https://doi.org/10.31925/farmacia.2019.4.6 **ORIGINAL ARTICLE**

EXPERIMENTAL PHARMACOLOGICAL RESEARCH REGARDING THE EFFECT OF SOME NEWLY SYNTHESIZED B-PHENYLETHYL AMINES ON THE MODIFIED PARAMETERS OF THE LIPID METABOLISM

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Abstract

The effect of some new β-phenylethylamine derivatives on some parameters of the lipid metabolism and lipid peroxidation was investigated. The research method used was based on inducing hyperlipidaemia in rats by administering orally 4% triton in a dose of 400 mg/kg bw. The new compounds were tested against fenofibrate, the reference substance. The results have shown that compounds coded C2 and A1 induced a decrease in the total cholesterol levels, that was higher than that generated by the reference substance, compared to the control group with hyperlipidaemia. Furthermore, compounds coded C2, A6 and C3 have lowered LDL-cholesterol levels more efficiently than fenofibrate, in the hyperlipidaemia control group. All compounds except for C1 increased HDL-cholesterol levels more than the reference substance in the hyperlipidaemia control group. All compounds lowered the malonyl dialdehyde liver concentration, as a biomarker of lipid peroxidation, compared to the hyperlipemic control group. According to our results, the newly synthesized β-phenylethylamine derivatives have a statistically significant positive impact on the parameters of the lipid metabolism, possibly due to a β_3- adrenergic receptors agonist mechanism.

Rezumat

A fost evaluat efectul unor compuși nou sintetizați cu nucleu beta-feniletilaminic asupra parametrilor metabolismului lipidic și asupra peroxidării lipidice. Metoda de cercetare folosită s-a bazat pe inducerea hiperlipemiei la șobolan prin administrarea orală a 400 mg/kg corp triton 4%. Compuși au fost administrați pe cale orală, comparativ cu fenofibratul, utilizat ca substanță de referință. Rezultatele au arătat că doi dintre compuși, notați C2 și A1 au determinat scăderi ale colesterolului total mai mari decât cele induse de fenofibrat la șobolanii hiperlipemici. Compușii C2, A6 și C3 au determinat scăderi ale LDL colesterolului mai mari decât cele induse de fenofibrat față de martorul hiperlipemic. Toate substanțele au determinat creșteri ale HDL colesterolului, respectiv scăderi ale trigliceridelor serice (cu excepția C1), mai mari decât cele induse de fenofibrat la șobolanii hiperlipemici. Toate substanțele au scăzut concentrația malonildialdehidei hepatice, ca marker al peroxidării lipidice, comparativ cu lotul martor hiperlipemic. Conform rezultatelor obținute, substanțele nou-sintetizate prezintă efecte benefice semnificative statistic asupra parametrilor metabolismului lipidic, posibil printr-un mecanism de tip agonist asupra receptorilor beta-3 adrenergici.

Keywords: β-phenylethylamine, triton, hyperlipidaemia, β_3 agonist

Introduction

Lipid metabolism dysfunctions that occur in obesity are increasing in Europe and North America, therefore the research in the direction of finding new remedies for these conditions are of maxim interest [6].

The involvement of the adrenergic transmission *via* β_3 receptors in modulating the lipid metabolism was demonstrated in preclinical researches [20]. Studies on obese rodents treated with β_3 adrenergic receptors

agonists [18, 19] have shown a significant decrease in their body mass and a reduction of adipose tissue without affecting the daily diet ratios. Comparative studies on normal and genetically modified mice without β_3 adrenergic receptors have revealed an inversely proportional correlation between the fat levels and the number of β_3 adrenergic receptors [15]. Furthermore, in models of genetically induced obesity in mice and rats, a decrease in RNAm levels within β_3 adrenergic receptors was observed [7, 8].

New potential β_3 adrenergic receptors agonists are being developed and investigated all over the world [2, 23]. Although at present several *in vitro* methods are available for preclinical trials [17] animal testing remains an essential step for introducing new drugs in therapy.

