Effect of Aspirin and Nicotinic Acid Derivative As Potential Hypocholesterolemic Agents

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SUMMARY :

An increase in fat enriched diet may lead to higher accumulation of lipid, lipoprotein and cholesterol, which in turn may lead to cardiovascular complications. Several lines of evidence suggest that coronary heart diseases and atherogenesis are more directly related to different classes of hyperlipidemia. During the course of present project attempt is made to study the effect of aspirin and a nicotinic acid derivative as antihyper-cholesterolesterolemic agents.

Male white rabbits were fed for 120 days on diets comprising of cholesterol 200 mg/day plus butter 2 g/day. Concentration of cholesterol is determined and compared with that of the blood serum of control and experimental animals to evaluate the effect of aspirin and nicotinic acid derivative.

This is evident from the results that these agents appreciably reduced blood cholesterol level.

INTRODUCTION :

Atherosclerosis is characterized by the deposition of cholesterol esters and other lipid in the connective tissues of the arterial wall.

Aspirin has long been used as an effective analgesic for pain, inflammation and fever (Tre acher, et. al., 1978; Vane, J. R., 1971).

Recent studies have proved that the effect of aspirin is to cause the inhibition of biosynthesis of prostaglandins in various organs (Klimov, et. al. 1969; Subissi, et. al. 1980).

It has recently been established that the biosynthesis of several prostaglandins proceeds through endoperoxide intermediate designated as PGG_2 and PGH_2 with specific enzymes in specific tissues. The most profound discovery is that in some cells the PGG_2 and PGH_2 endoperoxide intermediates are converted to two other products, Thromboxane and Prostacycline. Thromboxane formation occurs in blood platelets and promote blood clotting by aggregation of platelets. It also constricts the arteries.

On the other hand prostacycline formation occurs in cells lining the arteries and veins, and ibhibit blood clotting by preventing both the aggregation of platelets and the constriction of arteries. This strongly suggest that the relative activities of these two substances are the determining factors whether or not a clot will form (Bergman, et. al. 1981; Ekdund, et. al. 1981).

MATERIAL AND METHODS :

All experiments are carried out on healthy rabbits weighing 1.5 — 2.0 kg. Rabbits are classified into the following four groups, each group comprised of 4 rabbits :

GROUP - .1:

Control:— Nothing is given except normal food and water.

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GROUP - 2:

This group is used as Pathological Control. Pathological conditions (Hyperlipaemia) is produced by giving non-salted 2 gm butter fat (Glaxo) with 200 mg pure cholesterol (Merck) for 120 days.

GROUP – 3 :

Rabbits in this group, are treated in the same way as group 2 for 120 days and then 100 mg/day Aspirin is given orally for 20 days.

GROUP - 4 :

These rabbits are also treated as group 2 and after 120 days – p dibromoacetophenone nicotinate is given (100 mg/day) for 20 days.

All the groups of rabbits are given food and water adlibitum.

Blood samples are collected from all the rabbits from marginal ear vein and serum was separated taking following duration into consideration :

For controls and pathological controls interval between blood sampling is 20 days while in treated group blood is collected from rabbits after 5 days upto 20 days (4 collections).

Test for the level of cholesterol is done as follows: Serum is separated by centrifuging the blood at 4000 R.P.M., in Heraus centrifuge. 0.1 ml sample of serum was taken into tubes and treated according to instruction sheets for manual assay provided by Boheringer Mannheium, kit No. 124095 (West Germany) and measured on spectrophotometer (Spectronic-21). Control sera were used from Boheringer Mannheium (West Germany). All the chemicals used are of analytical and reagent grade supplied by Merck.

RESULT :

Results are summerized in Table-1 and 2. Table-1 shows the level of serum cholesterol (mg/100ml) in all the 4 groups of rabbit before the treatment with drugs i.e., for 120 days.

Group 1 (Control) showed a slight fluctuation in the level over the period of 120 days when compared from their own controls (32.98 ± 2.09). The maximum increase is found at 60 days 38.74 ± 2.48 mg/100 ml and then a progressive decrease is observed.

In Group 2 (Pathological control) there is a definite and progressive increase in cholesterol level with the passage of time. Maximum level is 322.25 ± 68.21 mg/100 ml which was highly significant (< 0.1) from controls (32.14 ± 1.44 mg/100 ml).

Similarly, two other groups (3rd and 4th) 326.64 ± 16.10 and 339.46–41,22 mg/100 ml shwed a highly significant and progressive increase over control values. These two groups are used later on to see the effects of drugs.

