r. Animals in subgroup E3 were treated with combined doses of SMX/TMP + LPV/r. All animals in this group were treated for 8 weeks.

**Collection of Sample for Analysis:** Animals were sacrificed using chloroform anesthesia at the end of 2, 4, 6 and 8 weeks of treatment respectively. About 2 ml of blood was collected directly via cardiac puncture, under aseptic conditions into sterile sample containers. The sample was allowed to clot and centrifuged at 1000 rpm for 5 mins using Uniscope centrifuge and serum separated for analysis. Rats were dissected and pancreas was collected for analysis. Blood samples were also collected from the tail artery of the rats at 2, 4, 6 and 8 weeks time interval for evaluation of blood glucose level.

**Determination of Cholesterol, Triglyceride and Low Density Lipoprotein:** Serum concentrations of total cholesterol, triglycerides and LDL cholesterol fractions were measured using standard methods with commercially available kits [13].

**Determination of Blood Glucose Levels:** Blood glucose level was evaluated with the aid of ONE TOUCH BASIC meter (LIFESCAN, Inc., 2001 Milpitas, CA 95035, USA) and results were expressed as mg/dl [14].

**Preparation of Tissue Homogenate:** Pancreas was quickly removed and washed in ice cold saline. The pancreas was sliced into pieces and homogenized in ice...
cold tri-hydrochloride buffer (pH 7.2). The homogenates were centrifuged at 3200 rpm for 10mins. Supernatant obtained was used for estimation of lipid malondialdehyde and enzymatic antioxidants.

**Determination Malondialdehyde Glutathione and Superoxide Dismutase:** Pancreatic malondialdehyde, superoxide dismutase and glutathione level were evaluated using the methods reported by Ahmed and Hassainein [15].

**Statistical Analysis:** Results were expressed as mean ± S.E.M. Statistical analysis was done with the aid of SPSS for windows; SPSS Inc., Chicago, Standard version 14.0 to determine difference between mean using one way Analysis of Variance (ANOVA).

**RESULTS**

**Effects on Cholesterol, Triglyceride and Low Density Cholesterol:** Treatment with SMX/TMP for 2-8 weeks didn’t produce any significant (p>0.05) change in cholesterol level. Animals treated with LPV/r showed a significant (45, 71% p<0.05) time dependent increase in cholesterol level in week 6 and 8 respectively when compared with the control. Animals exposed to SMX/TMP didn’t produce any significant (p>0.05) change in cholesterol level after treatment for 2-8 weeks with respect to the control. Combine doses of LPV/r and SMX/TMP significantly (p<0.05) increased cholesterol levels by 52% and 73% respectively in week 6 and 8 when compared with the control (Table 1).

Animals exposed to SMX/TMP didn’t produce any significant (p>0.05) change in triglyceride level after treatment for 2-8 weeks with respect to the control. Treatment with LPV/r produced a significant (45%, 68% p<0.05) time dependent increase in serum triglyceride level at week 6 and 8 respectively when compared with the control. Co administered doses of these agents increased serum triglyceride levels significantly (p<0.05) by 44% and 68% in week 6 and 8 with respect to the control (Table 2).

Treatment for 2-8 weeks with SMX/TMP didn’t produce any significant (p>0.05) change in LDL level with respect to the control. Animals exposed to LPV/r produced a time dependent increase in LDL level which becomes significant (40%, 59% p<0.05) at week 6 and 8 when compared with the control. Also co administration of LPV/r and SMX/TMP increased LDL level significantly (p<0.05) by 47% and 61% at week 6 and 8 with respect to the control (Table 3).

**Effects on Malondialdehyde, Superoxide Dismutase, Glutathione and Glucose:** Treatment with SMX/TMP for 8 weeks didn’t produce any significant change in SOD, MDA and GSH levels. Significant (p<0.05) decrease in SOD which represents 46% was observed in animals treated with lopinavir/ritonavir while 48% decrease was observed in animals treated with combined doses of LPV/r and SMX/TMP for 8 weeks respectively when compared with the control (Table 4).