We previously reported the synthesis and development of some new compounds that have a common β-phenylethylamine structure and different substituted radicals, which give each substance a different affinity for β_3 adrenergic receptors and a different diffusion level in various body compartments [1, 3, 12]. We coded the series of the new molecules as the A series (Figure 1) and the C series (Figure 2) [4, 5].

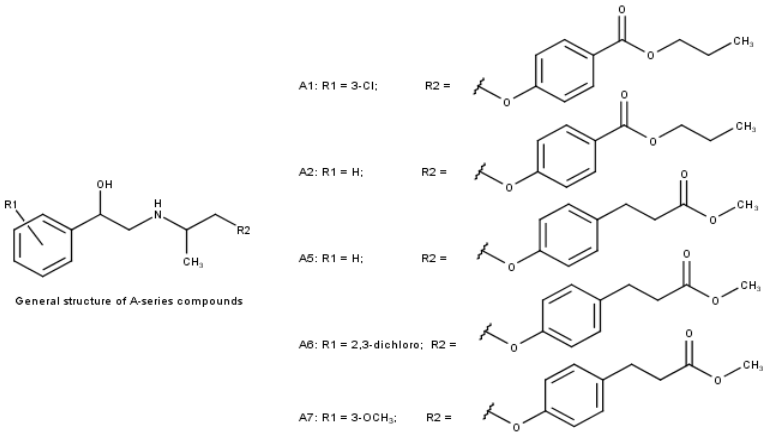

Figure 1.

Chemical structure of the tested compounds belonging to A-series

(A1: propyl-4-(2-((2-(3-chlorophenyl)-2-hydroxyethyl)amino)propoxy)benzoate, A2: propyl-4-(2-((2-hydroxy-2-phenyl-ethyl)amino)propoxy)benzoate, A5: methyl-3-(4-(2-((2-hydroxy-2-phenylethyl)amino)propoxy)phenyl)propanoate, A6: methyl-3-(4-(2-((2-(2,3-dichlorophenyl)-2-hydroxyethyl)amino)propoxy)phenyl)propanoate, A7: methyl-3-(4-(2-((2-hydroxy-2-(3-metoxyphenyl)ethyl)amino)propoxy)phenyl)propanoate)

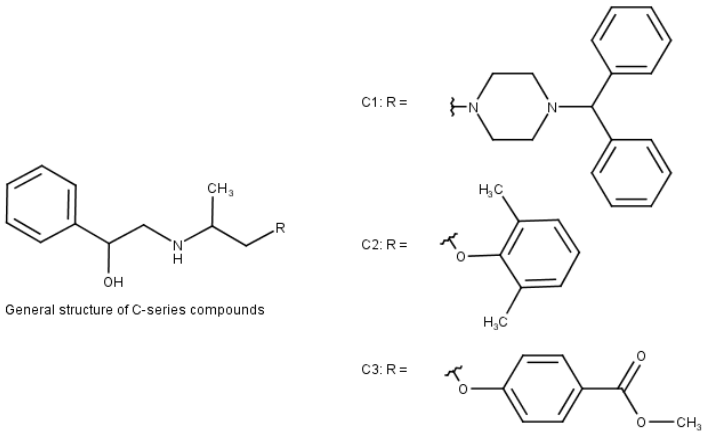

Figure 2.

Chemical structure of the tested compounds belonging to C-series

(C1: 2-((1-(4-benzhydrylpiperazin-1-yl) propan-2-yl)amino)-1-phenylethanol, C2: 2-((1-(2,6-dimethylphenoxy)propan-2-yl)amino)-1-phenylethanol, C3: methyl-4-(2-((2-hydroxy-2-phenylethyl)amino)propoxy)benzoate)

Based on suggestive literature data regarding the benefits of β_3 adrenergic agonists an modulating the lipid metabolism, we further investigated the potential pharmacological effects of our new molecules in this field.

Materials and Methods

Reagents

Fenofibrate (Terapia SA, Romania), triton WR-1339, thiobarbituric acid, trichloracetic, 4-aminoantipyrine, phenol, tris-buffer-saline were used. All chemicals were of analytical grade and used without further purification. The tested new molecules employed were A1, A2, A5, A6, A7 from the A-series and C1, C2, C3 from the C-series and were provided by the National Institute for Chemical - Pharmaceutical Research and Development (ICCF) Bucharest, Romania.

