# Treatment of hyperlipoproteinemia with low density lipoprotein antibodies in rats

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## ABSTRACT

**الهدف** : تهدف هذه الدراسة لاختبار تأثير الحقن بواسطة الأجسام المضادة للبروتين الدهني منخفض الكثافة على الجرذان المستحثة بتصلب الشرايين من خلال تناولها لوجبة بحثية .

**الطريقة** : تمت معالجة الجرذان التي قسمت إلى أربع مجموعات ( عدد الجرذان في كل مجموعة يتراوح ما بين ٨ إلى ١٤ ) لمدة ٨ أسابيع في الفترة من ( ١٣ / ١ / ٢٠٠٧ – ٩ / ٣ / ٢٠٠٧ ) و ذلك بوحدة حيوانات التجارب ،كلية الصيدلة ، جامعة الملك سعود المجموعات على النحو التالي : مجموعة ضابطة تناولت الوجبة المعملية القياسية ، مجموعة ضابطة تناولت الوجبة المعملية القياسية + الحقن بواسطة الأجسام المضادة للبروتين الدهني منخفض الكثافة، مجموعة تناولت الوجبة البحثية المستحثة لتصلب الشرايين،مجموعة تناولت الوجبة البحثية المستحثة لتصلب الشرايين + الحقن بواسطة الأجسام المضادة للبروتين الدهني منخفض الكثافة . تم قياس مستويات الدهون و عوامل أخرى في مصل دم الجرذان .

**النتائج** : انخفضت مستويات الكوليسترول الكلي في الجرذان المعالجة بواسطة الأجسام المضادة للبروتين الدهني منخفض الكثافة ( n=9 ) انخفاضاً معنوياً ( P<0.001 ) من 5.72 ± 0.21 mmol/l إلى 1.81± 0.08 mmole/l و كذلك كان الانخفاض معنوياً في مستويات كوليسترول ، البروتين الدهني منخفض الكثافة لنفس المجموعة ( P< 0.001 ) حيث كانت نسبة الانخفاض ٧٣٪ من 3.06± 0.09 mmole/l إلى 0.84± 0.04 mmole/l. أيضاً الدهون الفسفورية كان انخفاض مستوياتها في نفس المجموعة معنوياً ( P< 0.01 ) لم يلاحظ وجود اختلاف معنوي لمستويات كل من كوليسترول البروتين الدهني عالي الكثافة و الجلسريدات الثلاثية عند مقارنة مجموعات الإختبار مع المجموعات الضابطة .

**خاتمة** : تشير نتائج هذه الدراسة أن معالجة الجرذان المستحثة بتصلب الشرايين بواسطة الحقن بالأجسام المضادة للبروتين الدهني منخفض الكثافة له تأثير معنوي على مستويات الكولسترول الكلي و كذلك كوليسترول البروتين الدهني منخفض الكثافة في مصل الدم . كما تقترح الدراسة أن الحقن بالأجسام المضادة للبروتين الدهني منخفض الكثافة له تأثير واقي على الجرذان المستحثة بتصلب الشرايين .

**Objective:** To examine whether the vaccination with low density lipoprotein (LDL) antibodies could have a protective effect on rats developed atherosclerosis through the action of atherogenic diet.

**Methods:** Four groups of rats (ranged 8 and 14) were treated for 8 weeks (13 January 2007 to 9 March 2007) at the animal house, Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia, with normal control diet (NC), normal control diet+LDL-Ab (NC +I), atherogenic diet (AT), and atherogenic diet + LDL-Ab (AT+I). Lipid concentrations and other different parameters were measured in serum.

**Results:** The concentrations of total cholesterol in atherogenic rats treated with LDL-Ab (AT+I) (n=9) were significantly decreased from 5.72 ± 0.21mmol/l to 1.81 ± 0.08 mmol/l ($p$<0.001). Low density lipoprotein-cholesterol concentrations were also significantly decreased in AT+I group ($p$<0.001), as it dropped by 73% from 3.06 ± 0.09 mmol/l to 0.84 ± 0.04 mmol/l. Phospholipids concentrations were also decreased in AT+I group ($p$<0.01). The concentrations of both HDL-cholesterol and triacylglycerol were not significantly different from their matched groups.

**Conclusion:** Results of the present study showed that treatment of atherogenic rats with LDL-Ab significantly decreased the serum concentrations of TC and LDL-C. Our findings suggest that immunization with LDL-Ab has a protective effect on artherogenic rats.


