Effect of Some Antihypertensive Drugs on Alkaline Phosphatase and DNA of Mice

El-Khawaga OY*, El-Waseef A, Y.O. Ellazec, M.M. El-Naggar, M. Abd alla

1* Bachelor Of Science pharmaceutical.
2 First-degree specialist in Clinical Laboratorio.
3 Second-degree specialist in Clinical Laboratorio.
4 Master in scientific social studies.

Abstract

The dislipidemias are a risk factor well recognized of the cardiovascular diseases and constitute a problem of public health. A descriptive study in 150 patient elders of 30 years with diagnosis of Isquemic Cardiopathyes accomplished itself for the sake of identifying dislipidemias in patients of high cardiovascular risk that helped the high-technology General Medical Center state James Maribo Aragua, at the Republic Bolivariana of Venezuela, that you constituted the sign of study from October 2011 to October 2012. They used quantitative and qualitative variables like weight, age, sex, pathological personal background, risk factors cardiovascular associates, seric levels of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol VLDL cholesterol. 63 percent of patients with dislipidemias were detected, being hypercholesterolemia the more alteration frequently found. The ages understood between 41 and 60 years evidenced the bigger frequency.

Introduction

Cardiovascular diseases are one of the leading causes of death for people in developed countries world-wide. Hypertension and osteoporosis are frequent diseases among elders. Both are induced by interaction of many genetic and environmental factors (Zhang et al., 2007). Many metabolic and physiological changes occur in hypertensive individuals and some of these processes stem from the associated increased oxidative stress (Vasdev, 2006). The depression in antioxidant enzymes and increase in oxidants in the hypertensive state have been reported to increase the production of reactive oxygen species (Lassegue and Griendling, 2004; Manning et al., 2007). The reactive oxygen spe—cies/free radicals resulting from the oxidant-antioxidant imbalance tend to accumulate and are known to cause oxidative damage to the cellular macromolecules including the genetic material (Rao, 2009). The genetic instability can include mutations, chromosomal aberrations and unscheduled DNA synthesis (Prerara and Bapat, 2007; Shimizu et al., 2008). Amlodipine is a de—rivative of dihydropyridine and is one of calcium channel blockers. Its principal action is to inhibit calcium entry through voltage-gated transmembrane L-type channels, thus decreasing intercellular calcium concen—tration and in—ducing smooth muscle relaxation (Li et al., 2007; Devabhaktuni and Bangalore, 2009; Liu et al., 2011). Atenolol is a beta 1-selective drug that binds to the beta-receptors and prevents the stimu—lation by cat—echolamine resulting in lowering of heart rate and of the systemic blood pressure (Wadworth et al., 1991). Captopril is an angiotensins—converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. Captopril was the first ACE inhibitor developed and was considered a break through both because of its novel mechanism of action and also because of the revolutionary de—velopment process (Akif et al., 2010).

Alkaline phosphatase (ALP) is an enzyme that cata—lyzes the hydrolysis of organic pyrophosphate and in—hibitor of vascular calcification (Harney et al., 2004). Although ALP is expressed in a variety of tissues, its concentrations are highest in bone, liver and kidney (Schoppet and Shanahan, 2008). Accordingly, serum levels of ALP are used in clinical practice as a mark—er of hepatic , peripheral vascular and bony diseases (Regidor et al., 2008). No published data have been found in the relevant literature on the toxicity of anti—hypertensive drugs on the DNA of normal mice and thereby the present study was undertaken to determine the effect of chronic administration of recommended ; half recommended and one and half recommended doses of the antihy—pertensive drugs amlodipine, ateno—lol and captopril on the genomic DNA and on the ac—tivity of alkaline phosphatase (ALP) in the liver, spleen and kidney of Swiss albino mice.

Materials and Methods

Chemicals

Atenolol was purchased from Kahira Pharmaceutical and Chemical Industries Company (Egypt). Amlodi—pine was purchased from Pfizer (Egypt) and captopril was purchased from Bristol My—
Experimental animals

All experiments were performed using adult female Swiss albino mice, with an average body weight of 25 g purchased from Theodore Bilharz Research Institute, Giza, Egypt. The mice were housed in steel mesh cage and provided with commercial standard diet and tap water ad libitum.