Commercially available kits were used for the biochemical assays: total cholesterol (TC; Liquick Cor-Chol 60 No. 2-204; Cormay, Lublin, Poland), HDL-cholesterol (HDL-C; Olympus Diagnostica GmbH No. OSR 6287; Hamburg, Germany) and triacylglycerides (TG; Liquick Cor-TG 30 No. 2-262; Cormay, Lublin, Poland), as described by Kostogrys *et al*. The LDL and VLDL-cholesterol were calculated as the difference between TC and HDL-cholesterol [9, 10].

Animals

110 Wistar male white rats weighing 249.5 ± 48.5 g (M ± SD) was purchased from the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania animal facility complex. After an accommodation period of 5 days, the animals were divided in 11 groups (10 rodents/group) and housed in individual cages, separately ventilated, having *ad libitum* access to water and dry food granules. The temperature of the housing environment was 20 - 22°C and the relative humidity was maintained at 35 - 45%.

All experiments were conducted in accordance with EU Directive 63/2010 on the protection of animals used for scientific purposes. The protocol was approved by the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, Bioethics Committee.

The doses were chosen in accordance with the LD_{50} previously determined for each substance [12]. The new compounds were administered orally, as aqueous suspensions, in 4 consecutive days, as follows: 20 mg/kg bw C1 0.2%; 50 mg/kg bw C2 0.5%; 100 mg/kg bw C3, A1, A2, A5, A6 and A7 1%. The control group received distilled water 1 mL/100 g bw, while the reference group was treated with 100 mg/kg bw fenofibrate 1%. In the 2nd day, the hyperlipidaemia inducing agent was administered (400 mg/kg bw triton 4%) [9]. In the 4th day of the experiment, the animals were euthanized. Blood was collected from the neck into glass tubes and the serum was obtained from blood samples after centrifugation (1000 rpm, 10 min at 4°C). Liver samples were also harvested on ice. LDL-, HDL-, total cholesterol and triglyceride serum levels were assessed, as well as the hepatic malondialdehyde (MDA) concentration. Hepatic samples were fragmented and approximately 1 g of tissue was weighted and added in a glass homogenizer with 10 mL 0.1 M tris-KCl for homogenate preparation and the resulting liver homogenate was centrifuged for 10 minutes at 3000 rpm. MDA levels were determined (Halo DB-20 Dynamica, Great Britain), based on previously described methods [13, 16]. Results were expressed as nmols MDA/mg proteins in the studied samples [14]. The pharmacological effect was also quantified in terms of % effect *versus* the control group.

Statistical analysis

The statistical evaluation of the results was performed using the software – GraphPad Prism version 5.01. This software analyses populations with normal distribution using the Student's t test (for 2 groups) and the ANOVA test (for multiple groups). In case of statistical significance in the ANOVA test ($p < 0.05$), a further post-test was employed (Dunnett). The normality of the distribution was determined using the D`Agostino-Pearson (D&P) test.

Results and Discussion

The administration of our new β-phenylethylamine molecules, to the studied groups of animals, revealed important variations of the investigated lipidic parameters, as shown in Tables I - IV.

After administering a dose of 400 mg/kg bw triton 4%, a significant disruption of the normal equilibrium in the lipid metabolism parameters was induced: total cholesterol levels increased by 176.88%, LDL-cholesterol levels increased by 347.76%, HDL-cholesterol decreased by 19.57% and triglyceride levels increased by 155.31%.

Fenofibrate induced a high statistically significant decrease in total cholesterol levels compared to the hyperlipidaemia control group (by 59.99%). All tested compounds induced a significant decrease of this parameter compared to the hyperlipidaemia control group (Table I, Figure 3), but compounds C2 and A1 had the most intense effect, decreasing total cholesterol levels by more than 60%. Similar data were obtained for compounds C1, C3, A2, A6 and A7, that lowered this parameter by more than 45%, while A5 compound showed the lowest anti-cholesterol effect.

