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Hypolipidemic effects of nimesulide and celecoxib in experimentally induced hypercholesterolemia in rabbits

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Background/aim: Hypercholesterolemia plays an important role in the development of atherosclerotic disease, which is one of the leading causes of mortality in the world. Previous studies showed that cyclooxygenase (COX) inhibitors could be used in treating hypercholesterolemia. The present study was designed to test whether COX-2 inhibition can improve lipid profiles in hypercholesterolemia.

Materials and methods: Rabbits were fed a high-cholesterol diet to produce hypercholesterolemia. The role of COX-2 was evaluated using celecoxib and nimesulide. Rabbits were divided into 4 groups: the first with normal healthy rabbits, second with high-cholesterol diet and pretreatment with saline, third with high-cholesterol diet and pretreatment with celecoxib, and fourth with high-cholesterol diet and pretreatment with nimesulide. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride levels were measured at 2-week intervals in all the groups.

Results: Results show significantly high levels of serum cholesterol, LDL, and triglyceride but low levels of HDL in hypercholesterolemic rabbits pretreated with saline. Rabbits pretreated with nimesulide and celecoxib showed improvement compared to the saline-treated group.

Conclusion: Improvement of lipid profile by celecoxib in hypercholesterolemic rabbits indicates the detrimental role of COX-2 during atherogenesis. However, the observed effects of nimesulide and celecoxib in hypercholesterolemia may be independent of their ability to inhibit COX-2. Nevertheless, both celecoxib and nimesulide show lipid-lowering potential in experimental hypercholesterolemia.

Key words: Hypercholesterolemia, antiinflammatory, nimesulide, celecoxib

1. Introduction

Atherosclerosis, leading to myocardial infarction and stroke, is one of the most important causes of death due to cardiovascular diseases (1). The association of hypercholesterolemia with atherosclerosis is well known (2). Studies suggest that elevated lipoproteins in blood, and especially low-density lipoproteins (LDL), play an important role in the pathogenesis of atherosclerosis as indicated by their accumulation in atherosclerotic lesions (3,4). Therefore, reduction of LDL-cholesterol is an important target for the prevention and treatment of atherosclerosis.

Nonsteroidal antiinflammatory drugs (NSAIDs) are used clinically as antiinflammatory, antipyretic, and antirheumatic agents (5). They exert their effect by inhibiting cyclooxygenase (COX) activity with subsequent inhibition of the formation of prostaglandins, prostacyclins, and thromboxanes (6). Several studies have shown that indomethacin or ibuprofen reduces the plasma cholesterol levels in humans or laboratory animals (7,8). Indomethacin can lower the cholesterol content of atherosclerotic blood vessels and liver in monkeys and rabbits (9), and when combined with inhibitors of angiotensin converting enzymes, indomethacin lowers blood cholesterol levels in humans (8). NSAIDs, especially flufenamic acid and indomethacin, increase LDL binding, cell association, and degradation by increasing the expression of the mRNA of the LDL receptor protein in HepG2 cells (5). Celecoxib, a selective COX-2 inhibitor, lowers plasma cholesterol during carbon tetrachloride-associated hepatotoxicity in rats (10). Therefore, these studies suggest that COX inhibition may be useful in treating hypercholesterolemia.

Reduction in the LDL levels can be achieved by the use of statins, which are the currently employed widely for the

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treatment of lipid disorders. Statins are very effective in reducing LDL levels and have improved the quality of life and decreased mortality and morbidity in cardiovascular patients. However, the protection they provide is not complete and, therefore, a complementary drug that could enhance high-density lipoprotein (HDL) levels is required. Since pathogenesis of atherosclerosis is closely linked with prostaglandins, it is thought that COX-2 inhibitors may be beneficial in such a scenario. Therefore, in the present study, we employed two COX-2 inhibitors, nimesulide and celecoxib, to see their effects on HDL-cholesterol, LDL-cholesterol, total cholesterol, and triglycerides.