A total of 150 Swiss albino mice were divided into 10 groups, 15 mice each, according to the following scheme: group 1, control (untreated) mice; groups 2-4, mice received captopril in doses of 1.3 mg/kg b.w. (low dose; half the recommended dose), 2.6 mg/kg b.w. (the recommended dose) and 3.9 mg/kg b.w. (high dose; one and half times the recommended dose) respectively every other day; groups 5-7, mice received amiodipine in doses of 0.26 mg/kg b.w. (low dose; half the recommended dose), 0.52 mg/kg b.w. (the recommended dose) and 1.04 mg/kg b.w. (the recommended dose) respectively every other day; groups 8-10, mice received atenolol in doses of 1.3 mg/kg b.w. (low dose; half the recom-mended dose), 2.6 mg/kg b.w. (the recommended dose) and 15.6 mg/kg b.w. (high dose; one and half times the recommended dose) respectively every other day. Each of the nine groups of mice receiving the drugs were classified into subgroups, 5 animals each. The animals of the subgroups were sacrificed at 1, 3, 6 months of starting administration of the drugs while the animals of the control group were all killed at the end of the experimental period. Liver, spleen and kidney were immediately excised, washed in ice-cold saline, blotted dry and weighed for measuring various biochemical parameters.

Preparation of homogenates

An accurately weighed piece of each of liver, spleen and kidney tissues was homogenized in ice-cold 0.9 % saline using a Teflon pestle connected to a homogenizer motor. The homogenates were adjusted at a concentration of 5 % (w/v). The homogenates were centrifuged at 5000 rpm for 30 minutes at 4°C to remove cell debris and nuclei. The resulting supernatant was used for biochemical analysis.

Biochemical Analysis

Alkaline phosphatase activity was estimated by the method of Ali et al. (2006) using a commercial available assay kit (AAT Biochemicals, Inc., Egypt). Protein in the homogenates was determined by the method of Lowry et al. (1951).

Genomic DNA extraction and RAPD analysis

Total DNA from mice tissues was extracted and purified according to the Wizard® Genomic DNA Purification Kit supplied by Promega Corporation (USA). Among several primers used for screening and RAPD-PCR analyses of the studied samples only one primer OPE-13: 5’- CCCGATTCGG-3’ was chosen. Genomic DNA was analysed with random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) technique according to the method described by Williams et al. (1990). The PCR amplifications were performed in 25 μl of the reaction mixture containing 1 μl of template DNA, 3.5 μl primer and pure Taq Ready-To-Go PCR beads. The PCR conditions were as follows: denaturing at 94°C for 1.5 min, annealing at 37°C for 1.5 min and extension at 72°C for 3 min. The products were mixed with the loading buffer and were loaded in 2% agarose gels and electrophoresed at 100 V for 1h. The gels were stained with ethidium bromide and visualized under UV light.

Statistical analysis

The results are expressed as means ± SD. Statistical analysis was performed according to the method of Murray (1982). Data were analyzed using unpaired Student’s t-test. P values of < 0.05 were considered to be statistically significant.

Results

Table 1 illustrates the alkaline phosphatase (ALP) activities in mice of group 1. After administration of captopril for one month, three and six months in doses of 1.3, 2.6, 3.6 mg/kg b.w., highly significant elevations in the activities of ALP in the liver, spleen and kidney homogenates were found compared to its activities in the homogenates of the control group. Similarly, a highly significant increase in the activities of ALP in the liver and spleen homogenates of mice were observed after administration of 5.2, 10.4, 15.6 mg/kg b.w. atenolol for one, three and six months. On the other hand, a significant decrease was observed in ALP activity in the kidney homogenates after administration of 5.2 mg/kg b.w. atenolol for one month while no significant changes were found after three and six months from the administration of atenolol. Moreover, a significant decrease in the kidney ALP activity on the administration of 10.4 and 15.6 mg/kg b.w. of atenolol for six months was found compared to the control activity. The administration of 0.26, 0.52 and 0.78 mg/kg b.w. of amiodipine for one, three and six months caused highly significant increases in the activities of ALP in the liver, spleen and kidney homogenates compared to the corresponding activities of the controls.