All tested compounds induced a statistically significant decrease in LDL cholesterol levels compared to the hyperlipidaemia control group (Table II, Figure 4). Most of our compounds decreased this biomarker by more than 70%, only C1 molecule showed less than 60% effect.

Regarding HDL-cholesterol values, a significant effect was observed in compounds C3 and A5 that increased this biomarker by more than 130% and, respectively, almost 115% (Table III, Figure 5).

Total serum triglycerides were lowered by more than 60% (Table IV, Figure 6), compared to the hyperlipidaemia control group. In this case, the most effective derivatives proved to be A6 and A7 which developed the most significant effect (> 70%).

Table I

Variation of mean total cholesterol levels (mg/dL) compared to the control groups

Parameter / Group	Normal Control (NC)	Hyperlip Control (HC)	Ref	C1	C2	C3	A1	A2	A5	A6	A7
M ± SEM	54.4 ± 5.35	150.9 ± 6.95	60.09 ± 7.77	77.1 ± 3.01	53.78 ± 5.87	80.19 ± 5.02	57.26 ± 2.83	77.17 ± 4.75	88.32 ± 8.46	62.1 ± 6.64	79.33 ± 8.07
D&P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ANOVA	p < 0.0001 (***)										
Dunnett *vs*. NC	-	***	ns	ns	ns	*	ns	ns	**	ns	*
Dunnett *vs*. HC	-	-	***	***	***	***	***	***	***	***	***
Ef% vs. NC	-	176.88	10.97	41.72	-1.13	47.4	5.07	41.85	62.35	14.15	45.82
Ef% *vs*. HC	-	-	-55.99	-48.4	-64.36	-46.85	-64.1	-48.86	-24.79	-58.84	-47.42
Dunnett *vs*. Ref	-	-	-	**	ns	ns	ns	ns	ns	ns	ns

ND = normal distribution; ns = no statistical significance

Table II

Variation of mean LDL cholesterol levels (mg/dL) compared to the control groups

Parameter / Group	Normal Control (NC)	Hyperlip Control (HC)	Ref	C1	C2	C3	A1	A2	A5	A6	A7
M ± SEM	18.09 ± 3.04	81.00 ± 4.11	15.52 ± 1.59	34.37 ± 4.87	13.74 ± 1.82	15.24 ± 3.56	15.59 ± 2.75	22.37 ± 2.17	24.00 ± 1.36	14.66 ± 1.81	20.65 ± 2.81
D&P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ANOVA	p < 0.0001 (***)										
Dunnett *vs*. NC	-	***	ns	**	ns	ns	ns	ns	ns	ns	ns
Dunnett *vs*. HC	-		***	***	***	***	***	***	***	***	***
Ef% *vs*. NC	-	347.76	-14.20	89.99	-33.23	-15.74	-13.81	23.65	32.66	-18.96	14.15
Ef% *vs*. HC	-		-80.83	-57.6	-83.80	-81.85	-80.75	-72.38	-70.37	-82.90	-74.50
Dunnett *vs*. Ref	-			**	ns	ns	ns	ns	ns	ns	ns

ND = normal distribution; ns = no statistical significance

Table III

Variation of mean HDL cholesterol levels (mg/dL) compared to the control groups

Parameter / Group	Normal Control (NC)	Hyperlip Control (HC)	Ref	C1	C2	C3	A1	A2	A5	A6	A7
M ± SEM	28.40 ± 3.31	22.90 ± 1.17	32.01 ± 6.51	37.37 ± 4.88	40.46 ± 3.98	53.04 ± 7.27	39.53 ± 4.53	41.33 ± 2.43	49.22 ± 7.22	42.18 ± 3.70	45.18 ± 3.50
D&P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ANOVA	p < 0.0007 (***)										
Dunnett *vs*. NC	-	ns	ns	ns	ns	**	ns	ns	*	ns	ns
Dunnett *vs*. HC	-		ns	ns	ns	***	ns	ns	**	*	*
Ef% vs. NC	-	-19.57	12.71	31.58	42.46	86.76	39.19	45.52	73.30	50.42	59.08
Ef% *vs*. HC	-	-	39.78	63.18	76.68	131.61	76.62	80.48	114.93	84.19	97.29
Dunnett *vs*. Ref	-	-	-	ns	ns	ns	ns	ns	ns	ns	ns