EFFECT OF DRUGS :

Table–2 shows the effect of drug on the levels of serum cholesterol (Treated-A and Treated-B group). This table also shows the level of serum cholesterol of the pathological control rabbits without giving any medicine (untreated group).

Group-A is given Aspirin and it showed a progressive decrease in cholesterol levels at each time point which is statistically significant ($p < .05 - < .01$) and the level reached to near normal on 20th day.

Nicotinic acid derivative treated group (Group-B) also showed a progressive decrease in serum cholesterol level starting from the 5th day through 20th day though statistically not significant except on 20th day (< .05).

An interesting phenomenon was also observed in untreated pathological control rabbits (Table-2) that when cholesterol and butter is stopped the body itself started lowering the serum cholesterol level and it is lowered to 82.11 + 11.76 mg/100 ml from 322.25 + 68.21 mg/100ml on 20th day which is satistically highly significant (< .001).

Aspirin and Nicotinic derivative are of the value that they further reduced the serum cholesterol level at the same time period to 30.15 ± 1.54 mg/100 ml and 46.43 ± 5.54 mg/100 ml respectively.

These figures are found even lower than the lowest figures observed in the pathological untreated control rabbits (82.11 ± 11.76 mg/100ml) and are satistically significant for Aspirin and Nicotinacide derivatives (< .01) at the same time period.

TABLE · I

THE CONCENTRATION OF SERUM CHOLESTEROL IN CONTROL AND EXPERIMENTAL RABBITS (mg / 100 ml)

DAYS	CONTROL						PATHOLOGICAL CONTROL						
	1A	1B	1C	1D	MEAN ± SEM		2A	2B	2C	2D	MEAN	± SEM	P
00	34.12	38.14	31.40	28.28	32.98	2.09	29.13	35.71	33.12	30.61	32.14	1.44	
20	33.31	35.11	28.93	25.13	30.62	2.24	55.73	41.62	53.17	39.21	47.43	4.11	< .02
40	39.23	37.77	34.11	40.19	37.83	1.33	71.62	49.23	102.23	49.32	68.10	12.53	> .05
60	37.12	41.79	32.21	43.17	38.57	2.48	93.92	200.17	149.42	109.23	138.18	23.74	< .01
80	35.92	33.12	41.00	39.32	37.34	1.75	131.16	293.32	196.62	231.23	213.08	33.84	< .01
100	29.22	30.32	39.91	37.18	34.18	2.61	153.72	345.72	240.00	291.30	257.68	40.82	< .01
120	28.11	26.77	33.77	41.93	32.64	3.44	198.73	510.13	249.97	330.17	322.25	68.21	< .01

DAYS	GROUP "A"						GROUP "B"						
	3A	3B	3C	3D	MEAN ± SEM	P	4A	4B	4C	4D	MEAN ±	SEM	P
00	28.97	33.61	39.08	37.57	34.80	2.26	—	42.00	35.61	30.11	39.26	36.74	2.56	
20	71.53	85.43	81.26	67.20	76.35	4.21	< .001	78.31	90.56	75.10	92.14	84.02	4.29	< .001
40	139.29	146.35	154.17	115.16	138.74	8.42	< .001	136.27	146.50	119.64	153.51	138.98	7.35	< .001
60	196.15	184.21	189.41	149.22	179.74	10.46	< .001	178.39	193.24	156.81	236.07	190.97	16.84	< .001
80	245.09	250.11	248.36	185.09	232.16	15.72	< .001	235.01	254.11	181.00	329.08	249.80	30.62	< .001
100	289.00	315.64	288.54	236.43	282.40	16.58	< .001	266.57	301.44	234.43	389.19	297.90	33.36	< .001
120	332.17	367.60	316.16	290.65	326.64	16.10	< .001	293.16	347.41	265.22	452.06	339.46	41.22	< .001

TABLE – II

CHANGES PRODUCED BY ASPIRIN (A) AND NICOTINIC ACID DERIVATIVE (B) IN CHOLESTEROL LEVEL OF RABBITS SERUM (mg/10 ml)