Significant (p<0.05) decrease in pancreatic GSH level which represents 43% was observed in animals treated with LPV/r for 8 weeks. Forty four percent (44%) decrease was noted in animals co administered with LPV/r and SMX/TMP for 8 weeks with respect to control. Malondialdehyde level was significantly (p<0.05) increase by 67% in animals exposed to LPV/r for 8 weeks with respect to the control. Animals treated with combined doses of LPV/r and SMX/TMP showed 69% significant (p<0.05) increase in MDA level with respect to the control. Treatment with SMX/TMP did not produce any significant (p>0.05) change in MDA and GSH level when compared with the control (Table 4).

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**Table 1:** Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and their combination on total serum cholesterol (mg/dl) in rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK2</th>
<th>WK4</th>
<th>WK6</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>60.4±2.15</td>
<td>65.1±2.22</td>
<td>68.3±1.03</td>
<td>70.2±3.21</td>
</tr>
<tr>
<td>SMX/TMP (22.4/4.6mg/kg)</td>
<td>60.2±3.21</td>
<td>59.1±2.01</td>
<td>60.3±4.10</td>
<td>61.±2.03</td>
</tr>
<tr>
<td>LPV/r (22.8/5.8mg/kg)</td>
<td>65.1±3.00</td>
<td>72.2±3.10</td>
<td>99.1±2.50*</td>
<td>120.1±3.21*</td>
</tr>
<tr>
<td>SMX/TMP +LPV/r</td>
<td>68.1±3.14</td>
<td>74.8±3.25</td>
<td>103.4±2.20*</td>
<td>121.2±2.10*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at p<0.05 (ANOVA).

**Table 2:** Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and combination on serum triglyceride (mg/dl) in rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK2</th>
<th>WK4</th>
<th>WK6</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>47.3±3.10</td>
<td>47.7±2.21</td>
<td>48.3±1.14</td>
<td>50.1±4.10</td>
</tr>
<tr>
<td>SMX/TMP (22.4/4.6mg/kg)</td>
<td>47.4±6.10</td>
<td>47.2±5.22</td>
<td>48.5±2.24</td>
<td>49.2±2.14</td>
</tr>
<tr>
<td>LPV/r (22.8/5.8mg/kg)</td>
<td>49.7±5.11</td>
<td>50.3±3.14</td>
<td>69.1±3.31*</td>
<td>83.1±3.20*</td>
</tr>
<tr>
<td>SMX/TMP +LPV/r</td>
<td>50.9±3.35</td>
<td>52.5±2.14</td>
<td>69.8±1.40*</td>
<td>84.2±3.20*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at p<0.05 (ANOVA).
Table 3: Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and combination on low density lipoprotein (mg/dl) in rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK2</th>
<th>WK4</th>
<th>WK6</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>16.7±2.23</td>
<td>18.8±2.05</td>
<td>17.9±3.13</td>
<td>18.2±2.51</td>
</tr>
<tr>
<td>SMX/TMP (22.4/4.6mg/kg)</td>
<td>17.3±3.21</td>
<td>17.8±0.53</td>
<td>17.9±4.10</td>
<td>18.8±3.30</td>
</tr>
<tr>
<td>LPV/r (22.8/5.8mg/kg)</td>
<td>17.1±4.00</td>
<td>18.5±3.10</td>
<td>25.0±2.50*</td>
<td>28.9±3.41*</td>
</tr>
<tr>
<td>SMX/TMP +LPV/r</td>
<td>18.4±2.04</td>
<td>19.1±2.15</td>
<td>26.2±1.10*</td>
<td>29.2±2.10*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at \( p<0.05 \) (ANOVA).

