PHARMACOLOGICAL EFFECTS OF 3-METHOXY-PHENACYL-NICOTINIUM BROMIDE AND COMPARISON WITH THE EFFECTS OF ASPIRIN ON TOTAL SERUM CHOLESTEROL IN MALE RABBITS

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SUMMARY: Most of the cardiovascular diseases and complications are related with different types of hyperlipidemic conditions. During the course of present research, an attempt has been made to observe the effect of 3-Methoxy-phenacyl-nicotinium bromide as anti-cholesterolemic agent and comparison with the effect of Aspirin. These studies show that 3-Methoxy-phenacyl-nicotinium bromide and Aspirin significantly lowers total serum cholesterol in induced hypercholesterolemic rabbits as compared to untreated (Pathological group). The findings confirm our previous studies where the effects of 2-Methoxy-phenacyl-nicotinium bromide on total lipid, phospholipid, triglyceride, total serum cholesterol and transaminases were investigated on male rabbits (1-3).

Key Words: 3-Methoxy-phenacyl-nicotinium bromide, aspirin, total serum cholesterol.

INTRODUCTION

The increase of cholesterol and its deposition in the arterial wall of the circulatory system lead to atherosclerosis and arteriosclerosis and to the coronary heart diseases. It has been reported (5) that nicotinic acid and para-amino salicylic acid lowers cholesterol by inhibiting the synthesis of cholesterol.

Hypercholesterolemia developed in rabbits fed with cholesterol and coconut oil in the diet, was reduced when nicotinic acid was given (5, 6). The same decrement in concentration of cholesterol was observed in hypercholesterolemic patients (7). Despite these beneficial effects of these drugs it is important to note that some patients experience a harmless cutaneous vasodilatation, taxyphylaxis, pruritus, rashes, dry skin and acanthosis nigricans, nausea and abdominal discomfort, moderate elevation of transaminases and alkaline phosphatase (8), Hyperurecemia may precipitate gout. Hyperglycemia and arrhythmias have also been reported (9).

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We in this communication present further evidence for reduction of cholesterolemia in rabbits by nicotinic acid and aspirin.

MATERIAL AND METHODS

Animals and their feeding schedule

Twenty white male healthy rabbits supplied by local supplier, weighing 1 to 1.5 Kg were kept in individually marked cages throughout the experimental period of 150 days. Animals were divided at random into four (4) groups each comprising five (5) animals (n=5), receiving their respective diets and water *ad libitum*.

The feeding schedule during the experimental period of 0-150 days in shown in the given chart.

Material for feeding and treatment purposes

1. Cholesterol E. Merck (Germany)
2. Butter Lurpak (Denmark)
3. Aspirin (acetyl salicylic acid) Aspro Nicholas
4. Bread (Local supplier)
5. Chick pea (gram seed) (Local supplier)
6. Alfa Alfa (loosan or green grass) (Local supplier)

Materials and apparatus for biological testing

1. Electrical centrifuge 1000-4000 r. p. m (China)
2. Disposable syringes 10 ml, 24 guage JMS (Japan)
3. Disposable scalpvein 24 guage JMS (Japan)
4. Justor From 5 to 1000 μl with disposable tips Nichryo (Japan)
5. Photometric system 4010 from Boehringer Mannheim (W. Germany) is a filter photometer perating in the visible and UV range. All functions being controlled by Microprocessor having a special program for kinetic enzymatic method along with program for end point method. Consisted of photometer 4010, pump 4010 water bath (precitherm) type PFV.

Animals feeding schedule during the experimental period i. e. (0-150 days)

Days 0 ⟷ 120 ⟷ 150 Group 1 n = 5 Normal Control (N.C)	Normal diet*	
Days 0 ⟷ 120 ⟷ 150 Group 2 n = 5 Pathological Control (P.C)	Normal diet* + cholesterol 200 mg/day + butter 2 g/day	Normal diet*
Days 0 ⟷ 120 ⟷ 150 Group 3 n = 5 Treated 1 (T_1)	Normal diet* + cholesterol 200 mg/day + butter 2 g/day	Normal diet + 3 - methoxy - phenacyl-nicotinium bromide 30 mg/day
Days 0 ⟷ 120 ⟷ 150 Group 4 n = 5 Treated 2 (T_2)	Normal diet* + cholesterol 200 mg/day + butter 2 g/day	Normal diet* + Aspirin 50 mg/day

Reagents

All the reagent kits were supplied by Boehringer Mannheim W. Germany. For quality control and precision control sera Boehringer Mannheim (Cat no. 171735).

