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COMPARATIVE STUDIES ON NICOTINIC ACID DERIVATIVES AS HYPOLIPOPROTEINEMIC AGENTS

Z. S. SAIFY, M. ARIF, SHAMIM AKHTAR, NOUSHEEN MUSHTAQ AND FARAH MAZHAR*
*Department of Pharmaceutical Chemistry, *Department of Pharmacognosy*
Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

ABSTRACT

Nicotinic acid and its related compounds are well known lipid lowering agents but the use of nicotinic acid in clinical practice is limited because of its side effects. To reduce the unwanted effects, new derivatives of nicotinic acid have been synthesized and tested in control and pathological animals for their antilipoproteinemic effects. Two newly synthesized derivatives of nicotinic acid i.e. 3-methoxy phenacyl nicotinium bromide (T_1) and 2-methoxy phenacyl nicotinium bromide (T_2) have been screened for their hypolipoproteinemic effects in white male rabbits. The compounds were administered in the doses of 30 mg/day and the results were compared with aspirin taken as the reference drug. The present work revealed that both of these synthesize compounds exhibited marked effects on plasma lipoproteins assuming that these compounds exerted their action by inhibiting hepatic production of very low density lipoprotins (VLDL), ultimately leading to reduction in low density lipoprotein. Clofibrate and aspirin are taken as standards to evaluate the potentials of these agents for their effects on lipid.

INTRODUCTION

The increase in blood lipids is an important risk factor in the development of atherosclerosis (Stefan Niewiarowski and A. Koneti Rao, 1983). The incidence of coronary insufficiency and myocardial infarction is higher in the individuals with high lipid levels. The major components of plasma lipids are the triglycerides, phospholipids, free cholesterol esters in individuals with the highest levels of plasma lipids and lipoproteins. It is established that total serum cholesterol is the sum of the concentration of three components: Very Low-Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL), and High Density Lipoprotein (HDL). VLDL and LDL appear to promote the development of atherosclerosis, whereas HDL seems to exert a protective effect on the arterial wall and to counteract the effects of the VLDL and LDL (Gordon T. *et al.*, 1977; Whayne T.F. *et al.,* 1981 and Pearson T.A. *et al.,* 1979).

Increase in high fat diet may lead to higher accumulation of lipids and lipoproteins, which in turn may result in various types of complications in the cardiovascular system. Besides high fat diet, it is believed that the onset of hypercholesterolemia involves many factors such as age, sex, lack of physical activity, obesity, smoking, hypertension, nephrotic syndrome, diabetes mellitus, hypothyroidism, obstructive jaundice and pregnancy (Holmes D.R. Jr. *et al.*, 1981; Hjermann I. *et al.*, 1981 and Ross R.K. *et al*., 1981). These findings indicate that the above factors are also responsible for hypercholesterolemia. In this context, scientists are working in several directions for its treatment and cure.

Under the Lipid Research Clinic (LRC) Program an effective relationship between lipoprotein and atherogenesis was established. The LRC investigators determined that lowering the total and

low-density lipoprotein plasma cholesterol concentration could reduce significantly the risk of coronary heart disease (CHD) by diet and drug treatment (Lipid Research Clinics Program, 1984a, b).

Nicotinic acid is a well-known and potent lipid-lowering agent. Its hypercholesterolemic activity was first discovered by Altschul in 1955 (Altschull R. *et al.,* 1955) and further established by others (Parson W.B. Jr. *et al.,* 1955; Gurian H. & Alden Berg D. 1957 and Richard W.P. *et al*., 1958). However, nicotinic acid itself has side effects such as cutaneous flushing, gastrointestinal disturbances and increased risk for the development of gall stones; therefore its use is limited (Angelin B. *et al*., 1979). To overcome these problems several studies were carried out on nicotinic acid derivatives and found promising results (Miller O.N. *et al*., 1960, Hardy R.W.F. *et al.*, 1960 and Jaffrey M. Hoeg *et al.,* 1987).

MATERIAL AND METHOD

Instrument and Appartus

Electrical centrifuge, photometric system 4010 Boehringer Mannheim (W. Germany), disposable syringes, disposable scalpvein, justor.

Reagents

Reagent Kits were supplied by Boehringer Mannheim W. Germany.

Food Material and Drugs

Cholestrol, Butter (Lurpak), Aspirin, Clofibrate, Bezafibrate, Synthetic compounds: 3-methoxyphenacylnicotinium bromide (T_1), 2-methoxyphenacylnicotinium bromide (T_2), Bread, Chick -pea (gram seed), Alfa Alfa (*loosan* or green grass).

Animals And Feeding Schedule

150 days study involving 45 healthy white male rabbits of 1.0 to 1.5 kg. All of them received their respective diet and water ad libitum.

