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FEBS Letters 341 (1994) 86–90

FEBS 13761

FEBS LETTERS

Amiodarone-induced hypercholesterolemia is associated with a decrease in liver LDL receptor mRNA

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Received 1 February 1994

Abstract

Amiodarone decreases plasma and tissue triiodothyronine (T_3) and increases plasma cholesterol levels resembling changes seen during hypothyroidism. To elucidate the mechanism of amiodarone-induced hypercholesterolemia we investigated gene expression of three key proteins in cholesterol metabolism (cholesterol 7α-hydroxylase, LDL receptor, HMG-CoA reductase) in livers of rats. Animals were treated with amiodarone or propylthiouracil (to induce mild hypothyroidism). The LDL receptor mRNA was downregulated (≈50%) in both amiodarone-treated and hypothyroid animals, while the other mRNA remained unchanged after 14-day treatment. The results suggest that amiodarone-induced hypercholesterolemia is associated with decreased LDL receptor mRNA levels.

Key words: LDL receptor; Amiodarone; Thyroid hormone; Cholesterol; Messenger RNA

1. Introduction

A number of tissue effects in patients or experimental animals upon amiodarone medication are strikingly similar to those observed in hypothyroidism. For example, the lengthening of the cardiac action potential induced by amiodarone medication is similar to that observed in hypothyroidism [1]. Furthermore, hypothyroidism and amiodarone treatment are both associated with hypercholesterolemia. In patients, long-term amiodarone treatment induces a dose-dependent increase in plasma cholesterol [2]. The same increase was observed in rats [3]. Therefore it has been hypothesised that one of the mechanisms of action of amiodarone is the induction of a local 'hypothyroid-like' condition. Indeed, amiodarone treatment decreases plasma and tissue triiodothyronine (T_3) concentrations [4]. This is due to a reduction of the 5'-deiodination of thyroxine (T_4) into T_3 which is secondary to an inhibition of T_4 transport across the plasma membrane into the cell, resulting in increased plasma T_4 values. To elucidate the mechanism of amiodarone-induced hypercholesterolemia we investigated the gene expression of three key proteins in the metabolism of cholesterol: the LDL receptor (responsible for the uptake of cholesterol into the liver), HMG-CoA reductase (the rate-limiting enzyme in the cholesterol biosynthesis), and cholesterol 7α-hydroxylase (catalyses the rate-limiting step in the synthesis of bile acids from cholesterol). All these proteins are under thyroid hormone control. We studied the mRNA's in livers of amiodarone treated rats and pair-fed controls, because amiodarone has a slight anorectic effect, and mildly hypothyroid animals.

2. Materials and methods

2.1. Materials

Amiodarone was obtained from Sanofi B.V., Maassluis, The Netherlands. Propylthiouracil was obtained from Sigma Chemical Company, St Louis, MO, USA. Cesium chloride was from JT. Baker BV, Deventer, The Netherlands. Formaldehyde, formamide, β-mercaptoethanol and guanidine isothiocyanate were from Merck, Darmstadt, Germany. The cDNA probes for human LDL receptor (pLDLR3) [5] and HMG-CoA reductase (pHRED-102) [6] were generous gifts from Dr D.W. Russell (Dallas, TX, USA). The cDNA for rat cholesterol 7α-hydroxylase (p7α-11) was kindly provided by Dr M. Noshiro [7] (Hiroshima, Japan). A cDNA probe for rat 5'-deiodinase was a gift from Dr R. Larsen [8] (Boston, MA, USA). The C/EBP cDNA was kindly provided by Dr S.L. McKnight [9] (Baltimore, MD, USA).

2.2. Animals

Male wistar rats (weight 270–325 g) were housed under normal conditions with free access to standard lab chow (except when stated otherwise) and tap water. In experiment I, a time-course experiment, the rats (two per time point) received a daily aqueous suspension of amiodarone (10 mg/100 g BW) by gastric tube for 2, 4, 8 and 14 days. Controls received daily water by gastric tube for 14 days. Prior to amiodarone treatment 2 ml blood was taken from each rat by cardiac puncture to determine baseline plasma T_3 and cholesterol concentrations. In experiment II rats were divided into three groups of six rats. Group A was fed ad libitum on standard lab chow, receiving 10 mg/100 g BW amiodarone daily by gastric tube for two weeks. Group B were pair-fed controls of group A. Group C was fed ad libitum on standard lab chow, receiving propylthiouracil 4 mg/100 g BW daily by gastric tube for two weeks. Twenty-four hours after the last administration blood (EDTA) was collected by cardiac puncture and plasma stored at −20°C. The liver was removed and stored in liquid nitrogen. All animal experiments were approved by our ethics committee.

