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Effects of Vitamin D Combined with Aspirin or Atorvastatin on Plasma Lipid Profiles and Lipid Peroxidation in Triton-X Induced Hyperlipidemia in Rats

Aduragbenro Deborah Adedapo* and Olumuyiwa Ope Ogunfowora

Department of Pharmacology, University of Ibadan, Oyo State, Nigeria

***Corresponding author:** Adedapo AD

debyee1965@yahoo.co.uk

Department of Pharmacology, University of Ibadan, Oyo State, Nigeria.

Tel: +2348033635204

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Abstract

Vitamin D deficiency as an independent cardiovascular risk factor has been associated with increased risks of cardiovascular events. The present study assessed the modulatory effects of post-treatment of vitamin D alone and with aspirin or atorvastatin on triton-X-induced hyperlipidemia in rats. Forty-Nine Wistar rats were divided into seven experimental groups of seven per group. Group A, control negative group, received no treatment. Group B-G received triton (400 mg/kg) to induce hyperlipidemia. Groups C, D and E were post-treated with vitamin D only (200 IU/kg), aspirin only (1 mg/kg), atorvastatin only (10 mg/kg) respectively. Groups F and G were post-treated with vitamin D in combination with either aspirin or atorvastatin. Results obtained showed increased MDA (an indicator of lipid peroxidation) levels in group B animals [rats that received triton (400 mg/kg) only and not treated with any drug] by 77.4% and an elevation of low density lipoprotein (LDL) by 65.8% when compared with control negative group ($p<0.05$). Similarly, high density lipoprotein (HDL) deceased in this group of rats that received triton only ($p>0.05$). Vitamin D (200 IU/kg), aspirin (1 mg/kg) and atorvastatin (10 mg/kg) did not significantly ($p>0.05$) alter total cholesterol TC, TG, HDL, LDL and malondialdehyde (MDA) levels respectively when administered alone. However, Vitamin D plus aspirin or atorvastatin treated animals reduced triton-induced lipid profile and MDA, although not statistically significant ($p>0.05$). In conclusion, this present study suggested vitamin D possesses lipids and lipid peroxidation lowering activity. Thus, vitamin D supplementation could offer chemoprevention in this condition.

Keywords: Vitamin D; Triton; Hyperlipidemia; Lipid peroxidation

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Introduction

In the diversity of cardiovascular diseases, atherosclerosis and or hypertension are the most common [1]. Both physiological and morphological changes that alter cardiovascular function lead to increased risk of cardiovascular disease, even in healthy asymptomatic individuals [2]. Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis, begin in early life, making primary prevention efforts necessary from childhood [3]. There is also increased emphasis on preventing atherosclerosis from occurring or preventing the progress of atherosclerosis, by modifying risk factors, for example by healthy eating, exercise, and avoidance of smoking tobacco [4]. WHO in 2011 stated that cardiovascular diseases are the number one cause of death globally? An estimated 17.3 million people died from cardiovascular diseases in 2008, representing 30% of all global deaths as reported by WHO in 2011. Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million were due to stroke as reported by WHO in 2011 [5]. The number of people, who die from cardiovascular diseases (CVD), mainly from heart disease and stroke, will increase to reach 23.3 million by 2030 [6]. Vitamin D is classically known for its role in bone metabolism by modulating calcium homeostasis and ensuring physiologic