ND = normal distribution; ns = no statistical significance

Table IV

Variation of mean triglyceride levels (mg/dL) compared to the control groups

Parameter / Group	Normal Control (NC)	Hyperlip Control (HC)	Ref	C1	C2	C3	A1	A2	A5	A6	A7
M ± SEM	55.07 ± 2.22	140.6 ± 9.35	55.69 ± 8.99	62.30 ± 5.86	50.15 ± 5.81	47.26 ± 10.80	44.68 ± 3.35	49.31 ± 4.08	44.85 ± 2.69	41.37 ± 4.66	38.66 ± 3.35
D&P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ANOVA	p < 0.0001 (***)										
Dunnett *vs*. NC	-	***	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dunnett *vs*. HC	-		***	***	***	***	***	***	***	***	***
Ef% vs. NC	-	155.31	1.12	13.12	-8.93	-14.18	-18.86	-10.45	-18.55	-24.87	-29.79
Ef% *vs*. HC	-		-60.39	-55.7	-64.33	-66.38	-68.22	-64.92	-68.10	-70.57	-72.5
Dunnett *vs*. Ref	-			ns	ns	ns	ns	ns	ns	ns	ns

ND = normal distribution; ns = no statistical significance

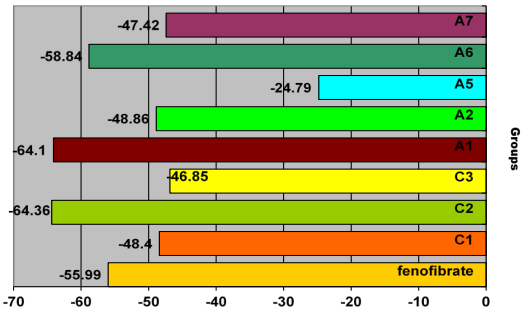

Figure 3.
Variation of average total cholesterol levels (%) in the groups treated with the new compounds and reference substance compared to the hyperlipidemia control group

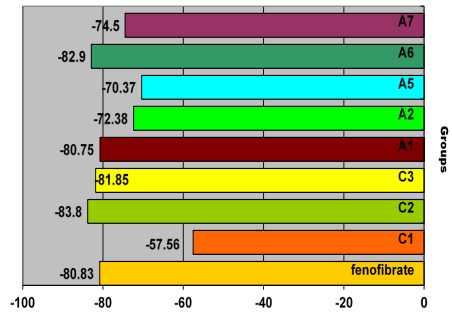

Figure 4.
Variation of average LDL-cholesterol levels (%) in the groups treated with the new compounds and reference substance compared to the hyperlipidemia control group

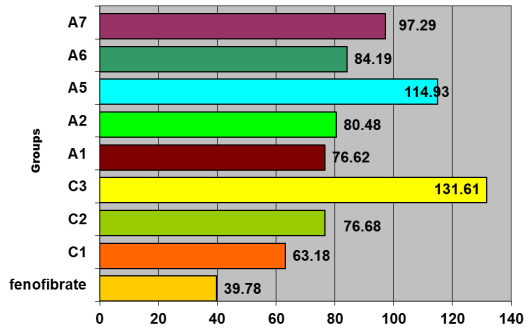

Figure 5.
Variation of average HDL-cholesterol levels (%) in the groups treated with the new compounds and reference substance compared to the hyperlipidemia control group

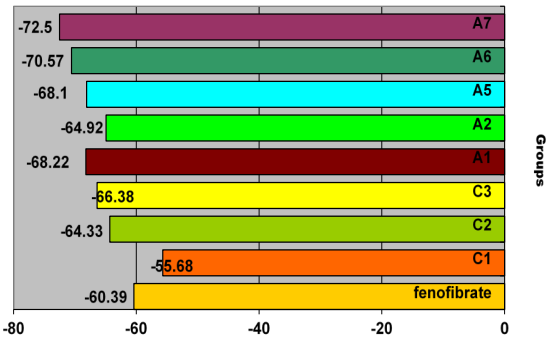

Figure 6.
Variation of average triglyceride levels (%) in the groups treated with the new compounds and reference substance compared to the hyperlipidemia control group

Regarding the lipid peroxidation, expressed as MDA levels, our data showed a modulating effect of β-phenylethylamines (Figure 7).

