Effect of nifedipine and glibenclamide addition in a model of high fat diet- induced hypertension and hyperlipidemia in rabbits receiving donepezil.

Salwa Abdeltawab Ibrahim¹, Maha Yehia Kamel¹ and Mina Thabet Kelleni¹*

¹Department of Pharmacology, Faculty of Medicine, Minia University, Minia 61511, Egypt

Abstract

Geriatric individuals suffering from Alzheimer’s disease are frequently co-treated for hypertension and/or diabetes. In the current research, we have explored the addition of nifedipine (1 mg/kg), glibenclamide (0.45 mg/kg) to donepezil (0.75 mg/kg) in a rabbit model of high fat diet- induced hypertension and hyperlipidemia. Nifedipine was shown to improve hypertension, lipid profile as well as the nitro-oxidative pathway when combined with donepezil. Glibenclamide has shown an improvement regarding hypertension and nitro-oxidative pathway when compared to the high fat diet receiving group.

Keywords: Donepezil; Nifedipine; Glibenclamide; Alzheimer’s disease; hypertension; hyperlipidemia

Introduction

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive cognitive deterioration and is the most common cause of dementia [1]. Donepezil; an acetylcholine esterase inhibitor, is one of the drugs commonly used worldwide in treatment of AD patients [2]. Patients suffering from AD patients belong mainly to the geriatric age group [3] who are frequently co-suffering from other chronic diseases like diabetes mellitus and hypertension and vice versa. In this research we’ve tested preliminarily the effect of the antihypertensive calcium channel blocker; nifedipine and the anti-diabetic sulfonylurea; glibenclamide in a model of high fat diet- induced hypertension in rabbits receiving donepezil.

Material and Methods

Animals

The present study was conducted on rabbits weighing from 1090–1300g. Rabbits were obtained from an animal market, Minia, Egypt and They were housed for one week as acclimatization period before conduction of the experiment; fed a standard diet of commercial rabbit chow, water was available ad libitum. All animal care and experimental procedures were in accordance with the protocols of the Research Advisory Ethical Committee of Faculty of Medicine, Minia University, Egypt.

Drugs and chemicals

Donepezil powder was obtained from Pfizer pharmaceuticals Company branch in Egypt. Nifedipine and glibenclamide powder was obtained from Egyptian pharmaceutical industries Co. (Eipico), Egypt; copper sulfate (El-Nasr Pharmaceuticals Chemicals Co., Egypt); N-naphthyl ethylenediamine (BDH, England); sulfanilamide (El-Gomhoria, Egypt); thiobarbituric acid (Sigma-Aldrich Chemical Co, USA); trichloroacetic acid (El-Nasr Pharmaceuticals Chemicals Co, Egypt). All other chemicals were of analytical grade and were obtained from commercial sources.

Experimental design

The rabbits(either sex) were divided into five groups (5 rabbits each): (1) control group received distilled water p. o. and normal diet (2) High Fat Diet (HFD) receiving group for 3 weeks [4] (3) HFD plus donepezil (0.75 mg/kg/day, p. o. for the last 5 days) receiving group (4) HFD plus donepezil as described plus nifedipine(1 mg/kg/day, p. o. for the last 5 days) receiving group (5) HFD plus donepezil plus glibenclamide (0.45 mg/kg/day, p. o. for the last 5 days). The doses of the drugs were selected according to previous studies [5-7].

Sampling and methodology

At the end of the experimental period, animal were anesthetized with l. p injection with urethane then systolic and diastolic blood pressure were measured by using (POLYGRAPH, 2006). Animals were scarified and blood samples were collected and prepared for the following biochemical analysis:

Serum levels of Malondialdehyde (MDA) were measured according to the thiobarbituric acid method; it depends on...
Effect of nifedipine and glibenclamide addition in a model of high fat diet-induced hypertension and hyperlipidemia in rabbits receiving donepezil.

measuring MDA, the breakdown products of lipid peroxides. Trichloroacetic acid was added to the sample for protein precipitation and then thiobarbituric acid was added. The mixture was heated for 10 min in a boiling water bath. One molecule of MDA reacted with two molecules of thiobarbituric acid and the intensity of the color developed in the supernatant was measured spectrophotometrically at 535 nm. The absorbance was read at 535 nm and the corresponding concentration was calculated from a standard curve using 1,1,3,3-tetraethoxypropane as a standard.

Nitric Oxide (NO) is rapidly oxidized to nitrite and/or nitrate by oxygen and thus the stable oxidation end products of nitric oxide, nitrite and nitrate were used as an index of NO production. The method used to determine NO level in serum depends on reduction of nitrate by copper–cadmium granules, followed by color development with Griess reagent (sulfanilamide and N-naphthylethylenediamine) in acidic medium and then measuring spectrophotometrically the total nitrites at 540 nm.

Reduced glutathione (GSH) was measured using colorimetric kit (Biodiagnostic, Egypt) according to kit instructions. The method based on the reduction of 5,5′dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance was measured at 405 nm using Beckman-DU-64 spectrophotometer (USA).

Serum triglycerides (TG) and High Density Lipoproteins (HDL) levels were determined using commercially available kits (Biodiagnostic, Giza, Egypt) and expressed as mg/dl according to the kit instructions and quantitated at 500 nm using Beckman-DU-64 spectrophotometer (USA).

