CHANGES IN BLOOD COUNT ASSOCIATED WITH MULTIPLE DRUG ADMINISTRATION IN RABBIT

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ABSTRACT

Hematological toxicities are very common and associated with the prolong use of drugs. The present study is performed to evaluate the comparison of individual drugs and cumulative effect of combinations of drugs on hematological parameters. These drugs are usually prescribed for the treatment of multiple disorders including hypertension, hyperlipidemia and hyperglycemia. In the present study, individual as well as different combinations of the drugs are given to the rabbits for the period 60 days. Blood samples were taken at the end of study to evaluate red blood cell count (RBC), white blood cell count (WBC), platelets and hemoglobin percentage. The results showed no any change in RBCs and hemoglobin percentage. However, as compared to control rabbits, Glibenclamide, Metformin, Lisinopril individually as well as those received combinations of Glibenclamide, Losartan and Atorvastatin and Metformin, Amlodipine and Atorvastatin decreased platelets count (P<0.05) while Amlodipine increased it (P<0.05).Glibenclamide and combinations of Glibenclamide, Losartan and Atorvastatin and Metformin, Amlodipine and Atorvastatin decreased WBCs (P<0.05).

KEYWORDS: Hematological toxicities, hyperlipidemia, hypoglycemia, hypertension, platelets, white blood cells and red blood cells.

INTRODUCTION

Many medicines have been shown to produce hematological disorders through different mechanisms. Among these some of the drugs are not in use now like penicillin, Quinidine etc. Recently the physicians have described drugs like hormones, heparin, COX-2 inhibitors etc. Drugs can affect RBCs, WBCs, platelets and coagulation process. [1]
Drug induced hematological disorders are not much frequent. In most of the patients using multiple drugs, it is quite difficult to identify which of the agents are producing blood targeted disease. It is a real challenge for the researcher to identify the suspecting medicine. Some of the drugs like quinidine, vancomycin, heparin which can be tested in vitro to identify blood related disease while for most of the commercially available agents assays techniques are not useful for standardization.\cite{2} As some of the toxic effects are may be the result of related metabolites.\cite{3}

Some of the older drugs which are now removed from market and no longer in use like penicillin, quinidine, chloramphenical are associated with blood toxicities, however, the new compound have also potential blood toxicities. These agents include ribavirin, rituximab, and clopidogeletc.\cite{4} Cytopenicas is the classical drug-induced effect however, thrombosis is increasingly found with the use of cox2 Inhibitors, hormones for hormone-replacement therapy (HRT), erythropoietin, thalidomides.\cite{5} It is necessary that physician must take care and understand the possible consequences of drugs related disease of blood cells.\cite{6}

**Type of Toxicities**
Spectrum of toxicities depending upon the type of cell affected due to drug induced toxicities. Table 1 summarizes the drug induced of hematological disorders.\cite{7-9}

### Red cell Disorders
- Nonimmune Hemolytic Anemias
- Immune Hemolytic Anemia
- Megaloblastic Anemia
- Methemoglobinemia
- Sideroblastic Anemia
- Red Cell Aplasia
- Aplastic Anemia

### White Cell Disorders\cite{10-12}
- Myelodysplasia and Acute Leukemia
- Eosinophilia
- Neutrophilia
- Agranulocytosis/Neutropenia
Platelets Disorders\textsuperscript{[13-15]}

- Immune Thrombocytopenia
- Thrombotic Microangiopathies
- Platelet Dysfunction
- Hypercoagulability
- Circulating Anticoagulant
- Hypoprothrombinemia