Discussion

The hypertension increases the risk for stroke and coronary heart disease and is a main contributor to premature death (Yadav et al., 2008). Toxic effects of drugs may be functional, biochemical, structural and specific. Such effects are evaluated by assessment of oxidant-antioxidant parameters in tissues and severity of DNA damage (Polat et al., 2010). The pathology of essential hypertension may involve one or more abnormalities in cardiovascular homeostatic mechanisms including endothelial dysfunction (Beever et al., 2001). The endothelial dysfunction is related to a deficiency or vasodilator nitric oxide (NO) synthesis. There may be an elevation of reactive oxygen species like superoxide anion, which inactivate the NO. Normally, the endogenous antioxidant mechanism involving enzymes like catalase and superoxide dismutase in addition to reduced glutathione scavenger and regulate the superoxide formed. This protective mechanism may be defective in essential hypertension cases (Touyz, 2004).
The present study examines the effects of treatment with antihypertensive drugs on alkaline phosphatase and DNA in mice. The study involved the administration of captopril, atenolol, and amlodipine, and the activity of ALP and its level in the organs of mice was investigated. Chronic administration of captopril significantly increased the activity of ALP compared to its level in control mice. Other antihypertensive drugs, atenolol, and amlodipine also showed significant increases in ALP activity in liver, spleen, and kidney of mice.

Figure 1: RAPD-PCR analysis of DNA samples isolated from livers (lanes 1-3); spleen (lanes 4-6) and kidney (lanes 7-9) of captopril-treated (A); atenolol-treated (B) and amlodipine-treated mice (C): Lane M: standard molecular weight marker. Lanes 1, 4, 7: samples after one month treatment; Lanes 2, 5, 8: samples after 3 month treatment; Lanes 3, 6, 9: samples after 6 month treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>L (IU/mL) 1m</th>
<th>S (IU/mL) 1m</th>
<th>K (IU/mL) 1m</th>
<th>L (IU/mL) 3m</th>
<th>S (IU/mL) 3m</th>
<th>K (IU/mL) 3m</th>
<th>L (IU/mL) 6m</th>
<th>S (IU/mL) 6m</th>
<th>K (IU/mL) 6m</th>
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<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>14.7 ± 1.81</td>
<td>3.88 ± 0.70</td>
<td>3.36 ± 0.947</td>
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<td>3.36 ± 0.947</td>
<td>14.7 ± 1.81</td>
<td>3.88 ± 0.70</td>
<td>3.36 ± 0.947</td>
</tr>
<tr>
<td>Group 2 (Treated with Captopril 1.3 mg/kg)</td>
<td>18.6 ± 0.55 **</td>
<td>11.59 ± 2.3 **</td>
<td>16.08 ± 0.55 **</td>
<td>17.13 ± 2.82</td>
<td>11.88 ± 0.11**</td>
<td>11.55 ± 1.06</td>
<td>54.93 ± 3.98**</td>
<td>8.6 ± 1.22 **</td>
<td>12.52 ± 1.18 **</td>
</tr>
<tr>
<td>Group 3 (Treated with Captopril 2.6 mg/kg)</td>
<td>25.14 ± 0.75 **</td>
<td>15.96 ± 0.54 **</td>
<td>15.31 ± 1.98 **</td>
<td>32.32 ± 4.009</td>
<td>16.9 ± 1.34 **</td>
<td>19.29 ± 1.4 **</td>
<td>26.24 ± 1.046</td>
<td>19.33 ± 1.27 **</td>
<td>16.14 ± 1.605 **</td>
</tr>
<tr>
<td>Group 4 (Treated with Captopril 3.9 mg/kg)</td>
<td>34.99 ± 1.65 **</td>
<td>31.39 ± 1.5 **</td>
<td>10.98 ± 0.92 **</td>
<td>61.55 ± 3.45 **</td>
<td>35.19 ± 2.21 **</td>
<td>16.76 ± 0.72 **</td>
<td>26.76 ± 1.18 **</td>
<td>6.4 ± 1.83 **</td>
<td>5.47 ± 1.025 **</td>
</tr>
<tr>
<td>Group 5 (Treated with Atenolol 3.2 mg/kg)</td>
<td>20.83 ± 0.48 **</td>
<td>8.84 ± 0.86 **</td>
<td>1.08 ± 0.176 **</td>
<td>43.9 ± 0.346 **</td>
<td>7.09 ± 0.72 **</td>
<td>3.98 ± 0.339 NS</td>
<td>17.58 ± 1.06 **</td>
<td>8.80 ± 0.78 NS</td>
<td>3.17 ± 0.784 NS</td>
</tr>
<tr>
<td>Group 6 (Treated with Atenolol 10.4 mg/kg)</td>
<td>25.34 ± 1.83 **</td>
<td>12.66 ± 1.4 **</td>
<td>18.57 ± 1.59 **</td>
<td>70.76 ± 1.89 **</td>
<td>3.93 ± 0.28 **</td>
<td>14.414 ± 1.166 **</td>
<td>18.74 ± 0.409 **</td>
<td>26.43 ± 0.905 **</td>
<td>1.86 ± 0.21 **</td>
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<tr>
<td>Group 7 (Treated with Atenolol 15.6 mg/kg)</td>
<td>29.81 ± 0.94 **</td>
<td>14.08 ± 1.59 **</td>
<td>12.51 ± 1.27 **</td>
<td>53.37 ± 1.26 **</td>
<td>9.916 ± 1.011 **</td>
<td>13.62 ± 0.33 **</td>
<td>24.014 ± 0.59 **</td>
<td>30.5 ± 0.558 **</td>
<td>1.93 ± 0.066 **</td>
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<tr>
<td>Group 8 (Treated with Amlodipine 0.26 mg/kg)</td>
<td>61.2 ± 0.593 **</td>
<td>10.25 ± 0.401 **</td>
<td>10.07 ± 0.402 **</td>
<td>51.9 ± 3.04 **</td>
<td>5.314 ± 0.021 **</td>
<td>18.5 ± 0.396 **</td>
<td>19.6 ± 1.73 **</td>
<td>10.97 ± 0.813 **</td>
<td>23.05 ± 0.629 **</td>
</tr>
<tr>
<td>Group 9 (Treated with Amlodipine 0.52 mg/kg)</td>
<td>9.23 ± 0.72 **</td>
<td>25.3 ± 0.014 **</td>
<td>6.23 ± 1.407 **</td>
<td>16.6 ± 0.84 **</td>
<td>6.48 ± 0.148 **</td>
<td>7.96 ± 1.42 **</td>
<td>17.69 ± 1.54 **</td>
<td>6.39 ± 0.417 **</td>
<td>16.6 ± 1.32 **</td>
</tr>
<tr>
<td>Group 10 (Treated with Amlodipine 0.78 mg/kg)</td>
<td>19.97 ± 0.806 **</td>
<td>9.48 ± 0.49 **</td>
<td>9.22 ± 2.33 **</td>
<td>18.6 ± 0.134 **</td>
<td>9.89 ± 1.25 **</td>
<td>9.54 ± 2.13 **</td>
<td>21.2 ± 0.226 **</td>
<td>19.7 ± 0.74 **</td>
<td>8.08 ± 0.95 **</td>
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</table>