calcium absorption by the gut [7,8]. It was recently reported that Vitamin D deficiency, CVD and related risk factors are highly prevalent worldwide and frequently co-occur [9]. This has led to extensive research on vitamin D as a potential influencing factor in the pathogenesis of several chronic non-skeletal diseases including infectious or autoimmune diseases as well as cancer or CVD [10]. Animal studies have revealed that the biologically active metabolite of vitamin D-1,25-dihydroxy-vitamin D (1,25[OH] 2D) can modulate various processes involved in the pathogenesis of CVD [9]. Also, numerous observational studies, prospective meta-analyses, as well as some interventional studies have addressed the possible linkage of vitamin D deficiency and the development of CVD and its risk factors [11]. While it seems plausible that vitamin D deficiency can be considered a surrogate marker for poorer health status, most notably observed in patients with chronic diseases, including cardiovascular risk factors and CVD, it remains to be proven whether vitamin D itself can directly impact on cardiovascular outcomes [12]. Presently, there is paucity of data on the effect of vitamin D supplementation on the lipid profile to elucidate the mechanisms between vitamin D and lipids [13]. Although, evidence abounds of potential benefits of vitamin D, but, its therapeutic roles in specific heart related conditions is unknown [14]. The solution of Triton X-100 has successfully been used to induce hyperlipidemia in rats in previous studies, and it was chosen as the hyperlipidemic model due to its convenience, reproducibility and availability [15]. The present study investigated the anti-atherogenic effects of vitamin D alone and in combination with atorvastatin or low dose aspirin in rats to ascertain if vitamin D supplementation can significantly alter atherogenic process as well as the probable mechanisms that may be involved.

Materials and Methods

Drugs and chemicals

Atorvastatin 10 mg (Lipitor®) produced by Pfizer was purchased from CCD Pharmacy in Ogudu, Lagos state Nigeria. Dispersible aspirin (Acetylsalicylic acid) 75 mg tablets was purchased from M&A Pharmchem Ltd. and Vitamin D3 (Cholecalciferol) 400 IU tablet was obtained from Nature Made. Triton-X-100, iso-octylpolyoxy ethylene phenol formaldehyde polymer was purchased from Sigma-Aldrich chemical Co. (USA). Total cholesterol (TC), Triglyceride (TG) assay kits were obtained from Randox Laboratory (Crumlin, UK). All other chemicals and reagents used were of analytical grade.

Animals

Forty-nine Wister Albino adult rats (of either sex) with average weight of about 200 g were obtained from the Animal House Unit of the Faculty of Basic Medical Science, University of Ibadan. The animals were housed under standard laboratory condition in plastic cages at 25°C (light and dark cycles were maintained). The animals were left for an initial adaptation period of 7 days before any experimental adaptation and supplied with commercially available standard pellet diet and water ad libitum. Animal experiments were conducted strictly according to ethical guidelines for use of animals in scientific research as stated in International Guiding Principles for Biomedical Research Involving Animals [16].

Experimental design

Forty-nine albino rats weighing between 180 g and 220 g of both sexes were used for the work. They were randomly distributed into seven groups of 7 rats per group. Group A, control negative group received distilled water. Group B, control triton group was administered 400 mg/kg triton-X orally (p.o) for 30 days to make them hyperlipidemic. Group C, Vitamin D only, were administered 400 mg/kg triton-X p.o. for 30 days and then treated with Vitamin D (200 IU/kg) for 15 days. Group D, Aspirin only, received 400 mg/kg triton-X p.o. for 30 days plus aspirin (1 mg/kg) for 15 days. Group E, Atorvastatin only, was administered 400 mg/kg triton-X p.o. for 30 days plus atorvastatin (10 mg/kg) for 15 days. Group F (Vitamin D and Aspirin) received 400 mg/kg triton-X p.o. for 30 days plus vitamin D (200 IU/kg) and aspirin (1 mg/kg) for 15 days. Group G (Vitamin D with Atorvastatin) was given 400 mg/kg triton-X p.o. for 30 days plus treated with vitamin D (200 IU/kg) and atorvastatin (10 mg/kg) for 15 days.

Collection of blood samples for assays

The animals were fasted for 24 h. after the last day of treatment, after which blood sample was collected via retro-orbital sinus into heparinized bottle before the animals were sacrificed and animals were sacrificed by cervical dislocation. The anticoagulant contained blood samples were centrifuged at 4200 rpm for 5 minutes to obtain the clear supernatant (i.e., serum) from which all biochemical assays were carried out.