Precipath-u (Cat No. 171760) special control serum for HDL cholesterol (Cat No. 225053) were used.

Group 1: Normal control (N.C) (n=5) received normal diet throughout the whole experimental period.

Group 2 : Pathological control (P.C) (n=5) rabbits. In this group rabbits received normal diet plus 200 mg of cholesterol and 2 gm of butter per day for 120 days (0-120th day) and then cholesterol and butter were withdrawn and the rabbits were kept on normal diet only (121st to 150th day).

Group 3 - Treated (T_1): This group received normal diet plus 200 mg cholesterol and 2 gm of butter per day (0-120th day) and then cholesterol and butter were withdrawn and the rabbits were kept on normal diet plus 30 mg of 3-Methoxy-phenacyl-nicotinium bromide for the remaining 30 days (121st to 150th day).

Group 4 - Treated (T_2): This group received normal diet plus 200 mg of cholesterol and 2 gm of butter per day (0-120th day) cholesterol and butter were withdrawn and the rabbits were kept on normal diet plus 50 mg of Aspirin for remaining 30 days (121st to 150th day).

Note: Normal diet: -Loosan (Alfa Alfa), Gram seeds (Chick pea) and bread. Cholesterol (Merck, West Germany), Butter (Lurpak, Holland).

Biochemical analysis

5.00 ml of blood from rabbits (non-fasting) was collected from the marginal ear vein on every 20th day up to 120 days (0, 20, 40, 60, 80, 100 and 120) and then on every 10th day (130, 140 and 150) the blood samples were centrifuged and serum was separated.

In these serum samples, total cholesterol was determined according to the instruction sheet provided with reagent kits. All the values are expressed as the mean and standard error. 'T' test is performed and 'P' values are observed (10).

The reagent kit and control sera were supplied by Boehringer Mannheim (W. Germany) for the determination of serum total cholesterol. Reagent kit having Cat. No. 237574 was used.

RESULTS

Results have been summarized in Tables 1-4 showing the concentration of total cholesterol in rabbits before and after treatment for 150 days. Pathological control Table 2 shows an increase in the concentration of total cholesterol in 120 days. The increase in concentration was from 27.00 mg/100 ml to 646.00 mg/100 ml. It has also been observed that in untreated pathological control (Table 2) when cholesterol and butter fat is stopped,

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

Days	1A	1B	1C	1D	1E	Mean	SE	P
00	25.00	27.00	21.00	34.00	35.00	28.40	8.39	
20	33.00	38.00	35.00	32.00	40.00	35.60	1.34	
40	38.00	45.00	49.00	39.00	37.00	41.60	2.07	
60	42.00	47.00	50.00	45.00	42.00	45.20	1.36	
80	50.00	53.00	76.00	60.00	57.00	59.20	4.05	
100	47.00	60.00	63.00	55.00	68.00	58.60	3.20	
120	59.00	65.00	90.00	65.00	80.00	71.80	5.10	
130	62.00	72.00	95.00	65.00	83.00	75.40	5.40	
140	61.00	57.00	98.00	53.00	87.00	71.20	7.20	
150	69.00	86.00	105.00	61.00	93.00	82.80	9.74	

the body itself started lowering the serum cholesterol level i.e., 646.06 mg/100 ml to 287.00 mg/100 ml (from 120th to 150th day).

