Amiodarone decreases gene expression of low-density lipoprotein receptor at both the mRNA and the protein level

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Amiodarone Decreases Gene Expression of Low-Density Lipoprotein Receptor at Both the mRNA and the Protein Level

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Amiodarone, a potent antiarrhythmic drug, decreases plasma and tissue triiodothyronine (T3) and increases plasma cholesterol levels, resembling changes seen during hypothyroidism. The increase of serum cholesterol during amiodarone medication is associated with a decreased expression of the hepatic low-density lipoprotein (LDL) receptor mRNA. To further elucidate the mechanism of amiodarone-induced hypercholesterolemia, we investigated whether the decreased mRNA levels are the result of decreased transcription or increased degradation or both, and whether protein expression is decreased accordingly. Relative to pair-fed controls, amiodarone treatment increased plasma cholesterol by 69% and decreased expression of the mRNA encoding for the hepatic LDL receptor by 45%. To study this decrease in mRNA, we performed a run-on assay, from which it appears that amiodarone acts by decreasing LDL receptor mRNA expression 2.5-fold at the transcriptional level. The decay rate of liver LDL receptor mRNA, measured at different time points after injecting actinomycin D, was not different between amiodarone-treated and control animals (116 ± 32 minutes and 84 ± 10 minutes, \( P = .44 \)).

Hepatocytes in primary culture isolated from amiodarone-treated and control animals were used to determine specific binding of [125I]-LDL to hepatic LDL receptors. Amiodarone decreased specific LDL binding and Scatchard analysis demonstrated that amiodarone treatment reduced the number of LDL receptors by 69%, without affecting the dissociation constant (Kd). In conclusion, amiodarone-induced hypercholesterolemia can be explained by decreased transcription of the LDL receptor gene, resulting in lower mRNA and protein levels.

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A MIODARONE IS AN IODINATED benzofuran derivative that is used as an antiarrhythmic drug. It contains 39.4% iodine on weight basis, and pharmacological doses of iodine are released during the biotransformation of the drug. This may cause hyperthyroidism or hypothyroidism in susceptible subjects.1 Apart from these effects on the thyroid gland itself, amiodarone profoundly affects extrathyroidal metabolism of thyroid hormones. It inhibits the transport of thyroxine (T4) across the plasma membrane into hepatocytes. The subsequent decreased availability of the substrate T4 in the liver cells results in a diminished 5′-deiodination of T4, which results in a decreased production of triiodothyronine (T3).2 Amiodarone thus appears to induce a hypothyroid-like condition in extrathyroidal tissues. The antiarrhythmic effect of amiodarone is related to a lengthening of the cardiac action potential, quite similar to what is observed in hypothyroid patients. It has been hypothesized that the mechanism of action of amiodarone is partly due to the induction of a local hypothyroid-like condition by inhibition of the extrathyroidal conversion of T4 into T3.

Thyroid hormone deficiency results in hypercholesterolemia, and a dose-dependent increase of plasma cholesterol has indeed been observed in patients on long-term amiodarone treatment.1 The amiodarone-induced increase of plasma cholesterol is largely caused by an increase in low-density lipoprotein (LDL) cholesterol, and has been reproduced in rabbits4 and rats.5 We hypothesized that the increase of plasma cholesterol by amiodarone is related to the induction of a hypothyroid-like condition in the liver by the drug. The hepatic LDL receptor plays a key role in maintaining cholesterol homeostasis, with lower levels being associated with a higher plasma cholesterol concentration due to diminished uptake of LDL cholesterol, partly in the liver. We have recently demonstrated that amiodarone decreases the LDL receptor mRNA in the liver, whereas mRNA’s encoding for hepatic hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase and cholesterol 7a-hydroxylase (the rate-limiting enzymes in cholesterol synthesis and degradation, respectively) remained unchanged.6 The present studies were designed to answer the following questions: (1) Are the decreased LDL receptor mRNA’s caused by decreased gene transcription or by increased mRNA degradation? (2) Does amiodarone treatment also reduce the expression of the LDL receptor protein?

MATERIALS AND METHODS

Materials

Amiodarone was a kind gift of Sanofi B.V. (Maassluis, The Netherlands). Formaldehyde, formamide, β-mercaptoethanol, and guanidine isothiocyanate were from Merck, Darmstadt, Germany. Collagenase (type H); actinomycin D, rat-tail collagen, and β-actin riboprobe (digoxigenin-labeled) were purchased from Boehringer Mannheim Biochemicals (Mannheim, Germany), and 125I (carrier-free) in NaOH from Amersham (Buckinghamshire, UK). Ham’s F10, Hanks balanced salt solution (HBSS) and fetal calf serum (FCS) were obtained from Biowithacker (Brussels, Belgium). Fatty acid-free bovine serum albumin (BSA) was from Sigma (St Louis, MO). The cDNA probe for human LDL receptor (pLDLR3) was a generous gift from Dr D.W. Russell (Dallas, TX).7 All other reagents were of the highest grade possible.