2. Materials and methods

High-cholesterol diet is known to cause hypercholesterolemia in rabbits (11). Before starting any experiments, male New Zealand rabbits with a mean age of 12 ± 2 weeks and weight of 2.150 ± 0.335 kg were kept for 1 week to acclimatize in the animal house. Rabbits were randomly distributed into 4 groups of 12 rabbits each. The first group consisted of normal healthy rabbits, the second (pretreated with saline) was fed a high-cholesterol diet, the third (pretreated with nimesulide in 5% DMSO) was fed a high-cholesterol diet, and the fourth (pretreated with celecoxib in 5% DMSO) was fed a high-cholesterol diet. The first two groups were given saline while the third group was pretreated with 25 mg/kg nimesulide and the fourth group with 25 mg/kg celecoxib daily for the entire experimental period (weeks 0–20). Doses of nimesulide and celecoxib used in this study correspond to their recommended doses used for various inflammatory conditions in humans. Food and water was freely available to all the rabbits. Each morning at selected time points, i.e. at weeks 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, blood was withdrawn from the marginal ear vein of the rabbits and tests were done within 2–3 h. Ethical approval of the study was obtained from the Kohat University of Science and Technology (AERC01092011). All the tested animals were treated in conformity with the highest ethical guidelines of the National Institutes of Health (USA). Experiments were carried out on the blood of all rabbits. The composition of the high-cholesterol diet (% w/w) used in this study diet is as follows: chokar (wheat bran) 30, flour 30, butter fat 5, cholesterol 2, cholic acid 0.5, Nutrivet L 0.25, salt 0.50, powdered milk 12, fish meal 14, oil 3.75, molasses 1, potassium meta bisulfate 1.

2.1. Total cholesterol

Total cholesterol levels were measured using enzymatic hydrolysis and an oxidation method with RANDOX kits according to the manufacturer's instructions. 4-Aminophenazone, hydrogen peroxide, and 4-chlorophenol through the action of peroxidase make quinone imine, which acts as an indicator in this assay.

2.2. HDL-cholesterol

Determination of HDL-cholesterol was based on the precipitation of low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) and the measuring of the remaining fraction of cholesterol. The kit was purchased from RANDOX and the assay was performed according to the manufacturer's instructions. In the presence of magnesium ions, phosphotungstic acid was used to quantitatively precipitate VLDL and LDL as well as chylomicrons. HDL fraction was found in the supernatant after centrifugation and determined quantitatively.

2.3. LDL-cholesterol

The principles of centrifugation and precipitation at the isoelectric point were used to determine LDL concentrations in the blood. The assay was performed according to the manufacturer's instruction (RANDOX). In brief, heparin was used to precipitate LDL at the isoelectric PH (5.04). Centrifugation was done to separate various fractions. LDL was determined by subtracting cholesterol in the supernatant from the total cholesterol.

2.4. Triglycerides

In brief, principles of enzymatic hydrolysis of lipases were used to determine triglyceride levels in the blood of the rabbits and were carried out using RANDOX kits according to the manufacturer's instructions. 4-Aminophenazone, hydrogen peroxide, and 4-chlorophenol through the action of peroxidase make quinone imine, which acts as an indicator in the reaction.

2.5 Statistical calculations

All the experiments were carried out in triplicate and data were statistical analyzed by one-way analysis of variance (ANOVA) followed by the Newman–Keuls test. $P < 0.05$ was considered significant.

3. Results

3.1. HDL-cholesterol

In group 1, there was no significant change in HDL-cholesterol throughout the 20-week period. In group 2, HDL-cholesterol increased from 0.71 ± 0.17 to an upper limit of 1.00 ± 0.21 mM at week 6. In group 3, HDL-cholesterol increased within 1 week from 0.70 ± 0.15 to 1.05 ± 0.12 mM and to a maximum of 1.20 ± 0.16 mM after week 20, with a net increase of 0.35 mM at week 10. In group 4, at week 20 HDL-cholesterol increased from 0.70 ± 0.16 to 1.15 ± 0.13 mM, with a net increase of 0.45 mM with maximum levels of 1.17 ± 0.21 mM observed at week 14 (Table 1). Data are shown as mean ± SD.

3.2. LDL-cholesterol

There was only a slight nonsignificant increase in LDL-cholesterol in group 1. In group 2, LDL-cholesterol increased from 5.00 ± 0.17 to 75.00 ± 12 mM at week 20 with a maximum level of 76.00 ± 21 mM observed at week

Table 1. HDL-cholesterol of rabbits in control, saline, nimesulide, and celecoxib groups at weeks 0–20 of the experimental period.