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

Days	2A	2B	2C	2D	2E	Mean	SE	P
00	19.00	28.00	34.00	24.00	30.00	27.00	2.29	
20	63.00	75.00	97.00	90.00	80.00	81.00	5.78	
40	110.00	220.00	171.00	110.00	225.00	167.20	19.20	
60	155.00	380.00	243.00	365.00	310.00	290.60	37.17	
80	236.00	507.00	357.00	487.00	368.00	391.00	43.99	
100	398.00	683.00	482.00	590.00	425.00	515.60	47.63	
120	551.00	612.00	655.00	800.00	615.00	646.60	46.60	
Showing the effect of normal diet								
130	391.00	684.00	459.00	563.00	403.00	500.00	49.30	
140	288.00	567.00	326.00	495.00	387.00	412.60	46.60	
150	219.00	355.00	308.00	341.00	212.00	287.00	27.00	

The other two groups of rabbits were used to study the effects of drugs.

In group of T_1 rabbits, 3-Methoxy-phenacyl-nicotinium bromide shows a marked decrease in the level of total cholesterol (Table 3) which is from 766.00 mg/100 ml to 220.2 mg/100 ml. In Group 4 rabbits (T_2) aspirin also shows a marked decrease in the total cholesterol level, i.e., from 606.20 mg/100 ml to 214 .60 mg/100 ml (Table 4).

Table 3: Showing the effect of cholesterol/butter rich diet on the serum total cholesterol mg/100 ml in group 3 treated 1 (T_1) rabbits.

Days	3A	3B	3C	3D	3E	Mean	SE	P
00	84.00	18.00	21.00	36.00	25.00	36.80	10.90	
20	279.00	102.00	81.00	45.00	39.00	109.20	39.30	
40	403.00	305.00	169.00	113.00	202.00	238.40	46.20	
60	612.00	465.00	201.00	223.00	305.00	361.20	69.70	
80	891.00	512.00	290.00	401.00	573.00	533.40	90.90	
100	1023.00	591.00	415.00	500.00	762.00	658.20	96.44	
120	1111.00	675.00	545.00	702.00	800.00	766.60	85.18	
Effect of 3-Methoxy-phenacyl-nicotinium bromide 30 mg/day								
130	925.00	457.00	345.00	584.00	410.00	544.20	92.06	NS
140	628.00	303.00	288.00	367.00	315.00	380.20	56.67	NS
150	364.00	139.00	195.00	253.00	150.00	220.20	38.80	NS

DISCUSSION

The lipid atherogenic complications have undergone a change from being initially focused on serum total cholesterol. As has been observed in case of normal control the values remain low with little change whereas in case of pathological control there is significant increase in the serum cholesterol compared to normal control. In the animals an increase in serum cholesterol level was observed when placed on high cholesterol diet (Pathological Control). Our findings are similar to (11,12) that high cholesterol diet causes an increase of total cholesterol. Newly synthesized compounds 3-Methoxy-phenacyl-nicotinium bromide and Aspirin decreases the amount of cholesterol from 776 mg/100 ml to 220 mg/100 ml whereas 50 mg of Aspirin reduced the level of serum cholesterol from 606 mg/100 ml to 214 mg/100 ml respectively. The effect of some other newly synthesized derivatives of Nicotinic acid have already been published (13,14).

Table 4: Showing the effect of cholesterol/butter rich diet on the serum total cholesterol mg/100 ml in group 4 treated 2 (T_2) rabbits.

Days	4A	4B	4C	4D	4E	Mean	SE	P
00	34.00	29.00	35.00	33.00	30.00	32.20	1.03	
20	65.00	73.00	67.00	49.00	51.00	61.00	4.10	
40	146.00	178.00	178.00	154.00	149.00	161.00	6.30	
60	254.00	310.00	299.00	237.00	293.00	278.60	12.50	
80	307.00	425.00	376.00	295.00	418.00	364.20	24.30	
100	428.00	539.00	481.00	446.00	575.00	493.80	24.80	
120	510.00	678.00	486.00	594.00	763.00	606.20	46.30	
Effect of Aspirin 50 mg/day								
130	321.00	390.00	377.00	395.00	459.00	388.40	19.70	<0.05
140	226.00	285.00	293.00	279.00	364.00	289.40	19.70	<0.02
150	194.00	237.00	185.00	198.00	259.00	214.60	12.70	<0.02

The structure elucidation of these derivatives was done with spectroscopic techniques (UV, IR, NMR and Mass) and published (15).

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