Animals

Male Wistar rats (weight, 270 to 325 g) were housed under normal conditions with free access to tap water. All animal experiments were approved by the local animal welfare committee. Rats were divided into two groups. Group A was fed ad libitum on standard laboratory chow, and treated daily with 10 mg/kg body weight (BW) amiodarone by gastric tube for 2 weeks. Group B was treated daily with water by gastric tube, and served as pair-fed controls of group A.

Experiment I was designed to evaluate the stability of the mRNA encoding for the LDL receptor. Twenty-four hours after the last gastric
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RESULTS

Amiodarone treatment, as compared with pair-fed controls, resulted in a significant increase of plasma cholesterol and T₄ and a decrease of plasma T₃ (Table 1). Plasma TSH did not increase significantly.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>T₄ (nmol/L)</th>
<th>T₃ (nmol/L)</th>
<th>TSH (ng/dL)</th>
<th>Cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>60 ± 3</td>
<td>1.28 ± 0.06</td>
<td>1.04 ± 0.14</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>16</td>
<td>130 ± 5*</td>
<td>1.13 ± 0.047</td>
<td>2.93 ± 1.04</td>
<td>2.7 ± 0.1*</td>
</tr>
</tbody>
</table>

NOTE: Values are means ± SE. *P < .0001, vcontrols. **P < .01 vcontrols.
The rate of LDL receptor mRNA disappearance after administration of the transcription inhibitor actinomycin D was determined by Northern blot analysis, using β-actin as internal standard (Fig 1A). The hybridization patterns of three identical experiments were analyzed. The relative mRNA expression at time zero of the pair-fed controls was taken as one. The amount of LDL receptor mRNA (log values) was plotted against time (Fig 1B). Treatment with amiodarone reduced the level of LDL receptor mRNA by approximately 45% relative to pair-fed controls, as evident from time-zero levels in Fig 2. The mRNA half-lives (t1/2) were calculated assuming first-order kinetics. The t1/2 of LDL-R mRNA in controls was not different from that in amiodarone-treated rats (84 ± 10 v 116 ± 32 minutes, respectively; P = .44, n = 3). We also performed a run-on experiment on nuclei isolated from livers of control rats and those treated with amiodarone. As can be seen in Fig 2, the transcription from the LDL receptor gene is reduced 2.5-fold in the amiodarone-treated animals compared with controls.

Figure 3 shows the specific binding of LDL to isolated hepatocytes. The binding of LDL to LDL receptors increased with increasing amounts of added LDL. The binding of LDL to hepatocytes isolated from amiodarone-treated rats was approximately two times lower than the binding to control hepatocytes. Data obtained from this experiment were also evaluated by Scatchard analysis. A representative plot is depicted in the insert of Fig 3. The slope and the intercept of the fitted line indicate the dissociation constant (Kd) of the binding of LDL to LDL receptors and the MBC or total number of LDL receptors, respectively. The Kd was not different between amiodarone-treated animals and pair-fed controls (Table 2), but the number of LDL receptors was decreased by 69% (Table 2).

**DISCUSSION**

In agreement with our previous study, amiodarone treatment resulted in an increase of plasma T4 and a decrease of plasma T3, explained by inhibition of T4 5'-deiodination in the liver. The
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Fig 2. Effect of amiodarone on LDL receptor gene transcription. Dots represent the result of a run-on experiment performed on nuclei purified from livers of control or amiodarone-treated rats. β-actin was used as a control to normalize the LDL receptor expression data.

Table 2. Effects of Amiodarone on Liver LDL Receptors

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MBC (ng/mg protein)</th>
<th>Kd (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>64 ± 7.5</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>4</td>
<td>20 ± 8.8*</td>
<td>41 ± 21</td>
</tr>
</tbody>
</table>

NOTE. Values are means ± SE.
*P < .01 vs controls.

reduction in plasma T₃ in this study is comparable to that previously published. The plasma and tissue concentrations of rats treated with 10 mg/100 g BW closely resemble those in patients on long-term amiodarone treatment. Plasma cholesterol increased by 69%; this cannot be explained by the mild increase of TSH, because levels up to 7.6 ng/dL in experimentally induced hypothyroidism do not increase plasma cholesterol as observed previously. In a previous study, we demonstrated that the increase in plasma cholesterol during amiodarone treatment is due to an increase in both LDL and HDL cholesterol, whereas triglyceride levels remained unchanged. In line with our previous study, the liver mRNA encoding the LDL receptor was decreased by approximately 45% after 2 weeks of amiodarone treatment. We evaluated the degradation of the LDL receptor mRNA by injecting actinomycin D at various time intervals before the animals were killed. The dose used was twice the dose shown to decrease total RNA transcription by more than 80% in rat liver. The rate of LDL receptor mRNA degradation was not different between the amiodarone-treated animals and the pair-fed controls, as evident by the similar slope of the regression lines (Fig 2). We calculated the t½ of the LDL receptor mRNA as 84 minutes in control rats. This figure is comparable to that obtained by other investigators using human cells. The only other report in the literature on the t½ of the LDL receptor mRNA in relation to thyroid hormone status is that by Ness et al. They observed a t½ of approximately 30 minutes in young hypophysectomized rats, which did not change after treatment with T₃. The discrepancy with our results might be due to difference in age (adult v
young) or hormonal status (intact vs hypophysectomized rats). The latter seems more plausible, because rats easily become hypercholesterolemic after hypophysectomy, by which they lose their natural resistance to develop high cholesterol levels when the cholesterol content of the diet is increased.20 Because the stability of the LDL receptor mRNA is not affected, whereas its gene expression is, we conclude that the amiodarone-induced decrease in LDL receptor mRNA is caused by decreased transcription.