Week	Control			Saline			Nimesulide			Celecoxib		
	M	SD	N	M	SD	N	M	SD	N	M	SD	N
0	0.70	0.19	12	0.71	0.17	12	0.70	0.15	12	0.70	0.16	12
2	0.80	0.19	12	0.67	0.23	12	0.78	0.18	12	0.72	0.12	12
4	0.87	0.26	12	0.98	0.25	12	0.76	0.26	11	0.88	0.11	11
6	0.67	0.23	12	1.00	0.21	12	0.88	0.20	11	0.96	0.14	11
8	0.65	0.25	11	0.98	0.19	11	0.98	0.19	11	1.06	0.15	11
10	0.74	0.21	11	0.94	0.26	11	1.20*	0.16	10	1.15	0.21	10
12	0.67	0.25	11	0.85	0.23	11	1.15*	0.12	10	1.14*	0.23	10
14	0.62	0.21	10	0.86	0.25	11	1.00	0.10	10	1.17*	0.21	10
16	0.78	0.19	10	0.89	0.21	11	1.12*	0.14	10	1.15*	0.17	10
18	0.66	0.26	10	0.87	0.19	10	1.10*	0.10	10	1.14*	0.15	10
20	0.67	0.23	10	0.89	0.26	10	1.05	0.12	10	1.15*	0.13	10

M: HDL-cholesterol in mM, SD: standard deviation, N: number of rabbits, *: $P < 0.05$ compared to saline group.

14. In group 3, LDL-cholesterol increased from 4.90 ± 0.16 at week 0 to 42 ± 16 at week 20 with a maximum of 43 ± 14 mM at week 8. In group 4, LDL-cholesterol increased from 4.9 ± 0.16 to a maximum of 49 ± 14 mM at week 20. In group 2, a net increase of 70.00 mM was observed from the baseline value while in groups 3 and 4 this increase in LDL-cholesterol was significantly lower. A net increase in LDL-cholesterol of 37.00 and 44.00 mM was observed in groups 3 and 4, respectively (Table 2). Data are shown as mean ± SD.

Table 2. LDL-cholesterol of rabbits in control, saline, nimesulide, and celecoxib groups at weeks 0–20 of the experimental period.

Week	Control			Saline			Nimesulide			Celecoxib		
	M	SD	N	M	SD	N	M	SD	N	M	SD	N
0	5.00	0.19	12	5.00	0.17	12	4.90	0.16	12	4.90	0.16	12
2	7.00	0.35	12	40.00	12.00	12	36.00	10.00	12	28.00*	9.00	12
4	5.00	0.62	12	55.00	17.00	12	45.00	15.00	12	34.00*	12.00	12
6	4.00	0.34	12	66.00	23.00	12	44.00*	18.00	11	35.00*	11.00	11
8	6.00	0.38	11	70.00	22.00	11	43.00*	14.00	11	39.00*	13.00	11
10	8.00	0.35	11	72.00	19.00	11	42.00*	18.00	11	43.00*	14.00	11
12	6.00	0.62	11	72.00	20.00	11	45.00*	13.00	10	47.00*	11.00	10
14	5.00	0.34	10	76.00	21.00	11	43.00*	16.00	10	48.00*	13.00	10
16	7.00	0.38	10	75.00	21.00	11	40.00*	14.00	10	48.00*	16.00	10
18	5.00	0.55	10	73.00	20.00	10	41.00*	17.00	10	46.00*	15.00	10
20	5.00	0.35	10	75.00	12.00	10	42.00*	16.00	10	49.00*	14.00	10

M: LDL-cholesterol in mM, SD: standard deviation, N: number of rabbits, *: $P < 0.05$ compared to saline group.

3.3. Total cholesterol

In group 1, total serum cholesterol remained roughly the same throughout 20 weeks; however, a nonsignificant increase was noted in the middle of the experimental period. In group 2, total serum cholesterol increased from 5.00 ± 0.17 at week 0 to 76 ± 12 mM at week 20, with a maximum value of 77.1 ± 23 mM at week 6. In group 3, total cholesterol increased from 5.12 ± 0.13 at week 0 to 57 ± 12 mM at week 20, with a maximum level of 62 ± 12 mM observed at week 12. In group 4, total cholesterol increased from 5.17 ± 1.23 at week 0 to 67 ± 21 mM at week 20. A net increase of 71.00, 52.00, and 62.00 mM was observed in groups 2, 3, and 4, respectively (Table 3). Data are shown as mean ± SD.

