ORIGINAL ARTICLE

THE INFLUENCE OF A NEW RUTIN DERIVATIVE IN AN EXPERIMENTAL MODEL OF INDUCED HYPERHOMOCYSTEINEMIA IN RATS

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Abstract

Cardiovascular diseases are a major cause of deaths today. It is generally accepted the involvement of three major risk factors: hyperlipidaemia, hyperhomocysteinemia and the molecular reactive species excess. Therapeutic approaches try to reduce the levels of lipids and homocysteine and to keep the balance between the oxidation/reduction system. We studied a new rutin derivative coded L103 that may determine the lowering of the lipid level and antioxidant properties. L103 contains a pyridine group attached to a rutin molecule. We studied the influence of L103 on the plasmatic levels of cholesterol, total antioxidant status and homocysteine in hyperhomocysteinemia experimentally induced in a rat model through methionine loading. The obtained data suggest that L103 succeeds to prevent the decline of the total antioxidant status, keeps cholesterol within normal ranges but has minor effects on the homocysteine levels. Despite the low influence on homocysteine, L103 confers protection against two of the major risk factors incriminated in cardiovascular diseases.

Rezumat

Bolile cardiovasculare sunt o cauză majoră a deceselor în prezent. Este general acceptată implicarea a trei factori de risc majori în bolile cardiovasculare: hiperlipidemia, hiperhomocisteinemia şi speciile reactive la nivel molecular. În terapie se încearcă scăderea nivelurilor lipidice şi homocisteinei precum şi menţinerea statusului antioxidant. Am studiat un nou derivat de rutin codificat L103, cu posibile proprietăţi antioxidante şi hipolipidice. L103 conţine o grupare piridinică ataşată la o moleculă de rutin. Am studiat influenţa L103 asupra nivelurilor plasmatice ale colesterolului, homocisteinei şi a statusului antioxidant total în hiperhomocisteinemia indusă experimental la şobolan prin încărcarea cu metionină. Datele obţinute sugerează că L103 previne declinul statusului antioxidant, menţine colesterolul în limite normale, dar are efect scăzut asupra nivelurilor de homocisteină. În ciuda influenţei slabe asupra homocisteinei, L103 conferă protecţie împotriva a doi dintre factorii de risc majori din bolile cardiovasculare.

Keywords: severe/intermediary hyperhomocysteinemia, total antioxidant status, cholesterol, rutin, rat model

Introduction

Atherosclerosis is considered a "hotbed" epidemic in developed countries, if we consider the increased number of people affected [3]. Proven major cardiovascular risk factors are hyperlipidaemia, hyperhomocysteinemia (HHcy) and excess reactive species. Elevated serum lipids initiate a series of reactions that finally generates excessive reactive species. Oxidised LDL fractions trigger the onset of the endothelial damage [15]. Elevated homocysteine (Hcy) levels, even slightly above the upper limit [8, 17] affect the endothelial function, cause vasoconstriction and initiate the coagulation cascade by generating reactive species after the autooxidation of homocysteine [7]. Thus, hyperhomocysteinemia is an aggravating factor for hyperlipidaemia. Therapy should be targeted to both lowering homocysteine and lipid levels and preserving the redox balance. Our study investigates the influence of a new rutin derivative coded L103, on the previous mentioned cardiovascular risk factors, in a rat model with experimentally induced HHcy through methionine loading. L103 contains a pyridine group (similar to niacin) chemically bound to a rutin molecule. Niacin exerts antihypertensive effects at low doses, hypolipidemic effects at high doses [13] and is used in the cardiovascular therapy [16]. Rutin is a citrus flavonoid glycoside presenting antioxidant activity and inhibiting platelet aggregation [4]. Based on the pharmacology of the initial molecules, niacin respectively rutin, we estimated for L103 hypolipidemic and antioxidant activities. The influence of L103 on the hyperhomocysteinemia-induced rats was determined by measuring plasma concentration of cholesterol, homocysteine and by the evaluation of the total antioxidant status (TAS). The obtained data suggest

that L103 prevents the increase of cholesterol, preserves the total antioxidant status, but has negligible effect on hyperhomocysteinemia levels.

Materials and Methods

Synthesis and analysis of the new rutin derivative

The new compound (L103) was synthesized from rutin and 2-amino-pyridine, using a previously described method [14]. The melting point was measured using an Electrothermal Mel-Temp apparatus and it is uncorrected. The IR spectrum was recorded on a FT/IR Jasco 670 Plus spectrometer. The 1H-NMR spectrum was recorded on a Bruker AC-300F, 300 MHz instrument using DMSO-d6 as solvent. The elemental analysis was performed on an Exeter Analytical CE-440 elemental analyser.