Our second set of experiments demonstrates that amiodarone also decreases the expression of the LDL receptor at the protein level; the number of LDL receptors in the liver of amiodarone-treated animals were on average 69% lower than that of pair-fed controls. This is evident from Scatchard analyses of the binding of LDL to isolated hepatocytes in primary culture. The experiments were performed at 4°C to prevent internalization of LDL receptors. Although the number of LDL receptors is in the same order of magnitude as reported by others,28,29 the observed $K_d$ is higher than reported. This could (partly) be the result of the collagenase treatment during the preparation of the primary hepatocytes. It is interesting to note that amiodarone did not decrease the affinity of the LDL receptor for LDL binding, although the variation in the $K_d$ between the experiments was rather large. Given the inherent variability of the assays, we believe that amiodarone decreases the LDL receptor mRNA and protein levels about equally. An additional mechanism by which the number of cell surface receptors is decreased could be via a amiodarone-induced decrease in growth hormone concentration. It has been shown that growth hormone can restore LDL receptor expression in livers of hypophysectomized rats.30,31 On the other hand, we have recently obtained evidence using transfection experiments that points to a thyroid hormone-responsive element in the promoter of the LDL receptor gene (F. Hudig, unpublished observations, 1997) and we therefore suggest that at least a large part of the amiodarone effect is direct via the LDL receptor promoter. Another way in which amiodarone could influence the number of LDL receptors on the cell surface could be a nongenomic, effect on LDL receptor recycling, as has been shown for the $\beta$-adrenergic receptor.32

The present results strengthen the hypothesis that a local hypothyroid-like condition in the liver is connected to the amiodarone-induced hypercholesterolemia. Hypothyroidism in experimental animals is, in addition to the increase of plasma cholesterol, associated with a decrease in hepatic LDL receptor mRNA, which is not caused by changes in mRNA stability.25 The expression of the LDL receptor protein in hepatocytes in culture from hypothyroid rats is also decreased relative to euthyroid controls.33 Amiodarone mimics these effects of thyroid hormone deficiency. The mechanism by which amiodarone exerts these hypothyroid-like effects remains to be elucidated. Thyroid hormones increase the expression of LDL receptors in rat liver both at mRNA and at the protein level.33 Putative thyroid hormone-responsive elements are present in the promoter region of the gene encoding for the LDL receptor.34 It might thus be that the potention inhibition of extrathyroidal T3 production induced by amiodarone, which causes a very low T3 content of several tissues, including the liver,35 which results in a low T3 receptor occupancy and thereby decreases transcription of the LDL receptor gene. The recently observed inhibition of the binding of T3 to its nuclear $\alpha_t$ and $\beta_t$ receptor by desethylamiodarone, the major metabolite of amiodarone, could reduce the occupancy of T3 receptors even further.36,37 We have recently shown that T3 counteracts the amiodarone-induced increase in plasma cholesterol and decrease in liver LDL receptor gene expression, which suggests that the inhibitory effect of amiodarone on LDL receptor gene expression is mediated via T3-dependent pathways.38 However, other mechanisms are also possible. It has been shown that amiodarone, a lipophilic drug that accumulates to a great extent in liver and adipose tissue, stiffens membranes.39 This could interfere with the internalization of membrane-bound receptors and affect the number of membrane receptors, like the LDL receptor.40

We conclude that the amiodarone-induced increase of plasma cholesterol levels can be explained by a reduced transcription of the LDL receptor gene, which results in a decrease of the LDL receptor protein on hepatocytes. It is likely that the reduced transcription is related to an amiodarone-induced hypothyroid-like condition in the liver.

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REFERENCES

2. de Jong M, Docter R, van der Hoek J: Different effects of amiodarone on transport of T4 and T3 into perfused rat liver. Am J Physiol 266:E44-E49, 1994


16. Wiersinga WM, Chopra IJ: Radioimmunoassays of thyroxine (T4), 3,3',5'-triiodothyronine (T3) and 3,3'-diodothyronine (T2). Meth Enzymol 84:272-303, 1982


36. Bakker O, van Beeren HC, Wiersinga WM: Desethylamiodarone is a noncompetitive inhibitor of the binding of thyroid hormone to the thyroid hormone receptor receptor protein. Endocrinology 134:1665-1670, 1994

37. van Beeren HC, Bakker O, Wiersinga WM: Desethylamiodarone is a competitive inhibitor of the binding of thyroid hormone to the thyroid hormone receptor protein. Mol Cell Endocrinol 112:15-19, 1995