3.4. Triglycerides

In group 1, there was no significant change in the triglyceride levels. In group 2, triglycerides increased from 1.10 ± 0.16 to 8.99 ± 2.67 at week 20, with a maximum elevation of 9.0 ± 2.13 mM observed at week 12. In group 3, triglycerides levels increased from 1.21 ± 0.23 at week 0 to 4.68 ± 1.47 at week 20, with a maximum of 5.34 ± 0.78 mM at week 12. In group 4, triglyceride levels increased from 1.21 ± 0.166 at week 0 to 5.46 ± 1.430 mM at week 20, with a maximum of 5.65 ± 1.670 mM at week 14. A net increase of 7.89, 3.50, and 4.26 mM was observed in groups 2, 3, and 4, respectively (Table 4). Data are shown as mean ± SD.

4. Discussion

This investigational work in hypercholesterolemic animals discloses a significant increase in serum cholesterol levels along with a comparable increase in serum triglycerides and LDL-cholesterol and a drop in serum HDL-cholesterol, which is in accord with previous studies (12). Both preventive and therapeutic effects were confirmed as lipid profile was improved in hypercholesterolemic rabbits after treatment with COX-2 inhibitors. Among the inhibitors of COX enzyme, aspirin is known to show hypolipidemic effects such as decreased levels of cholesterol and triglycerides during hypercholesterolemia, which are mediated primarily through the actions of aspirin on insulin secretion (13). Similarly, positive effects on myocardium during hypercholesterolemia have been reported with selective inhibition of COX enzymes (14). Peroxidative changes that take place during liver injury as well as hypercholesterolemia have responded positively with selective inhibition of COX-2 (15). ApoE-deficient mice show similar response to xanthohumol, a prenylated chalconoid with antiinflammatory activities (16).

Lowering the levels of LDL-cholesterol is one of the keystones in the prevention of cardiovascular disease and statins are the drug of choice to achieve the goal, but unfortunately it was reported that even at the highest dose of administration only limited additional lowering may be attained with increased incidence of side effects (17).

Table 3. Total cholesterol of rabbits in control, saline, nimesulide, and celecoxib groups at weeks 0–20 of the experimental period.

Week	Control			Saline			Nimesulide			Celecoxib		
	M	SD	N	M	SD	N	M	SD	N	M	SD	N
0	5.00	0.19	12	5.21	0.26	12	5.12	0.13	12	5.17	1.23	12
2	7.00	0.35	12	50.0*	12.00	12	33.00	10.00	12	31.00	11.00	12
4	5.00	0.62	12	55.0*	17.00	12	35.00	10.00	12	37.00	13.00	11
6	4.00	0.34	12	77.0*	23.00	12	37.00	15.00	11	48.00	16.00	11
8	6.00	0.38	11	75.0*	22.00	11	45.00	13.00	11	52.00	17.00	11
10	8.00	0.35	11	72.00	19.00	11	55.00	16.00	11	60.00	19.00	10
12	6.00	0.62	11	76.00	31.00	11	62.00	12.00	10	62.00	18.00	10
14	5.00	0.34	10	73.00	21.00	11	62.00	14.00	10	64.00	21.00	10
16	7.00	0.38	10	75.00	21.00	11	56.00	10.00	10	65.00	18.00	10
18	5.00	0.55	10	74.00	21.00	10	58.00	11.00	10	65.00	19.00	10
20	5.00	0.35	10	76.00	12.00	10	57.00	12.00	10	67.00	21.00	10

M: Total cholesterol in mM, SD: standard deviation, N: number of rabbits, *: $P < 0.05$ compared to nimesulide and celecoxib groups.

Table 4. Triglycerides of rabbits in control, saline, nimesulide, and celecoxib groups at weeks 0–20 of the experimental period.

Week	Control			Saline			Nimesulide			Celecoxib		
	M	SD	N	M	SD	N	M	SD	N	M	SD	N
0	1.08	0.980	12	1.10	0.166	12	1.21	0.230	12	1.21	0.166	12
2	1.10	0.570	12	2.50*	0.760	12	1.23	0.410	12	1.56	0.340	12
4	1.27	0.760	12	2.10*	0.790	12	1.25	0.240	12	1.42	0.560	11
6	1.09	0.650	12	2.90*	0.980	12	1.21	0.650	11	1.45	0.640	11
8	0.98	0.480	11	5.00	2.100	11	4.43	0.480	11	3.45	1.100	11
10	0.92	0.760	11	8.00*	2.400	11	4.56	0.980	11	4.45	1.240	10
12	1.18	0.570	11	9.00*	2.130	11	5.34	1.450	10	5.11	2.100	10
14	1.20	0.480	10	8.45*	2.340	11	4.89	1.670	10	5.65	1.670	10
16	1.06	0.760	10	8.78*	2.160	11	5.10	1.840	10	5.34	1.890	10
18	0.99	0.450	10	9.10*	3.100	10	5.12	1.560	10	5.36	1.660	10
20	0.97	0.760	10	8.99*	2.670	10	4.68	1.470	10	5.46	1.430	10

M: Triglycerides in mM, SD: standard deviation, N: number of rabbits, *: $P < 0.05$ compared to nimesulide and celecoxib groups.