TABLE 1: Drugs Induced Hematologic Toxicity\textsuperscript{[16-17]}

<table>
<thead>
<tr>
<th>Ref</th>
<th>Name of Drugs</th>
<th>Hematological Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18]</td>
<td>Pencillins, cephaloporins, alpha-methyl-DOPA, oxaliplatin, fludarabine, anti-Rh D antiglobulin</td>
<td>Immunohemolytic anemia</td>
</tr>
<tr>
<td>[2]</td>
<td>Ribavirin, phenazopyridine, chloroquine</td>
<td>Nonimmune hemolytic anemia</td>
</tr>
<tr>
<td>[8]</td>
<td>Phenazopyridine, dapsone, benzocaine, prilocaine</td>
<td>Methemoglobinemia</td>
</tr>
<tr>
<td>[12]</td>
<td>Rimethoprim, pyrimethamine</td>
<td>Megaloblastic anemia</td>
</tr>
<tr>
<td>[19-20]</td>
<td>Isoniazid, chloramphenicol, linezoldediphenhydantoin</td>
<td>Sideroblastic anemia</td>
</tr>
<tr>
<td>[21]</td>
<td>Chloramphenical, gold, NSAIDs</td>
<td>Aplastic anemia</td>
</tr>
<tr>
<td>[22-23]</td>
<td>Diphenylhydantoin, azathioprine, chloropropamide, isoniazid, erythropoietin</td>
<td>Pure red cell aplasia</td>
</tr>
<tr>
<td>[24]</td>
<td>Quinine, quinidine, heparin, vancomycin, sulfas, penicillins, glycoprotein IIb-IIIa inhibitors</td>
<td>Immune thrombocytopenia</td>
</tr>
<tr>
<td>[25]</td>
<td>Quinine, quinidine, clopidogrel, ticlopidine, cyclosporine A, mitomycin-C, cisplatin</td>
<td>Thrombotic microangiopathy</td>
</tr>
<tr>
<td>[26]</td>
<td>Pencillins, beta-lactam antibiotics, aspirin, NSAIDs</td>
<td>Platelet dysfunction</td>
</tr>
<tr>
<td>[27]</td>
<td>Estrogens, tamoxifen, asparaginase, heparin, bevacizumab, thalidomide/lenalidomide, COX-2 inhibitors, erythropoietin</td>
<td>Hypercoagulability</td>
</tr>
<tr>
<td>[26]</td>
<td>Isoniazid, hydralazine, procainamide</td>
<td>Circulating anticoagulants</td>
</tr>
<tr>
<td>[28]</td>
<td>Cephalosporins, pencillins, sulfas</td>
<td>Hypoprothrombinemia</td>
</tr>
<tr>
<td>[28]</td>
<td>Antithyroid drugs, procainamide, sulfas, captopril, phenothiazines, diphenylhydantoin, rituximab</td>
<td>Neutropenia</td>
</tr>
<tr>
<td>[29]</td>
<td>Glucocorticoids, lithium, G- and GM-CSF</td>
<td>Neutrophilia</td>
</tr>
<tr>
<td>[30]</td>
<td>Pencillins, sulfas, allopurinol, diphenylhydantoin</td>
<td>Eosinophilia</td>
</tr>
<tr>
<td>[31]</td>
<td>Erythropoietin, anabolic steroids, diuretics</td>
<td>Polycythemia</td>
</tr>
<tr>
<td>[31]</td>
<td>Alkylating agents, topoisomerase II inhibitors</td>
<td>Acute leukemia/myelodyplasia</td>
</tr>
</tbody>
</table>
MATERIAL AND METHODS
Rabbits of either sex from 1.2 to 1.5 kg were purchased from the local animal supplier and kept for a conditioning period of a week in the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Karachi. The animals were given free access to normal diet and water. Animal house environment was balanced with the temperature of 22 to 25 °C, with an equal distribution of illumination time in 12 hours ON and OFF sets per day.

**Hematological Testing**
Blood samples were collected in EDTA.K3 tubes for hematological examination of RBC, WBC, hemoglobin and platelet count by automatic Humacount plus (3 part differential with histogram, Hematology analyzer. model # 6400/S, Human Germany).

**Statistical Analysis**
All values were compared with control by taking mean and standard error (SE) to the mean using Two-way analysis of variance (ANOVA) followed by post hoc. Data was reported as mean ± SE with 95% confidence interval and p-values were noted. Values of P<0.05 were considered as significant and P<0.005 as highly significant.\[32\]

**RESULTS AND DISCUSSION**
Table 2 shows the comparison of hemoglobin concentration, platelet, WBC and RBC count between control animals and animals kept on individual drugs and their combinations for duration of 60 days in normal therapeutic doses.

Hematological parameters including RBC, platelets, WBC and hemoglobin were comparable to control in groups received acarbose, losartan, and atorvastatin alone at the end of dosing.

Animals kept on glibenclamide, metformin and lisinopril individually revealed significant decrease in platelet count i.e. 337±27x10⁵/mm³, 367±18x10⁵ /mm³, 345±12.10x10⁵/mm³ respectively with respect to control i.e. 428±25x10⁵/mm³. However, amlodipine showed significant increase in platelet count i.e. 772±10x10⁵/mm³ with respect to control. Conversely there was no significant change in other hematological parameters at the end of dosing, while animals received glibenclamide alone showed significant decrease in WBC count i.e. 2.88±0.23 x10³/mm³ with respect to control i.e. 7.70±0.24x10³/mm³. On the other hand there was no significant change in other hematological parameters in these animals groups.
Animals received GlLAt, GLoAt combinations revealed significant decrease in WBC count i.e. $1.78\pm0.01\times10^3/mm^3$ and $1.04\pm0.54\times10^3/mm^3$ with respect to control i.e. $7.70\pm0.24\times10^3/mm^3$. However, animals kept on GLoAt and MAAt combination revealed significant decrease in platelets count i.e. $215\pm18\times10^5/mm^3$ and $210\pm24\times10^5/mm^3$ with respect to control i.e. $428\pm25\times10^5/mm^3$. Conversely there was no significant alteration in other hematological parameters at the end of dosing in these groups of animals.