Biochemical assays

Serum total cholesterol (TC) and Triglyceride (TG) concentration were estimated following the principle described by Trinder using commercial kits obtained from Randox Laboratories Ltd. (Crumlin, UK) [17]. High Density Lipoprotein (HDL) was estimated according to Warnick and Albers [18] while serum low density lipoprotein (LDL) was calculated using Freidewald formula [19]. Atherogenic index was also determined using the formula described by Haglund et al. [20]. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale [21].

Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Significant differences among the group were determined by one-way analysis of variance (ANOVA) and student T-test using

the statistical analysis programmed for social sciences (SPSS). Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) as reported by Levin in 1991. Results were significant at $p \leq 0.05$.

Echocardiography and genetic studies were requested to be done in central hospitals of Kathmandu and standard international lab center SRL. Variables were predesigned according to WHO Performa where gender, weight, gestational age, mode of delivery, consanguinity, maternal age, antenatal visit record and family history were recorded. Post-mortem investigation of neonates was not done due to religious and social factors. Oral consent from the parents for the involvement in the study was taken. The results then were analyzed using Excel 2016.

Results

In **Table 1** there was an elevation of total cholesterol (TC) and Triglyceride (TG) in triton (400 mg/kg) treated rats when compared with control distilled water group, this was however, not statistically significant. Vitamin D (200 IU/kg), aspirin (1 mg/kg) and atorvastatin (10 mg/kg) did not significantly ($p>0.05$) reduce TC and TG levels when administered alone. Also, animals post-treated with Vitamin D plus aspirin (1 mg/kg) or atorvastatin (10 mg/kg) co-administration (200 IU/kg) for 15 days after triton-induced hyperlipidemia showed decreased levels of TC and TG though not statistically significant ($p>0.05$) when compared with triton group, no difference between aspirin or atorvastatin plus Vitamin D treated animals and vitamin D group as observed in this study.

In **Table 2** there was a decrease of high density lipoprotein (HDL) in triton (400 mg/kg) treated rats by 7.6% when compared with control distilled water group ($p>0.05$). Vitamin D (200 IU/kg), aspirin (1 mg/kg) and atorvastatin (10 mg/kg) when administered alone showed elevated HDL levels when compared with triton (400 mg/kg) group, this is however not significant ($p>0.05$). Though not significant ($p>0.05$), there was an increase in HDL level for aspirin or atorvastatin plus Vitamin D treated animals when compared with triton only treated group. Triton-induced rats showed significant ($p<0.05$) elevation of low density lipoprotein (LDL) when compared with control distilled water group. Aspirin, Vitamin D or atorvastatin animals when administered alone did not significantly alter LDL levels. Also, Vitamin D plus aspirin or atorvastatin co-administration decreased LDL ($p>0.05$) levels by 33% and 28% respectively when compared with triton treated rats.

Table 3 showed a significant increase ($p<0.05$) of lipid peroxidation (Malondialdehyde, MDA) in triton (400 mg/kg) treated rats by 77.4% when compared with control distilled water group. Although, Vitamin D (200 IU/kg), aspirin (1 mg/kg) and atorvastatin (10 mg/kg) did not alter MDA levels when administered alone on comparison with control distilled water group, the drugs decreased ($P>0.05$) MDA when compared with triton treated group. Also, aspirin or atorvastatin plus Vitamin D treated animals reduced ($p>0.05$) triton-induced MDA 18.2% and 12.7% respectively. Atherogenic index was reduced across all treatment groups, though there was only a significant ($p<0.05$) reduction in the aspirin plus Vitamin D group.

Table 1 Effect of Vitamin D and Aspirin on serum total cholesterol (TC) and triglycerides (TG) levels in normal and triton-treated rats.