Therefore, novel approaches of prescribing statins with other drugs that further reduce LDL-cholesterol levels are of interest. Our results showed that LDL-cholesterol level was reduced in hypercholesterolemic rabbits after treatment with nimesulide or celecoxib at recommended doses. Lowering LDL-cholesterol levels with various statins or the same statin at various doses is connected with a reduction in rates of cardiovascular events (18,19). Our studies showed that a net decrease of LDL-cholesterol with nimesulide and celecoxib pretreatment may help decrease the frequency and severity of cardiovascular events. A previous study in atherosclerotic animals reported a number of beneficial effects of ibuprofen, a nonselective inhibitor of COX, including antiatherosclerotic effects (20). Similarly, effects of other substances such as phytosterol also showed potential to reduce cholesterol levels in susceptible groups (21).

A major risk factor for adverse events related to coronary atherosclerosis is low-serum concentrations of HDL-cholesterol, which is prevalent in patients with acute coronary syndromes (22). Our results suggest that COX-2 inhibition by celecoxib or nimesulide is an effective therapy for the treatment of low HDL-cholesterol. Earlier investigative work indicates an increase of HDL-cholesterol (0.03 mM) with reduction (2% to 4%) in coronary heart disease (23). Our results show a net increase of 0.45 mM for cholesterol and 0.35 mM for HDL from week 0 to 20 after celecoxib and nimesulide pretreatment, respectively. The National Cholesterol Education Program proposes less than 40 mg/dL as low levels of HDL-cholesterol for a coronary risk factor for treatment of hyperlipidemia (24). The current study shows a significant increase in HDL-cholesterol after pretreatment with celecoxib and nimesulide, and these may increase the low levels of HDL-cholesterol that are connected with dyslipidemia. Patients treated with antiinflammatory medicinal plants like garlic and turmeric have HDL elevation and a reduction in LDL levels (25). Protection afforded by 17-β-estradiol during hypercholesterolemia is also mediated through selective inhibition of the COX-2 enzyme (26).

Statins are highly effective in lowering serum cholesterol concentrations and preventing ischemic heart disease (27,28). Atorvastatin decreases plasma LDL-cholesterol and decreases triglyceride levels in ApoE/LDL receptor-double-knockout mice fed an atherosclerotic diet (29). Similarly, simvastatin decreases aortic cholesterol accumulation in ApoE-/- mice (30). Fluvastatin, the first fully synthetic statin, is a well-established drug for the treatment of hypercholesterolemia, primarily due to its marked lowering action of LDL-cholesterol (31). However, the protection afforded by statins is not complete and therefore a complementary drug that could enhance HDL levels is required. Our investigation indicates that COX-2 inhibitors may be used as adjunct hypolipidemics with more established statins.

The available clinical data on COX-2 inhibitors pertaining to cardiovascular endpoints have been summarized (32). These data suggest the risk of cardiovascular events associated with the use of NSAIDs, except for naproxen. High doses of NSAIDs are associated

with increased risk of acute myocardial infarction (32) and, although short-term treatment with the COX-2 specific inhibitor rofecoxib does not impair endothelium-dependent or -independent vascular function in healthy volunteers (33), in patients with coronary artery disease, cardiovascular adverse effects are observed both at low and high doses (32). Although nimesulide and celecoxib are relatively safer drugs at the doses used in this study, we are cautious in generalizing our findings to other COX-2 inhibitors.

The results of our study also suggest that celecoxib and nimesulide may be used in combination with atorvastatin or other drugs. In this way, both nimesulide and celecoxib will increase levels of HDL-cholesterol as well decrease levels of LDL-cholesterol. This approach of treating dyslipidemia is quite novel and fruitful instead of using high dose of statins with increased probability of adverse effects, especially in the elderly. Further studies are required to establish the hypolipidemic mechanism of COX-2 inhibitors in hypercholesterolemia.

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