**Table 2: Comparison Of Hematological Parameters Following 60 Days Administration Of Individual Drugs And Their Combinations.**

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>RBC ($x10^6/mm^3$)</th>
<th>Platelet ($x10^5/mm^3$)</th>
<th>WBC ($x10^3/mm^3$)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.65±0.12</td>
<td>428±25</td>
<td>7.70±0.24</td>
<td>9.34±0.34</td>
</tr>
<tr>
<td>Acarbose</td>
<td>5.28±0.048</td>
<td>393±4.06</td>
<td>3.06±0.24</td>
<td>10.01±0.28</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>4.75±0.15</td>
<td>337±27*</td>
<td>2.88±0.23*</td>
<td>9.82±0.49</td>
</tr>
<tr>
<td>Metformin</td>
<td>6.08±0.72</td>
<td>367±18*</td>
<td>5.29±0.53</td>
<td>9.96±0.34</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>4.99±1.05</td>
<td>345±12.10*</td>
<td>3.47±0.65</td>
<td>9.48±0.96</td>
</tr>
<tr>
<td>Losartan</td>
<td>6.77±0.35</td>
<td>492±54</td>
<td>4.40±1.75</td>
<td>11.86±0.21</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>4.64±0.26</td>
<td>413±35</td>
<td>3.46±0.63</td>
<td>7.96±0.19</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>3.99±0.61</td>
<td>772±10*</td>
<td>3.13±0.35</td>
<td>7.59±0.29</td>
</tr>
<tr>
<td>GlLAt</td>
<td>4.68±0.79</td>
<td>417±7.98</td>
<td>1.78±0.01*</td>
<td>8.94±1.23</td>
</tr>
<tr>
<td>GLoAt</td>
<td>4.34±0.38</td>
<td>215±18*</td>
<td>1.04±0.54*</td>
<td>7.91±0.12</td>
</tr>
<tr>
<td>MAAt</td>
<td>5.20±0.33</td>
<td>210±24*</td>
<td>3.15±0.01</td>
<td>7.88±1.26</td>
</tr>
</tbody>
</table>

GlLAt: Acarbose, Lisinopril and Atorvastatin, GLoAt: Glibenclamide, Losartan and Atorvastatin and MAAt: Metformin, Amlodipine and Atorvastatin.

n=10.
Mean ± S.E.M.
*p < 0.05 significant with respect to control.
**p <0.005 highly significant with respect to control.

Drug interactions are of great concern because doctors and patients are unaware of the risks of toxicities due to simultaneous administration of drugs. It is therefore necessary to investigate such combinations that are less likely to interact with each other. Several studies have been done previously to determine the toxicities associated with the use of various combinations. But little work has done to evaluate toxicities associated with the simultaneous use of antihypertensive, antidiabetic and anti hyperlipidemic drugs. The present work has been therefore specially design to assess toxicity of not only individual drugs but various
combinations that may be probably used in case of multiple disorders and to suggest a combination safer for the patients.

In present study only animals received glibenclamide alone and GLoT combination showed significant increase in WBCs count which may be the side effect of glibenclamide already reported in literature,\[34\] while other groups did not show any change in WBCs count. The exact mechanism is still under investigation however, the increase may be associated with severe liver injury or infection which was not observed in present study when glibenclamide used alone. However chronic inflammation was reported in hepatic tissue of animals used in the combination.\[35\]

In the present study platelets count in all the groups remained normal with respect to control however, the animals received GLoAt and MAAt combinations showed significant decrease in platelets count although the drugs used have no individual effect on the platelet count. It may be associated with the idiosyncratic reaction of atorvastatin,\[36\] and its metabolites as well as it may be due to metformin as several studies confirmed chronic use is associated with decrease platelets count and causes diopathic thrombocytopenic purpura.\[37\] It is therefore necessary that atorvastatin and metformin should be used with caution with regular platelets count when taken for prolong period of time.\[38-39\]

CONCLUSION
From above study it is found that multiple drugs those are used for prolong period of time should be taken with regular blood count of the patient as in the above study it is clearly found the drugs in combination may produce synergetic effect to change the numbers of cell.

REFERENCES