DAYS	CONTROL						PATHOLOGICAL CONTROL					
	1A	1B	1C	1D	MEAN ± SEM		2A	2B	2C	2D	MEAN ± SEM	
00	28.11	26.77	33.77	41.93	32.64	3.44	198.73	510.13	249.97	330.17	322.25	68.21
05	40.29	28.73	34.29	41.00	36.07	2.87	169.23	490.23	201.37	299.93	290.19	72.24
10	42.90	31.18	31.17	40.87	37.03	2.86	120.00	321.62	169.28	205.18	204.02	42.91
15	45.62	35.18	33.87	38.77	38.36	2.63	79.13	222.03	101.13	172.21	143.62	32.82
20	37.77	25.18	32.87	35.92	32.93	2.75	50.00	101.97	79.24	97.23	82.11	11.76

DAYS	Aspirin 100mg/day							Nicotinic acid derivative 100mg/day						
	TREATED "A"							TREATED "B"						
	3A	3B	3C	3D	MEAN ± SEM		P	4A	4B	4C	4D	MEAN ± SEM		P
00	332.17	367.60	316.16	290.65	326.64	16.10		293.16	347.41	265.22	452.06	339.46	41.22	—
05	175.09	185.43	176.20	124.19	165.22	13.87	N. S.	189.36	267.29	190.67	294.39	235.42	26.79	N. S.
10	103.67	110.72	96.31	62.43	93.28	10.69	< .05	93.66	162.11	112.51	191.54	139.95	22.45	N. S.
15	66.31	71.29	51.10	40.21	57.22	7.14	< .05	65.81	69.77	76.24	99.01	77.70	7.41	N. S.
20	34.25	30.51	28.89	26.97	30.15	1.51	< .01	39.76	48.06	36.52	61.39	46.43	5.54	< .05

DISCUSSION :

It has been shown that during the feeding of an atherogenic diet containing 2% cholesterol and 10% butter for 5 months caused narrowing of the coronary and peripheral arteries which may produce lesion (Ross et. al. 1976) due to the subendothelial injury, platelet aggregation may result by the action of enzyme prostaglandin synthetase.

In this process an enzyme cyclo-oxygenase which catalyses the transformation of arachidonic acid into cylic endoperoxide PGG_2 was considered to be extremely sensitive to aspirin inhibition, (Ellis et. al. 1980; Bughanan et. al. 1980). It is observed that single dose of aspirin will lead to a platelet defect that last for well over a week when aspirin was given in low doses i.e., 0.3 gm daily and 0.6 gm twice a day for one week both prolong the bleeding time (Koesis et. al. 1973; Hanley et. al. 1981).

In rabbits kept on a diet containing 1 gm/day cholesterol for 12 weeks, the nicotinic acid derivative displayed greater hypolipidemic and antiatherogenic activity than an equidose of plain nicotinic acid (Subissi et. al. 1980). Various derivatives of nicotinic acid have therefore, been synthesized and tested. During the course of our study, it is confirmed that the new compound-P dibromoacetrophenone nicotinate is a potent antilipolytic agent.

REFERENCES :

1. Baenzinger, N.L., Dillender, M.J. and Majerus, P.W., Biochem. Biophys. Res. Commun., 78, 294- 301 (1977) ;
2. Bergman, G., Atkinson, L. and Richardson, P. J., Lancet, 1, 569—572 (1981).
3. Buchanan, M. R., Dejana, E., Cazenane, J. P. and Richardson, M., Thromb. Res., 20, 447- 460. (1980).
4. Eklund, B., Joretag, T. and Kaijser, L., Clinical Physiology, 1, 123—130 (1981).
5. Ellis, E. F., Wright, K. F., Jones, P. S., Richardson, D. W. and Ellis, C. K., J. Cardiovasc. Pharmacol., 3, 387—397 (1980).
6. Hanley, S. P., Bevan, J., Cockbill, S. R., and Haplinstall, Lancet 1, 969—971 (1981).
7. Klimov, A. N., Dokusova, O. K. and Poliakova, E.D., Biochem. Pharmacol., 18, 1251 (1969).
8. Koesis, J. M., Hermandovich, J., Silver, M. J., Smith, J. B. and Ingerman, C., Prostaglandin 3, 141- 144 (1973).
9. Ross, R., Glomset, J. A. N., New Engl. J. Med., 259, 369 (1976).
10. Subissi, A., Schiantarelli, P., Biazi and Sardelli, G., J. Atherosclerosis, 36(1), 135- 148 (1980).
11. Treacher, D., Warlow, C. and McPherson, K., Lancet, (ii), 1378, (1978).
12. Vane, J. R., Nature, New Biol., 231, 232- 235 (1971).