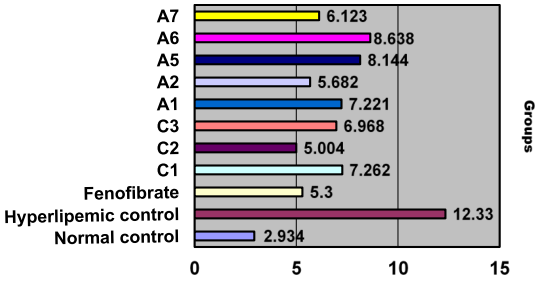

Figure 7.
MDA levels in the control, reference and test groups

All tested compounds reduced MDA levels compared to the hyperlipemic control group, some of them (C2, C3, A2, A7) showing an effect similar to the reference substance ($p < 0.001$) (Figure 8).
Additionally, a Pearson correlation coefficient of 0.0409 was determined, a significant positive correlation between hepatic MDA and HDL-cholesterol levels, for C3 derivative (Figure 9).

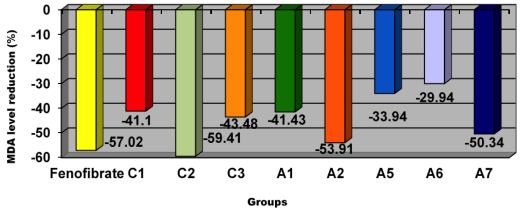

Figure 8.
MDA levels (%) within the reference and test groups, compared to the hyperlipemic control group

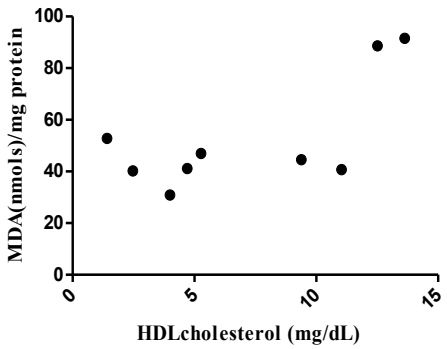

Figure 9.
MDA/HDL cholesterol - Pearson correlation ($p = 0.0409$) for the C3 group (100 mg/kg bw)

MDA, a marker of oxidative stress was shown to be altered in different stages of hyperlipidaemia in animal models [11, 21] as well as in humans [22]. HDL, a molecule with protective effect against oxidative stress, declines in hyperlipemia. Yang R *et al*. [22] revealed a negative correlation between these 2 parameters in hyperlipemic subjects, in accordance with our results. Our analysis u revealed a similar correlation between HDL and oxidative stress. Our study brings new approaches regarding the potential anti-lipidic effects of β_3 agonists and also as possible modulators of lipid peroxidation, results confirmed by other literature data [19, 20].

Conclusions

The newly β-phenylethylamine derivatives studied had a positive impact on the biomarkers of lipid metabolism disrupted by triton administration.

The molecules coded C2, A1 and A6 lowered the total cholesterol higher than the reference substance. The effect was similar on LDL-cholesterol values. C3 and A5 significantly increased HDL-cholesterol, while triglycerides were highly lowered compared to with reference substance by all tested compounds, except for C1.

C2, C3, A2 and A7 had the most important effect in preventing lipid peroxidation. C2 reduced MDA levels higher than the reference substance. Furthermore, C3 molecule showed a positive correlation between HDL variation and MDA activity.

Our results confirmed the β-phenylethylamines therapeutic potential in treating lipid metabolism dysfunctions, therefore additional research is required in order to confirm these findings.

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