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It is well accepted that plasma low density lipoprotein (LDL) plays a principal role in the process of atherogenesis, as the higher concentrations of LDL are correlated with the risk of the disease.[1] Several studies have reported that lowering LDL-cholesterol reduces the risk of coronary heart disease (CHD) and decreases CHD morbidity and mortality.[2,3] Many different new therapies have been investigated for the treatment of atherosclerosis and hyperlipidemia and they have showed good chances of success. Among these therapies is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), which are effectively reduce LDL and prevent atherosclerosis.[3,4] Although, statins are effective LDL lowering agent, however, they may not be the standard therapy for all dyslipidemic patients. This is particularly true for those patients whose their primary lipid abnormality is due to a low level of HDL-cholesterol with or without hypertriglycridemia, this group of patients represent almost one half of patients with CHD.[5] One of the most recent strategies for disease management is vaccination against atherosclerosis. It was reported that immunization of rabbits with a peptide containing a region of cholesteryl ester transfer protein (CETP) changed lipoprotein concentrations.[6] Nilson et al[7] reported that hypercholesterolemic rabbits treated with homologous oxidized LDL have a significant reduction of neo-intimal area after balloon injury despite severe hypercholesterolemia. Apolipoprotein E knock-out mice that had been immunized with either homologous plaque homogenates or homologous malondialdehyde (MAD)-LDL showed reduction of lesion development.[8] Palinski et al[9] demonstrated that immunization of LDL receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces the formation of atherosclerotic plaques. A recent study carried out by Caligiuri et al[10] concluded that immunization of apolipoprotein E knock-out mice with phosphorylcholine showed a protection against atherosclerosis. Although several strategies have been applied for the treatment of atherosclerosis, however, little is known about vaccination strategy against the disease. The present study explored whether the vaccination with LDL antibodies could have a protective effect on rats developed atherosclerosis through the action of atherogenic diet.

**Methods.** Atherogenic diet (for atherogenesis induction) was purchased from ICN pharmaceuticals (Costa Mesa,CA-USA). Low-density lipoprotein (LDL) antibodies, (LDL-Ab)(for LDL vaccination) were obtained from Sigma (Poole, UK). Kits for the assay of total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG), phospholipids (PL), and glucose were obtained from BioSystems (Costa Brava,Barcelona, Spain). This study was approved by the Institutional Research and Ethical Committee. Male Wistar rats (110-120 g) were obtained from the animal house, Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Rats were divided into 4 groups. Group 1 consisted of rats that were kept on standard laboratory diet. This group was used as normal controls (NC). Group 2 consisted of normal rats that injected with LDL-antibodies (NC+I). This group was used to monitor the effect of injection with LDL-antibodies on group 1. Group 3 consisted of rats that were kept on atherogenic diet (AT). This group was used as atherogenic controls. Group 4 consisted of rats that being kept on atherogenic diet and injected with LDL-antibodies (AT+I ). This group was used to monitor the effect of injection with LDL-antibodies on group 3. All diets were given for 8 weeks (13 January 2007 to 9 March 2007) with free access to water. Body weight and food intake of controls and LDL-antibodies treated rats were recorded daily. At the end of the period, aliquots of blood samples from all groups were collected in plain tubes. Serum samples were separated from cells by centrifugation at 5000 g in a refrigerated centrifuge. Low density lipoprotein in samples was precipitated and solubilized using reagents supplied with LDL-cholesterol kit. Invitro cross-reactivity of the solubilized pellets of LDL with LDL-Ab was examined after a series of serial dilutions of the pure anti-human lipoprotein (LDL Ab). Dilutions were 1:8, 1:16, 1:32, 1:64, 1:128, 1:256. Rats of groups 2 and 4 were being vaccinated with the determined dilution of LDL-Ab (1:16). Both groups were being injected interpretonialy with 0.1ml of diluted LDL-Ab for 3 times a week. Total cholesterol, LDL-C, HDL-C, TG, and PL were estimated by standard enzymatic methods using BioSystems kits.[11-13] The concentrations of these parameters were measured in a Boehringer Mannheim spectrophotometer model 4020.

***Data analysis.*** The data presented in this investigation are expressed as means±standard error of the mean (SEM) and comparison between experimental and corresponding control rats was made by using student's t-tests. The probability value of $<0.05$ were considered significant.