Table 4: Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and combination for 8 weeks on pancreatic SOD GSH and MDA in rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>SOD</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>5.20±1.26</td>
<td>7.85±4.16</td>
<td>1.65±2.21</td>
</tr>
<tr>
<td>SMX/TMP (22.4/4.6mg/kg)</td>
<td>5.01±3.30</td>
<td>7.83±3.41</td>
<td>1.75±2.30</td>
</tr>
<tr>
<td>LPV/r (22.8/5.8mg/kg)</td>
<td>2.82±2.51*</td>
<td>4.54±3.17*</td>
<td>2.75±1.40*</td>
</tr>
<tr>
<td>SMX/TMP +LPV/r</td>
<td>2.74±2.24*</td>
<td>4.43±2.15*</td>
<td>2.79±3.11*</td>
</tr>
</tbody>
</table>

SOD (u/g/protein), GSH (µ/mg/protein), MDA (nmol/mg/protein)

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at \( p<0.05 \) (ANOVA).

Table 5: Effects of lopinavir/ritonavir, sulfamethoxazole/trimethoprim and combination on glucose level (mg/dl) in normotensive rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK2</th>
<th>WK4</th>
<th>WK6</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>60.8±2.15</td>
<td>65.5±3.20</td>
<td>70.3±1.17</td>
<td>73.5±2.27</td>
</tr>
<tr>
<td>SMX/TMP (22.4/4.6mg/kg)</td>
<td>66.0±3.30</td>
<td>64.6±2.51</td>
<td>73.5±4.20</td>
<td>50.1±3.21*</td>
</tr>
<tr>
<td>LPV/r (22.8/5.8mg/kg)</td>
<td>62.7±141</td>
<td>68.4±1.55</td>
<td>83.1±3.18</td>
<td>100±2.31*</td>
</tr>
<tr>
<td>SMX/TMP +LPV/r</td>
<td>62.9±2.15</td>
<td>69.2±1.10</td>
<td>83.5±2.32</td>
<td>101.4±0.25*</td>
</tr>
</tbody>
</table>

Results are expressed as mean± SEM, the superscript (*) means significant difference with respect to the control at \( p<0.05 \) (ANOVA).

Fig. 1: Photomicrograph of H&E stained section of the pancreas of control animals treated with 1% ethanol showing normal exocrine and endocrine components (Mag x 400)

Fig. 2: Photomicrograph of H&E stained section of the pancreas of animals treated with 22.4/4.6mg/kg of sulfamethoxazole/trimethoprim showing normal exocrine and endocrine components (Mag x 400)

Fig. 3: Photomicrograph of H&E stained section of the pancreas of animals treated with 22.8/5.8mg/kg of lopinavir/ritonavir showing normal exocrine and endocrine glands with patchy infiltration of the interstitium by mononuclear inflammatory cells. (Mag x 400)

Fig. 4: Photomicrograph of H&E stained section of the pancreas of animals treated with combined doses of lopinavir/ritonavir and sulfamethoxazole/trimethoprim showing normal exocrine and endocrine glands with patchy infiltration of the interstitium by mononuclear inflammatory cells (Mag x 400)
SMX/TMP produced a significant ($p<0.05$) decrease in glucose level after treatment for 8 weeks with respect to the control. Treatment with LPV/r produced time-dependent increase in glucose level which was significant ($p<0.05$) in week 8 with respect to the control. Combination of LPV/r and SMX/TMP also produced time-dependent increase in glucose level which was significant ($p<0.05$) in week 8 with respect to the control (Table 5).

**Effect on Histopathology of Pancreas:** Pancreas of animals treated with SMX/TMP, LPV/r and their combination showed normal exocrine and endocrine components with patchy infiltration of the interstitium by mononuclear inflammatory cells when compared with the control (Figs. 1-4).

**DISCUSSION**

Our finding shows that cholesterol level remained normal after therapy with SMX/TMP which is at variance with some reported observations [16]. Elevation in cholesterol level observed with LPV/r in our study is in agreement with the work of Monte et al. [17] who documented elevated cholesterol level in association with LPV/r therapy. Concurrent use of SMX/TMP and LPV/r may not have any deleterious effect on cholesterol level due to lack of synergistic increase in cholesterol level when they were co-administered. Also our finding with respect to LPV/r induced elevation in LDL agrees with the work of Mencarelli and co-researchers who reported elevation in LDL induced by ritonavir [18]. Concurrent use of SMX/TMP and LPV/r may not have deleterious effect on LDL with respect to observation in our work. Lee and Colleagues who evaluated the metabolic effects of LPV/r in HIV – negative men reported increase in triglyceride level which is consistent with our findings [19]. In this study we observed that there wasn’t any synergistic increase in triglyceride level when LPV/r and SMX/TMP were co-administered which shows that these agents could be used concurrently without any adverse impact on triglyceride level.