The procedure from feeding schedule to the biochemical analysis and determination of LDL and HDL-cholesterol were explained comprehensively by Saify *et al* (1985).

RESULTS AND DISCUSSION

During the course of work the effects of synthesized compounds and on HDL and LDL were studied and the results are shown in table 1-8.

Nicotinic acid is one of the drugs of choice for lowering the lipid levels, but its side effects limit its use as a therapeutic agent. To overcome this problem, its derivatives were synthesized. Nicotinic acid when quaternised with phenacyl bromides, resemble the molecular makeup of catecholamines. They were then used to study their hypolipoproteinemic agents.

All the values of normal control (N.C.) remained low throughout the dietary period with slight fluctuations. While in pathological control (P.C.) and treated (T) groups, all the lipid parameters changed significantly as compared to the normal control group.

Our observations are in support of the investigation that rabbits has propensity to develop a massive increase in plasma cholesterol levels within days after being placed on a high cholesterol

diet (Petri T. Kovanen, 1987). Aspirin reduced the level of serum cholesterol from 606mg/100ml – 214 mg/100ml ($P<0.02$).

It is also assumed that these newly synthesized derivatives exert their action by inhibiting hepatic production of VLDL, ultimately leading to the reduction in low-density lipoprotein. It is in accordance with the observation that nicotinic acid presumably acts in part by inhibiting production of LDL cholesterol and its precursor (Joseph L., Witzun Lojolla Calif., 1987). Our observations also support the findings that when nicotinic acid was used orally in dose 3-6 gm/day in human for 1-3 years a significant reduction in serum cholesterol and LDL cholesterol was observed (Carlson L. *et al.,* 1968).

Among the synthetic compounds 2-methoxyphenacylnicotinium bromide (T_2) has been found more effective as compared to 3-methoxyphenacylnicotinium bromide (T_1). In the case of HDL, 3-methoxyphenacylnicotinium bromide (T_1) is more active than 2-methoxyphenacylnicotinium bromide (T_2).

These results lead to the conclusion that synthetic compounds show pronounced (in LDL) or equal effect (in HDL) with early onset of action all the time. It is concluded that the synthetic compounds have significant effects on HDL and LDL. For the treatment of hyperlipidemia, first regimen should always be a controlled diet with increased physical activity and drugs should be used as the second regimen.

Table 1
Showing the effect of normal diet on the serum LDL cholesterol mg/100 ml in group 1 normal control (N.C) rabbits

Days	1A	1B	1C	1D	1E	Mean	SE	P
00	9.00	12.00	7.00	15.00	16.00	11.80	1.50	
20	11.00	15.00	13.00	12.00	14.00	13.00	0.63	
40	10.00	17.00	15.00	18.00	15.00	15.00	1.20	
60	13.00	19.00	20.00	22.00	13.00	17.40	1.70	
80	15.00	20.00	29.00	24.00	21.00	21.80	2.10	
100	16.00	23.00	34.00	23.00	27.00	24.60	2.60	
120	18.00	30.00	55.00	24.00	33.00	32.00	5.60	
130	19.00	28.00	60.00	27.00	32.00	33.20	6.30	
140	23.00	30.00	61.00	28.00	34.00	35.20	5.90	
150	24.00	27.00	55.00	31.00	37.00	34.80	4.90	

Table 2a
Showing the effect of cholesterol/butter rich diet on the serum LDL cholesterol mg/100ml in group 2 pathological control (P.C) rabbits

Days	2A	2B	2C	2D	2E	Mean	SE	P
00	10.00	15.00	17.00	17.00	12.00	14.20	1.20	
20	42.00	39.00	51.00	72.00	42.00	49.20	5.60	
40	65.00	143.00	105.00	174.00	169.00	131.20	18.40	
60	117.00	308.00	165.00	246.00	271.00	221.40	31.40	
80	163.00	386.00	231.00	347.00	301.00	285.60	35.80	
100	327.00	528.00	360.00	424.00	366.00	401.00	31.60	
120	430.00	626.00	487.00	580.00	467.00	518.00	32.70	

Table 2b
Showing the effect of normal diet

130	339.00	540.00	382.00	471.00	334.00	413.20	35.90	
140	237.00	393.00	245.00	327.00	298.00	300.00	25.60	
150	134.00	209.00	217.00	228.00	143.00	186.20	17.70	

Table 3a
Showing the effect of cholesterol/butter rich diet on the serum LDL cholesterol mg/100 ml in group 3 treated 1 (Tl) rabbits