**Results.** As shown in **Table 1** the body weights of atherogenic rats were significantly higher than those of matched controls. The increase in the last week of treatment was 48 g ($p<0.001$) for athrogenic rats and 34 g ($p<0.001$) for athrogenic rats treated with LDL-Ab. In contrast, food consumption by atherogenic rats

was significantly lower. The decrease in the last week of treatment was 17% ($p$<0.001) and it was 14% for atherogenic rats treated with LDL-Ab. **Table 2** shows the changes in serum lipid concentrations among NC, NC+I, AT, and AT+I rats. The concentrations of total cholesterol in atherogenic rats treated with LDL-Ab (AT+I) were significantly decreased ($p$<0.001). This decrease was from 5.72 ± 0.21mmol/l to 1.81± 0.08 mmole/l. Low density lipoprotein cholesterol concentrations were also significantly decreased in AT+I group ($p$<0.001), as it dropped by 73% from 3.06 ± 0.09 to 0.84 ± 0.04 mmol/L. Phospholipids concentrations were also decreased ($p$<0.01) after treatment of atherogenic rats with LDL-Ab. The concentrations of both high density lipoprotein cholesterol and triacylglycerol were not significantly different from their matched groups (**Table 2**). **Table 3** shows serum concentrations of some clinical parameters in the 4 groups. Serum glucose concentrations were significantly increased when NC and AT groups were treated with LDL-Ab ($p$<0.05, $p$<0.02) respectively. Serum alkaline phosphatase levels were markedly

**Table 1 -** General characteristics of control and atherogenic rats treated with low-density lipoprotein-antibodies (LDL-Ab).

| Characteristics | Standard laboratory diet only | Standard laboratory diet +LDL-Ab | Atherogenic diet only | Atherogenic diet+LDL-Ab |
|---|---|---|---|---|
| Initial body wt. (g) | 120 ± 2.4 (12) | 116 ± 2.6 (14) | 114 ± 2.5 (15) | 110 ± 2.2 (12) |
| Fial body wt. (g) | 391.8 ± 3.2 (12) | 404.1 ± 2.8 (14)* | 439.8 ± 2.7 (12)† | 425.6 ± 3.0 (11)† |
| Food intake (g/day) | 23.3 ± 0.77 (14) | 22.4 ± 0.64 (11) | 19.4 ± 0.75 (11)† | 20.1± 0.67 (12)† |

Data are expressed as mean±SEM, with number of rats given in parentheses. As compared with control rats, *$p$<0.01, †$p$<0.001 (student t test).

**Table 2 -** Serum concentrations of total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), phospholipids (PL) in control and atherogenic rats treated with low-density lipoprotein-antibodies (LDL-Ab).

| Parameters | Standard laboratory diet only | Standard laboratory diet + LDL-Ab | Atherogenic diet only | Atherogenic Diet+LDL-Ab |
|---|---|---|---|---|
| Total cholesterol (mmol/L) | 1.46 ± 0.08 (12) | 1.52 ± 0.07 (14) | 5.72 ± 0.21 (9) | 1.81 ± 0.08 (10)† |
| Triglycerides (mmol/L) | 1.02 ± 0.08 (12) | 1.29 ± 0.06 (14) | 0.75 ± 0.05 (9) | 0.93 ± 0.07 (9) |
| LDL -C (mmol/L) | 0.59 ± 0.02 (14) | 0.56 ± 0.02 (11) | 3.06 ± 0.09 (11) | 0.84 ± 0.04 (12)† |
| HDL -C (mmol/L) | 0.33 ± 0.01 (12) | 0.30 ± 0.01 (12) | 0.28 ± 0.01 (11) | 0.24 ± 0.01 (11) |
| Phospholipids (mmol/L) | 1.38 ± 0.04 (12) | 1.38 ± 0.07 (10) | 1.63 ± 0.08 (11) | 1.33 ± 0.06 (10)* |

Data are expressed as mean±SEM, with number of rats given in parentheses. As compared with matched groups, *$p$<0.01, †$p$<0.001 (student t test).