Observed increase in LDL in this study could be attributed to increased hepatic hydroxyl-3-methylglutaryl-CoA reductase (hmger) expression which may have enhanced VLDL production and with a corresponding elevation in LDL-cholesterol as reported by Reyskens et al. [20]. Also ritonavir was reported to attenuate chymotrypsin and trypsin like activities of the 20S UPS proteasome subunit in hepatocytes. As a result, degradation of apolipoprotein B (major determinant of plasma lipid levels) may be diminished thus providing a potential mechanism for LPV/r induced hyperlipidemia [21, 22]. Furthermore, activating sterol regulatory element binding protein (SREBPs) are ubiquitinated and degraded by the ubiquitin-proteasome system (UPS) [23, 24] raising the possibility that an inhibition of this system may also contribute to development of dyslipidemia in HIV-infected individuals treated with PIs.

The decrease in glucose level observed in SMX/TMP is in agreement with some reported observation by some researchers who have attributed it to increase in insulin production induced by this agent [25]. Some studies have observed increase in glucose level with LPV/r therapy. One of these observations was reported by Cavenaghi and colleagues who documented increase in glucose level in LPV/r treated animal which agrees with our findings [26]. Also authors have implicated LPV/r in insulin resistance, new onset diabetes and complicating existing diabetes [27]. It is noteworthy that SMX/TMP had no significant effect on LPV/r associated elevation in glucose level when co-administered as observed in this study hence could be used concurrently without recourse to glucose level. Elevation in glucose level by LPV/r could be attributed to selective and potent inhibition of the intrinsic transport activity of the insulin-regulated glucose transporter isoform Glut4 in fat and muscle which may alter insulin sensitivity accounting for LPV/r ability to cause hyperglycemia [28]. Increase in glucose level observed in this study may have also occurred through the direct action of LPV/r on pancreatic beta cells leading to decrease function as reported by Woerle et al. [29].

Malondialdehyde is a product of lipid peroxidation and its elevation is a marker of oxidative stress [30]. This suggests that increase in pancreatic MDA level induced by LPV/r is a marker of pancreatic oxidative stress which was not potentiated when LPV/r was administered with SMX/TMP. Enzymatic antioxidants such as catalase, glutathione peroxidase and superoxide dismutase protect organs from drug induced oxidative stress; impairment in the functions of these enzymatic antioxidants could occur through oxidative stress [31]. There was no synergistic decrease in GSH and SOD levels observed in this study when LPV/r and SMX/TMP were co-administered.

Scholars have reported morphological changes in pancreas of rats treated with LPV/r which is consistent with our findings which showed inflammatory cells infiltration of the pancreas [32]. One of the factors that
could potentiate LPV/r induced pancreatic damage is the ability of pancreas to concentrate LPV/r as reported by Denissen et al. [33]. Also inflammatory cells infiltration of the pancreas as observed in this study could be associated with oxidative stress through the generation of reactive oxygen species which could be correlated with observed increase in malondialdehyde level and decreased enzymatic antioxidants. Concurrent use of SMX/TMP and LPV/r may not have deleterious effect on the pancreas due to observation in this study.

**CONCLUSION**

In this study LPV/r induced elevation in lipid profile, glucose level, malondialdehyde and decrease in pancreatic enzymatic antioxidants wasn’t potentiated when co administered with SMX/TMP. This shows that the concurrent use of antiretroviral containing LPV/r with SMX/TMP in the management of HIV/AIDS associated and co-infection may not have any adverse metabolic effect.

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