Group	Group Treatments	TC	TG
A	Control (DW, 10 ml/kg)	51.0 ± 5.2	31.8 ± 5.8
B	Triton (400 mg/kg)	59.5 ± 11.9 (-16.7)[a]	41.0 ± 16.8 (-28.9)[a]
C	Vitamin D (200 IU/kg)	54.0 ± 8.3 (-5.9)[a]	23.8 ± 4.3 (25.2)[a]
D	Aspirin (1 mg/kg)	51.3 ± 2.7 (-0.59)[a]	31.7 ± 7.7 (0.3)[a]
E	Atorvastatin Vitamin D (200 IU/kg)	54.0 ± 8.0 (-5.9)[a]	34.5 ± 8.5 (-8.5)[a]
F	+ Aspirin (1 mg/kg)	57.8 ± 9.3 (2.9)[b] (-7.0)[c] (-13.3)[a]	21.8 ± 5.6 (46.8)[b] (8.4)[c] (31.4)[a]
G	+ Atorvastatin (10 mg/kg)	55.0 ± 13.1 (7.6)b (-1.9)c (-7.4)a	32.8 ± 8.9 (20.0)[c] (-22.0)[c] (-3.1)[a]

Result expressed as Mean ± SEM. Values in parenthesis represent % change; (-)=increase; (+)=decrease, (a) % change relative to control distilled water (DW) group, (b) % change relative to control triton group, (c) % change relative to vitamin D only group. *p<0.05 when compared with control distilled water group.TC= Total cholesterol, TG=Triglycerides.

Table 2 Effect of Vitamin D and Aspirin on serum high density lipoprotein (HDL) and low-density Lipoprotein (LDL) levels in normal and triton-treated rats.

Group	Group Treatments	HDL	LDL
A	Control (DW, 10 ml/kg)	26.6 ± 6.1	19.6 ± 4.0
B	Triton (400 mg/kg)	19.0 ± 2.0 (7.6)[a]	32.5 ± 9.7* (-65.8)[a]
C	Vitamin D (200 IU/kg)	27.0 ± 6.8 (-1.5)[a]	25.0 ± 3.6 (-27.6)[a]
D	Aspirin (1 mg/kg)	23.7 ± 4.1 (10.9)[a]	21.3 ± 3.8 (-8.7)[a]
E	Atorvastatin Vitamin D (200 IU/kg)	25.5 ± 9.5 (4.1)[a]	21.5 ± 0.5 (-9.7)[a]
F	+ Aspirin (1 mg/kg)	32.0 ± 8.0 (-68.4)[b] (-18.5)[c] (-20.3)[a]	21.5 ± 2.6 (33.8)[b] (14.0)[c] (-9.7)[a]
G	+ Atorvastatin (10 mg)	25.3 ± 6.7 (-33.2)[b] (6.3)[c] (4.9)[a]	23.3 ± 5.3 (28.0)[b] (6.8)[c] (-18.9)[a]

Result expressed as Mean ± SEM. Values in parenthesis represent % change; (-)=Increase; (+)=Decrease, (a) % change relative to control distilled water (DW) group, (b) % change relative to control triton group, (c) % change relative to vitamin D only group. *p<0.05 when compared with control distilled water group. LDL=Low Density Lipoprotein; HDL=High density lipoprotein.

Table 3 Effect of Vitamin D and Aspirin on serum Atherogenic Index (AI) and Malondialdehyde levels in normal and triton-treated rats.

Group	Group Treatments	Atherogenic Index	Malondialdehyde
A	Control (DW, 10 ml/kg)	4.0 ± 1.0	3.1 ± 0.5
B	Triton (400 mg/kg)	6.4 ± 2.2 (-60.0)[a]	5.5 ± 0.6[*] (-77.4)[a]
C	Vitamin D (200 IU/kg)	3.5 ± 0.9 (12.5)[a]	3.4 ± 0.6 (-9.7)[a]
D	Aspirin (1 mg/kg)	4.4 ± 1.7 (-10.0)[a]	3.0 ± 0.2 (3.2)[a]
E	Atorvastatin Vitamin D (200 IU/kg)	4.7 ± 2.5 (-17.5)[a]	3.5 ± 0.7 (-12.9)[a]
F	+ Aspirin (1 mg/kg)	2.4 ± 0.6[**] (62.5)[b] (22.9)[c] (40.0)[a]	4.5 ± 0.8 (18.2)[b] (-32.4)[c] (-45.2)[a]
G	+ Atorvastatin (10 mg/kg)	3.9 ± 0.6 (39.1)[b] (-11.4)[c] (2.5)[a]	4.8 ± 0.6 (12.7)b (-41.2)[c] (-54.8)[a]

Result expressed as Mean ± SEM. Values in parenthesis represent % change; (-)=increase; (+)=decrease, (a) % change relative to control distilled water (DW) group, (b) % change relative to control triton group, (c) % change relative to vitamin D only group. *p<0.05 when compared with control distilled water group. **P<0.05 when compared with the control triton group. AI=Atherogenic Index; MDA=Malondialdehyde.