**Table 3 -** Serum concentrations of some clinical parameters in control, and atherogenic rats treated with low density lipoprotein-antibodies (LDL-Ab).

| Parameters | Standard laboratory diet only | Standard laboratory diet + LDL-Ab | Atherogenic diet only | Atherogenic diet+LDL-Ab |
|---|---|---|---|---|
| Glucose (mmol/L) | 7.96 ± 0.25 (11) | 7.07 ± 0.24 (13)* | 6.70 ± 0.16 (10) | 8.59 ± 0.29 (10)† |
| Total protein (g/L) | 60.75 ± 0.73 (12) | 64.02 ± 0.70 (9)‡ | 68.55 ± 1.15 (11) | 66.63 ± 1.13 (9) |
| Albumin (g/L) | 39.92 ± 0.42 (12) | 39.14 ± 0.68 (9) | 40.55 ± 0.73 (11 ) | 39.04 ± 0.78 (9) |
| Alkaline phosphatase (U/L) | 291.17 ± 7.55 (12) | 257.20 ± 6.70 (9)† | 334.32 ± 8.46 (11) | 292.07 ± 8.05 (8)† |
| Aspartate aminotransferase (U/L) | 110.5 ± 4.33 (11) | 112.43 ± 6.15 (9) | 126.25 ± 5.35 (10) | 109.53 ± 4.41(9) |
| Alanine aminotransferase (U/L) | 81.50 ± 2.98 (10) | 83.78 ± 2.38 (9) | 105.70 ± 4.01 (10) | 117.02 ± 3.45(9) |
| Urea (mmol/L) | 7.97 ± 0.24 (9 ) | 8.07 ± 0.29 (9) | 8.74 ± 0.17 (11) | 8.71 ± 0.23 (9) |
| Creatinine (µmol/L) | 25.92 ± 0.99 (10) | 27.11 ± 1.40 (9) | 31.18 ± 1.50 (11) | 27.25 ± 1.19 (9* |
| Uric acid (µmol/L ) | 45.33 ± 2.12 (11) | 43.78 ± 3.10 (9) | 57.06 ± 3.08 (11) | 57.14 ± 3.36 (8) |
| Calcium (mmol /L) | 2.66 ± 0.02 (12 ) | 2.73 ± 0.02 (9)† | 2.76 ± 0.06 (10) | 2.78 ± 0.02 (8) |
| Phosphorous (mmol/L) | 2.02 ± 0.05 (12 ) | 2.30 ± 0.06 (9)‡ | 2.32 ± 0.05 (11) | 2.03 ± 0.14 (9)* |

Data are expressed as mean±SEM, with number of rats given in parentheses.
As compared with mached group *$p$<0.05, †$p$<0.02, ‡p<0.002 (student t test).

decreased ($p$<0.02 ) by 13% after treating NC and AT with LDL-Ab. Serum creatinine concentrations were significantly decreased in atherogenic rats treated with LDL-Ab ($p$<0.05) whereas no significant change was detected in the same parameter when NC rats were treated with LDL-Ab. A significant increase ($p$<0.002) was observed in serum total protein concentrations when NC rats were treated with LDL-Ab ($p$<0.02) (**Table 3**). Both groups treated with LDL-Ab were showed a significant increase in their serum phosphorous concentrations, on the other hand, serum calcium concentrations were significantly increased when NC rats were treated with LDL-Ab.

**Discussion.** Results of the present study show that treatment of atherogenic rats with LDL-Ab significantly decreased the serum concentrations of TC by 69% and LDL-C by 73%. Although, many investigators reported that immunization with ox-LDL induces antibody formation (both IgG and IgM) and protects against atherosclerosis development;[14-16] however, from this view, our findings are consistent with the above mentioned findings. Also our findings are in a full agreement with Fredrikson et al[17] who reported that immunization with apo-ß peptide sequences in apo E-deficient mice, against which IgG and IgM titers are developed in man, resulted in a 60% decrease in atherosclerosis development and a concomitant increase in IgG antibodies. Serum glucose concentrations were significantly increased in atherogenic rats treated with LDL-Ab ($p$<0.02). This increase could be due to the action of LDL-Ab immunization on insulin as in immunization both humoral and cellular immune responses have been implicated.[18] The significant decrease in serum phospholipids concentrations with concomitant significant increase in serum phosphorous in the AT+I group, both are correlated to the significant decrease in TC concentrations in the same group. Account on the fact that most of the circulating cholesterol is found in esterified form (cholesterol esters), based on this view, our findings were coincided with Shaw et al[19] study.

In conclusion, the present study suggests that immunization with LDL-Ab has a protective effect on artherogenic rats. As the pathophysiological role of antibodies in atherosclerosis is still unclear, this work supports the hypothesis that the physiological role of antibodies is to trigger removal of the compounds from the circulation and possibly also from the arterial wall.[20] Although our objectives in the present investigation were targeted on the effect of LDL-Ab on serum lipid profile, however our future work will be focused on its effect on apo B100 protein expression to elucidate the immunological mechanism. The possibility of applying such immunization strategy to human is still controversial and difficult to answer at this early stage of the study.

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