The analysis of genomic alterations in liver, spleen and kidney of mice treated with atenolol and captopril using RAPD-PCR fingerprinting showed that these drugs did not produce significant changes in the RAPD-PCR fingerprints of the liver, spleen and kidney of the studied antihypertensive drugs dantrolene and antioxidant levels due to hypertension which resulted in oxidative stress of the liver, spleen and kidney DNA and caused chromosomal aberrations (Telex et al., 2000; Telex et al., 2010). On the other hand, the present results are in consistent with the results of other investigators (Kang et al., 1997 & Liu et al., 2006) who reported that atenolol and captopril did not show any significant antiproliferative effect on the cultured rat aortic smooth muscle cells through the action of any of the studied doses. Many studies indicated that the calcium channel blocker amlodipine influences DNA and affect bone tissues resulting in decrease in bone resorption (Rejnmark et al., 2006; Ushijima et al., 2010; Subash et al., 2010). In these previously reported data suggested that this calcium channel blocker (amlodipine) has antiinflammatory-antioxidant and antiapoptotic properties besides its antithyroid action (Yamagata et al., 2004 & Yoshii et al., 2006). To summarize, our results clearly indicated that the chronic administration of amlodipine affected the genome DNA and the activity of ALP. The present results may be attributed to: [1] the balance between inorganic pyrophosphate and serum phosphate which regulates vascular calcification. [2] imbalance in oxidant and antioxidant levels due to hypertension which resulted in accumulation of DNA damage with time (Khanna et al., 2008). In addition it is concluded that the studied antihypertensive drugs may induce inflammation by increasing the activity of ALP.

References


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