Animals

The pharmacological study was performed on three groups of 10 adult Wistar male rats, weighing 150 - 200 g. The rats received standard food (containing folic acid and vitamin B12) and water *ad libitum*. All procedures were performed according to the European legislation concerning the care and use of animals for scientific purposes (Directive 86/609/ EEC).

Experimental

Hyperhomocysteinemia was experimentally induced to all groups by oral administration of methionine 1.8 g/kg body weight (b.w.) single dose daily, for 30 days [10]. Group I served as control and received only methionine 1.8 g/kg b.w., for 30 days; Group II received methionine similar to Group I and niacin orally 50 mg/kg b.w. single dose daily, for 30 days; Group III received methionine similar to Group I and orally L103 in 36.76 mg/kg b.w. single dose daily, for 30 days. The dose for L103 represents 1/20 from 50% lethal dose (LD_{50}) in rats. The blood samples were taken from the retro-orbital plexus. TAS, homocysteine and cholesterol concentrations were determined at the beginning and at the end of the experiment thus each group had its own control in the initial values. TAS and cholesterol concentrations were measured using a Randox kit. Total plasma homocysteine was determined by a HPLC validated method [2].

Statistical analysis

The statistical analysis used the analysis of variance (ANOVA-one-way) and Turkey-Kramer multiple comparisons; a coefficient $p < 0.05$ was considered to indicate a statistically significant difference within or between groups.

Results and Discussion

Chemistry

The chemical name for L103 is 3-[[6-O-(6-deoxy-α-L-manopyranosyl)-β-D-glucopyranosyl]-oxy]-2-(3,4-dihydroxy-phenyl)-5-hydroxy-7-(oxy-(β-hydroxy-propyl-(amino-pyridin-2-yl))-4H 1-benzopyran-4-one. The chemical structure of L103 consisting in a pyridine group attached to rutin molecule is presented in Figure 1.

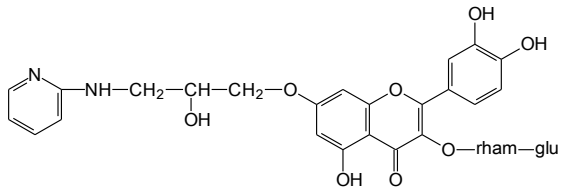

Figure 1.
Chemical structure of the rutin derivative coded L103

L103 characterization: Yellow crystalline powder (yield 70.32%), m.p. = 215 - 217°C, IR (KBr, cm^{-1}): 3310 (linked OH), 2993 (N-H), 2922 (C-H), 1657 (C=O aromatic ring), 1610 (aromatic structure), 1505 (aromatic C=C), 1362, 1312, 1215, 1060 (C-O-C), 979 (pyridine ring), 812 (aromatic substitutes); 1H-NMR (DMSO-d6) δ ppm: 8.10 (s, 1H, pyridine ring), 8.00 (s, 1H, pyridine ring), 7.92 (s, 1H, pyridine ring), 7.82 (s, 1H, pyridine ring), 7.44 (s, 1H, aromatic), 6.70 (d, 1H, aromatic), 6.30 (s, 1H, aromatic), 6.14 (s, 1H, aromatic), 5.40 (s, 1H, H-1 glucosyl), 4.56 (s, 1H, H-1 ramnosyl), 3.88 (s, 2H, CH2N), 1.21 (s, 1H, NH); Elemental analysis: calculated for $C_{35}H_{40}N_2O_{17}$: C: 55.26; H: 5.29; N: 3.68; Found: C: 55.19; H: 5.35; N: 3.72.

Pharmacological study

Homocysteine concentrations determined in rat plasma are presented in Table I.

Table I
Homocysteine concentrations determined in rat plasma

N = 10 animals/group	Hcy (μmol/L); Mean ± SD	
	Initial	Final
Group I - only methionine	9.692 ± 0.692	31.056 ± 0.616*
Group II - methionine plus niacin	10.555 ± 0.668	28.06 ± 0.540*Ψ
Group III - methionine plus L103	10.167 ± 0.852	26.828 ± 0.505*Ψ

* – Statistical difference within group; Ψ – Statistical difference between groups

The obtained data showed significant increase in homocysteine level for all groups at the final moment compared to the initial moment, which confirm HHcy status.