Days	3A	3B	3C	3D	3E	Mean	SE	P
00	45.00	6.00	7.00	14.00	11.00	16.60	6.50	
20	190.00	79.00	40.00	20.00	18.00	69.40	28.70	
40	295.00	240.00	93.00	61.00	150.00	167.80	39.40	
60	453.00	287.00	165.00	150.00	230.00	257.00	48.90	
80	700.00	426.00	297.00	184.00	470.00	415.40	71.90	
100	795.00	495.00	339.00	370.00	570.00	513.80	73.22	
120	880.00	525.00	463.00	531.00	636.00	607.00	65.90	

Table 3b
Effect of 3-methoxy phenacyl nicotinium bromide 30 mg/day

Days	3A	3B	3C	3D	3E	Mean	SE	P
130	700.00	367.00	273.00	441.00	260.00	408.20	71.60	N.S.
140	450.00	212.00	198.00	225.00	154.00	247.80	46.50	N.S.
150	228.00	72.00	99.00	1288.00	75.00	352.40	25.70	< .05

Table 4a
Showing the effect of cholesterol/butter rich diet on the serum
LDL cholesterol mg/100 ml in group 4 treated (T2) rabbits

Days	4A	4B	4C	4D	4E	Mean	SE	P
00	12.00	15.00	17.00	11.00	10.00	13.00	1.20	
20	30.00	43.00	31.00	39.00	26.00	33.80	2.80	
40	105.00	117.00	133.00	129.00	123.00	121.40	4.30	
60	200.00	221.00	297.00	245.00	266.00	245.80	15.20	
80	257.00	315.00	403.00	310.00	309.00	318.80	16.40	
100	396.00	359.00	516.00	363.00	505.00	427.80	30.80	
120	483.00	403.00	576.00	414.00	697.00	514.60	49.20	

Table 4b
Effect of 2-methoxy phenacyl nicotinium bromide 30 mg/day

130	261.00	217.00	420.00	285.00	466.00	329.80	22.90	N.S.
140	51.00	94.00	153.00	190.00	221.00	141.80	27.70	< .001
150	19.00	35.00	82.00	90.00	120.00	69.20	16.60	< .001

Table 5
Showing the effect of normal diet on the serum HDL cholesterol mg/100 ml
in group 1 normal control (N.C) rabbits

Days	1A	1B	1C	1D	1E	Mean	SE	P
00	6.00	7.00	4.00	11.00	9.00	7.40	1.08	
20	9.00	11.00	12.00	7.00	12.00	10.20	0.86	
40	12.00	18.00	11.00	9.00	10.00	12.00	1.41	
60	13.00	21.00	16.00	15.00	16.00	16.20	1.17	
80	14.00	17.00	23.00	20.00	24.00	19.60	1.66	
100	15.00	23.00	23.00	18.00	28.00	21.40	2.01	
120	17.00	19.00	23.00	20.00	27.00	21.20	1.55	
130	20.00	23.00	21.00	22.00	31.00	23.40	1.75	
140	18.00	20.00	21.00	22.00	33.00	22.80	2.35	
150	21.00	17.00	22.00	19.00	33.00	22.40	2.49	

Table 6a
Showing the effect of cholesterol/butter rich diet on the serum HDL cholesterol mg/100 ml
in group 2 pathological control (P.C) rabbits

Days	2A	2B	2C	2D	2E	Mean	SE	P
00	6.00	8.00	9.00	6.00	7.00	7.20	0.52	
20	7.00	11.00	11.00	12.00	9.00	10.00	0.79	
40	14.00	28.00	21.00	30.00	17.00	22.00	2.75	
60	19.00	41.00	29.00	63.00	32.00	36.80	6.64	
80	24.00	61.00	45.00	77.00	51.00	51.60	7.84	
100	31.00	67.0	53.00	80.00	53.00	56.80	7.31	
120	39.00	74.00	62.00	88.00	57.00	64.00	7.35	

Table 6b
Showing the effect of normal diet

130	38.00	79.00	65.00	80.00	52.00	62.80	7.19	
140	35.00	7700	58.00	61.00	45.00	55.20	6.41	
150	31.00	70.00	50.00	52.00	43.00	49.20	5.69	

Table 7a
Showing the effect of cholesterol/butter rich diet on the serum HDL cholesterol mg/100 ml in group 3 treated 1 (Tl) rabbits

Days	3A	3B	3C	3D	3E	Mean	SE	P
00	22.00	5.00	4.00	11.00	6.00	9.60	2.97	
20	42.00	7.00	7.00	15.00	11.00	16.40	5.80	
40	50.00	25.00	21.00	23.00	29.00	29.60	4.71	
60	73.00	42.00	39.00	47.00	44.00	49.00	5.49	
80	82.00	51.00	55.00	61.00	65.00	62.80	4.80	
100	110.00	65.00	57.00	74.00	72.00	75.60	8.13	
120	132.00	76.00	59.00	85.00	90.00	88.40	10.83	