Results

Effect on Blood Pressure (table 1, 2)

<table>
<thead>
<tr>
<th>Table 1: Effect on systolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>HFD + donepezil</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E.M. a, b, c, d Significant (P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

Effect on nitro oxidative stress biomarkers (table 3, 4, 5)

<table>
<thead>
<tr>
<th>Table 2: Effect on diastolic blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>HFD + donepezil</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
</tr>
</tbody>
</table>

Data represent the mean ± S. E. M. a, b, c, d Significant (P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

<table>
<thead>
<tr>
<th>Table 3: Effect on serum malondialdehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>HFD + donepezil</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
</tr>
</tbody>
</table>

Data represent the mean ± S. E. M. a, b, c, d Significant (P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

<table>
<thead>
<tr>
<th>Table 4: Effect on serum nitric oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>HFD + donepezil</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E.M. a, b, c, d Significant (P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

<table>
<thead>
<tr>
<th>Table 5: Effect on serum GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>HFD</td>
</tr>
<tr>
<td>HFD + donepezil</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E.M. a, b, c, d Significant (P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.
Effect on lipid profile (Table 6, 7)

Table 6: Effect on serum triglycerides

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.47±1.02</td>
</tr>
<tr>
<td>HFD</td>
<td>193.9±1.24</td>
</tr>
<tr>
<td>HFD + donepezil</td>
<td>192.2±2.63</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
<td>82.05±1.76</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
<td>191.9±0.89</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E.M. Significant (*P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

Table 7: Effect on serum high density lipoproteins

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum HDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.79±1.01</td>
</tr>
<tr>
<td>HFD</td>
<td>16.90±0.42</td>
</tr>
<tr>
<td>HFD + donepezil</td>
<td>16.73±0.64</td>
</tr>
<tr>
<td>HFD + donepezil + nifedipine</td>
<td>21.02±0.58</td>
</tr>
<tr>
<td>HFD + donepezil + glibenclamide</td>
<td>17.04±0.52</td>
</tr>
</tbody>
</table>

Data represent the mean ± S.E.M. Significant (*P < 0.05) difference from control, HFD, HFD + donepezil, HFD + donepezil + nifedipine, respectively.

Discussion

Cerebrovascular disease and ischemic brain injury secondary to cardiovascular disease are common causes of dementia and cognitive decline in the elderly [11]. It’s common to find geriatric individuals treated from multiple diseases like Alzheimer’s, diabetes, and hypertension. Further, hypertensive patients with concomitant diabetes must take both antihypertensive and hypoglycaemic medications, for which there is a lack of experimental and clinical guidelines [12]. In this research we’ve explored the possible interactions that may be encountered when donepezil is used together with nifedipine or glibenclamide.

In the current research, the addition of nifedipine to donepezil significantly improved both systolic and diastolic blood pressure compared with HFD, HFD + donepezil as well as HFD + donepezil + glibenclamide groups. This effect was predicted since nifedipine is a well-known antihypertensive drug. However, Donepezil +/- glibenclamide groups have also significantly improved systolic and diastolic blood pressure as compared to HFD.

Donepezil was shown to attenuate the development of hypertension in spontaneously hypertensive rats with a mechanism probably involving anti-inflammatory effects, indicating that acetyl cholinesterase inhibition yields beneficial effects for antihypertensive therapy [13]. Glibenclamide was also suggested to act as a competitive antagonist of thromboxane receptors inhibiting vasoconstriction [14].

Our research has shown that the addition of nifedipine to donepezil has improved the nitro-oxidative stress pathway; decreased serum MDA and increased serum NO and GSH compared with HFD, HFD + donepezil as well as HFD + donepezil + glibenclamide groups. Donepezil +/- glibenclamide groups have also decreased serum MDA and increased serum NO compared with HFD group.

Donepezil was previously shown to decrease lipid peroxidation (MDA); increase the contents of endogenous antioxidant (GSH) and the activities of antioxidant enzymes (catalase and SOD) in stroke rat model [15]. It was also shown that calcium disarrangement and free radical formation play a role in hepatotoxicity and nifedipine was shown to decrease MDA, increase GSH and SOD in diethyl dithiocarbamate-induced hepatic toxicity in rats [16]. Additionally, Nifedipine was shown to increase the activity of superoxide dismutase and catalase; elevate the contents of GSH and nitrates; and decrease the MDA levels [17].

Moreover, glibenclamide was shown to possess antioxidant effect contributing to the protective effect against oxidative stress-induced damage during diabetic complications in alloxan-induced diabetic rats [18]; further glibenclamide decreased MDA and increased GSH in streptozotocin-nicotinamide induced diabetic rat model [19].

The current research has also shown a favorable effect on serum TG and HDL when nifedipine was added to donepezil; an effect that was not shown by donepezil alone or donepezil + glibenclamide. Nifedipine was previously shown to decrease total cholesterol, LDL-cholesterol, TG, atherogenic index and elevated HDL level in rats under ethanol and sucrose feeding (dzeuliet). Nifedipine has also significantly decreased fasting TG level and increased HDL-C in the elderly group and was shown to have favorable metabolic effects that are beyond the known enhancement of insulin sensitivity [20].

Conclusion

Administration of both donepezil and the calcium channel blocker nilvadipine was shown to attenuate hyperhomocysteinemia-induced memory deficits and neuropathology’s in rats and the combination was suggested as a promising therapeutic candidate for AD [21]. In the current research we further confirm the favorable metabolic effect of nifedipine on the lipid profile as well as on the nitro-oxidative pathway when combined with donepezil. Glibenclamide has shown a significant improvement as regard to the nitro-oxidative pathway when compared to HFD. Further studies to test the combination and to explore other potentials are encouraged.

References

Effect of nifedipine and glibenclamide addition in a model of high fat diet-induced hypertension and hyperlipidemia in rabbits receiving donepezil.