Data are consistent with the literature [11] and our previous studies [5]. Hcy levels in groups II and III were significantly lower as comparing to the control group at the end of the experiment (< 0.001 for both groups), but Hcy is still over the upper limit (up to 0.7 μmol/L [1]). Between Group II and Group III there is no statistical difference in Hcy levels in the end. Hcy concentrations in blood are classified as follows: normal range 5 - 15 μM; moderate 16 - 30 μM; intermediary 31 - 100 μM; severe above 100 μM [9]. According to this classification, groups II and III belong to moderate hyperhomocysteinemia, and the control group in the intermediary one. Thus for the situation of moderate /intermediary HHcy, as in our case, L103 fails to prevent the increase of homo-cysteine levels. Even so, lowering Hcy level from intermediary to moderate hyperhomocysteinemia, might be a benefit for L103 administration.

The total antioxidant status determined in rat plasma is presented in Table II.

TAS concentrations were significantly decreased at the end as compared to the beginning of the experiment, within all groups ($p < 0.0001$, $p = 0.0064$ respectively $p < 0.0001$).

Table II

TAS status determined in rat plasma

N = 10 animals/group	TAS (mmol/L plasma); Mean ± SD	
	Initial	Final
Group I - only methionine	1.810 ± 0.111	1.064 ± 0.095*
Group II - methionine plus nicotinic acid	1.737 ± 0.134	1.237 ± 0.203*
Group III - methionine plus L103	1.859 ± 0.049	1.469 ± 0.116*Ψ

* – Statistical difference within group; Ψ – Statistical difference between groups

Regarding TAS status, between groups II and control there are no statistical differences at the end. The fact that TAS levels are slightly higher in Group II *versus* control suggests a minor antioxidant effect attributed to niacin. Literature shows that the oxidant/ antioxidant activity of niacin depends on the time of exposure as follows: the antioxidant activities of niacin in the later phases (3 weeks) of lipid per-oxidation are much stronger than those in the earlier phases (1 week) [10]. Even if niacin has been administered for 4 weeks, we assume that its antioxidant capacity was exceeded by the free radicals generated through the autooxidation of Hcy high concentrations [6, 12]. Group III presents significantly higher TAS concentration as compared to the control group at the end of the experiment ($p = 0.0011$). In fact, the drop in TAS concentrations was the smallest among all three groups, suggesting a protective antioxidant activity attributable most likely to the rutin moiety. The comparison between groups II and III showed no statistical difference at the final moment.

Cholesterol concentrations determined in rat plasma are presented in Table III.

Table III

Cholesterol concentrations determined in rat

N = 10 animals/group	Cholesterol (mg/dL); Mean ± SD	
	Initial	Final
Group I - only methionine	85.94 ± 3.31	97.43 ± 4.16*
Group II - methionine plus nicotinic acid	89.27 ± 5.11	88.07 ± 3.07Ψ
Group III - methionine plus L103	86.47 ± 9.79	87.30 ± 2.72Ψ

* – Statistical difference within group; Ψ – Statistical difference between groups

Cholesterol concentrations were significantly increased at the end of the experiment as compared to the initial one in the control group ($p = 0.0013$). Literature shows [18, 19] that after methionine administration, cholesterol level increases because of the stimulation of its hepatic synthesis, our data being consistent to that. Within groups II and III no statistic difference in cholesterol concentrations was found when comparing initial to final moment. For Group II, this result is justified because niacin is a well-known hypolipemiant drug. The similar behaviour for Group III suggests a nicotinic acid-like activity for L103. Both groups II and III exhibit significant decreased cholesterol levels as compared to the control group at the end of the experiment ($p = 0.0035$ respectively $p = 0.0008$) suggesting the lipid-lowering activity for niacin as well as for L103.

Conclusions

The new rutoside derivative L103 presents anti-oxidant properties and lowers the cholesterol levels in the experimental induced hyperhomocysteinemia in rats. We assume that in the case of the moderate/ intermediary hyperhomocysteinemia, the decrease of two aggravating risk factors (cholesterol and reactive species) justifies the use of L103. Since L103 exerts similar lipid-lowering effect as niacin, its administration might avoid the niacin side effects being a benefit in dyslipidaemia. The fact that L103

pulls back homocysteine levels, even in a very small amount, may be useful in cardiovascular disease.

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