Discussion

Vitamin D deficiency has emerged as a potential risk factor for cardiovascular diseases [22-24]. We investigated on the anti-atherogenic effects of vitamin D alone and in combination with atorvastatin or low dose- aspirin in rats. Hyperlipidemia was achieved through administration of the solution of Triton X-100 as previously reported by Ghule et al. [15]. In epidemiological studies of the general population, lower vitamin D levels have been associated with cardiovascular diseases, hypertension, and diabetes. High density lipoprotein cholesterol (HDL) and low-density lipoprotein (LDL) cholesterol are surrogate markers of cardiovascular risk such as coronary artery calcification and carotid intima medial thickness [24,25]. From the result obtained, there were increased plasma lipid profiles and lipid peroxidation. This indicates the suitability of Triton X-100 to induce hyperlipidemia with increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism [26]. In this study, Vitamin D was selected to screen for its anti-hyperlipidemic activity in Triton X-100 (100 mg/kg) induced hyperlipidemic rats in the presence of aspirin and atorvastatin as standards during a post-treatment. There is a large body of research from clinical studies in humans indicating that low levels of serum 25-hydroxy vitamin D are associated with atherosclerosis [27-31]. There are however contrasting reports showing evidence that vitamin supplementation has no effect on vascular disease mortality or all-cause mortality [32]. From the result obtained, total cholesterol and triglyceride increased in triton (400 mg/kg) treated rats **(Table 1)**, although, insignificantly. Animals post-treated with aspirin and atorvastatin plus Vitamin D co-administration following triton-induced hyperlipidemia showed decreased levels of TC and TG. Also, high density lipoprotein (HDL) decreased insignificantly in triton (400 mg/kg) treated rats **(Table 2)**. Vitamin D administration and co-administration with aspirin and atorvastatin increased HDL, though not significantly when compared with hyperlipidemic triton treated rats. Triton-induced rats showed significant elevation of low density lipoprotein (LDL) when compared with control distilled water group. Atorvastatin plus Vitamin D co-administration decreased LDL when compared with hyperlipidemic triton treated rats. Triton-induced hyperlipidemia presented significantly elevated MDA level, indicating the occurrence of lipid peroxidation and an increase in atherogenic index was seen. Vitamin D, Aspirin and Atorvastatin at the doses used in this present study did not alter Malondialdehyde (MDA) levels when administered alone. However, aspirin or atorvastatin plus Vitamin D insignificantly depleted elevated MDA levels in treated animals. Atherogenic index was reduced across all treatment groups. Co-administration of atorvastatin and Vitamin D significantly reduced atherogenic index. It has been demonstrated that 1α, 25-dihydroxy vitamin D3 reduced macrophage adhesion and migration as well as foam cell formation in monocytes isolated from type 2 diabetic patients [33]. This was evident with the aid of a mouse model of vitamin D deficiency facilitated atherosclerosis [34]. Further evidence showed that calcitriol treatment on atherosclerosis development was achieved from an oral calcitriol treatment which decreased the production of pro-inflammatory chemokines and reduced amount of inflammatory effector cells in atherosclerotic plaques [35]. Evidence of similar link between vitamin D, T cell modulation, and atherosclerosis has also been published in humans [36].

Conclusion

Thus, our present study further strengthens the anti-atherogenic effects of vitamin D alone and in combination with atorvastatin or low dose- aspirin in rats, although, the probable mechanisms that may be involved still require further investigation.

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