Table 7b
Effect of 3-methoxy phenacyl nicotinium bromide 30 mg/day

130	125.00	77.00	60.00	85.00	95.00	88.40	9.93	< .05
140	123.00	75.00	63.00	82.00	99.00	88.40	9.32	< .01
150	121.00	70.00	65.00	81.00	103.00	88.00	9.41	< .001

Table 8a
Showing the effect of cholesterol/butter rich diet on the serum HDL cholesterol mg100 ml
In group 4 treated (T2) rabbits

Days	4A	4B	4C	4D	4E	Mean	SE	P
00	5.00	7.00	6.00	8.00	6.00	6.40	0.45	
20	9.00	11.00	11.00	14.00	13.00	11.60	0.77	
40	17.00	23.00	29.00	28.00	31.00	25.60	2.25	
60	36.00	29.00	35.00	37.00	48.00	37.00	2.75	
80	49.00	31.00	42.00	41.00	62.00	45.00	4.58	
100	55.00	41.00	47.00	43.00	76.00	52.40	5.69	
120	62.00	48.00	59.00	57.00	83.00	61.80	5.17	

Table 8b
Effect of 2-methoxy phenacyl nicotinium bromide 30 mg/day

130	65.00	51.00	70.00	60.00	90.00	67.20	5.80	N.S.
140	59.00	55.00	77.00	64.00	97.00	70.40	6.35	N.S.
150	53.00	57.00	81.00	72.00	102.00	73.00	7.90	<.02

REFERENCES

Angelin, B. Einarsson, K. and Barbro, L. (1979). *J. Clin. Invest.* **9**(3): 185-190.

Altschull, R; Iioffer, A; and Stephen, J. D. (1955). *Arch. Biochem. Biophys.* **54**: 558-559.

Carlson, L. A; Oro, L; and Ostman, J. (1968). *J. Atheroscler Res*. **8**: 667-673.

Carlson, L. A; Oro, L; and Ostman, J. (1968). *Acta. Med. Scand.* **183**: 457-465.

Gordon, T; Castelli, W. P; Hjortland M. C; Annel W. B; Wawber, T. R. (1977). *Am. J. Med.* **62**: 707-715.

Gurian, H.; Alden Berg D. (1957). *Am. J. Med. Soci.* **237**: 12-22.

Hardy, R.W.F.; Baylor, J. L; and Bauman, C.A. (1960). *J. Nutr.* **71**: 159-170.

Hjermann, I.; Byrek. R.; Halme, I. and Leren P. (1981). *Lancet.* **(ii)**: 1303-1310.

Holmes, D.R. Jr.; Elveback L. R.; Frye, Kottke B.A. and Ellefson, R.D. (1981). *Circulation.* **63**: 293-305.

Jaffrey, M. Hoeg; Martha B.; Maher B. S. N.; Kent R.; Bailey and Bryn Brever H. Jr. (1987). *Am. J. Cardio.* **59**: 812-815.

Joseph, L.; Witzun; Lojolla Calif (1987). *Am. Heart J*. **113**(2): 603-609.

Lipid Research Clinics Program (1984): The Lipid Research Clinics Coronary prevention Trial Result I, *JAMA,* **251**(3): 351-364.

Lipid Research Clinics Program (1984): The Lipid Research Clinics Coronary prevention Trial Results II, *JAMA*, **251**(3): 365-374.

Miller, O.N., Hamilton, J.G. and Goldsmith, S.A. (1960). Am. J. Clin. Nutr. **8**: 480-495.

Parson W. B. Jr.; Jhone J. and Filunn (1955). *Circulation.* **16**: 449-458.

Pearson T. A; Bulkley, H. B. and Achieff, S.C. *et al.* (1979). *Am. J. Epidemiol*; **109**: 285-297.

Petri T. Kovanen (1987). *Am. Heart J.* **113**: 464-469.

Richard W. P. Achor, Kenneth Berg; Nelson W; Barker and Bernard Mackenzio (1958). *Circulation.* **17**: 497-510.

Ross R. K; Paginini. Hill A. and Mack T. M. *et al.* (1981). *Lancet.* **1**(8225): 858-860.

Saify *et.al.* (1985). *Pakistan Heart Journal.* **18**: 48-62.

Stefan Niewiarowski and A. Koneti Rao (1983). Contribution of Thrombozenic Facters is the Pathogenesis of Atherosclerosis Progress in Cardio Vascular Diseases Vol. XXVI, No. 3, Nov / Dec. 1983.

Whayne, T.F., Alaupoviep; Curry M. D.; Lee, E.T; Anderson P. S. and Schecter E. (1981). *Atherosclerosis*, **39**: 41-54.