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SSDI 0014-5793(94)00146-M

2.3. Assays

Plasma cholesterol and triglyceride concentrations were determined using a fully enzymatic, kinetic UV method (Cobas Bioanalyzer, Boehringer, Mannheim, Germany). Quantification of different lipoproteins was carried out by precipitation. HDL cholesterol was determined by measuring cholesterol concentrations in the supernatant after precipitation with Heparin/$MnCl_2$, HDL/LDL cholesterol was determined by measuring cholesterol concentration in supernatant after precipitation with 10% SDS in 0.15 M NaCl pH 9.0. LDL cholesterol levels were calculated from HDL/LDL minus HDL cholesterol levels. The free fatty acids in plasma were determined by an enzymatic colorimetric method (NEFA C kit, Wako Chemicals GmbH, Neuss, Germany). Thyroid hormones and TSH were measured by radioimmunoassay. Reagents used in the specific rat TSH-RIA were r-TSH-RP-2 (AFP-5153B) as reference preparation, anti r-TSH-S-5 (C 21381) as antibody and r-TSH-I-8 (AFP-8334B) as antigen for iodination (courtsey of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA). RNA was isolated from liver by the guanidine isothiocyanate method [10]. mRNA was isolated from RNA using the polyAT tract mRNA isolation system of Promega (Madison, WI, USA). 2 μg aliguots of poly A^+ RNA were denatured and electrophoresed in 1% agarose/formaldehyde vertical gels. The separated RNAs were transferred to Nytran NY13 membrane (Schleicher and Schuel, Dassel, Germany) by pressure-blotting (Posiblot pressure blotter, Stratagene, La Jolla, CA, USA) and membranes were fixed using UV cross-linking (1 J/cm^2). The RNA probes were labelled with digoxigenin using a DIG RNA labelling kit (Boehringer Mannheim Biochemica, Mannheim, Germany). Labelled RNA probes were synthesized by in vitro transcription of DNA, cloned downstream of T7 or T3 promoters, with T7/T3 polymerases according to the manufacturers protocol. Dig-labelled probes were detected by an enzyme catalyzed chemiluminescence reaction which can be documented on X-ray film. The hybridization and detection conditions were as described in the DIG-luminescence detection protocol (Boehringer Mannheim Biochemica, Mannheim, Germany). The X-ray films were scanned by densitometry using XRS/3CX scanner and Whole band analysis (version 2.4 1991) from Millipore Corporation (Ann Arbor, MI, USA) to determine relative RNA levels. As internal standard blots were hybridized with a rat probe for C/EBP, a transcription factor that is not regulated at the mRNA level by several hormones and second messengers [11]. All probes were hybridized at 68°C except the HMG-CoA reductase probe which was hybridized at 42°C. Routine quantification of relative mRNA levels was preformed by dotblot analysis on positively charged membrane (Boehringer Mannheim Biochemica, Mannheim, Germany) using probes labelled as above.

2.4. Statistical analysis

To evaluate differences between groups the Mann–Whitney test was used.

3. Results

3.1. Experiment I. Time-course of the effect of amiodarone

Amiodarone treatment for 14 days resulted in a 0.35 nM decrease of plasma T_3 and a 0.9 mM increase of plasma cholesterol (Fig. 1). In control rats, treated with water by gastric tube for 14 days, plasma T_3 decreased slightly by 0.12 mM, but plasma cholesterol remained unchanged. Northern blots showed a decrease in the LDL receptor mRNA from day 8 onwards (insert of Fig. 2). Further quantification of LDL receptor mRNA was preformed on polyA$^+$ RNA by dotblot analysis. Amiodarone caused a marked (about 50%) decrease in hepatic LDL receptor mRNA concentrations after 2 weeks (Fig. 2). No changes were observed in mRNA levels of HMG-CoA reductase, cholesterol 7α-hydroxylase and 5′-deiodinase (data not shown).

3.2. Experiment II. Amiodarone versus propylthiouracil treatment

Daily food intake in the propylthiouracil-treated rats was not different from control rats who were pair-fed to the amiodarone-treated animals. A slight increase in body weight was observed in all groups. Propylthiouracil treatment decreased plasma T_4 and T_3 concentrations and increased plasma TSH; it had no significant effect on plasma cholesterol. In contrast, amiodarone treatment resulted in a 60% increase of plasma cholesterol (HDL cholesterol as well as LDL cholesterol), despite a less pronounced rise of plasma TSH and a smaller decrease of plasma T_3 when compared to propylthiouracil treated animals (Fig. 3). No changes in plasma triglycerides and free fatty acids were observed. Northern blot analysis demonstrated a decrease of liver 5′-deiodinase mRNA in the propylthiouracil treated but not in the amiodarone treated animals (Fig. 4). Neither treatment resulted in a change in liver HMG-CoA reductase or cholesterol 7α-hydroxylase mRNA levels. The hepatic LDL receptor mRNA levels were ≈50% lower in both amiodarone and propylthiouracil treated animals compared to controls.

4. Discussion

From the time-course studies we concluded that two weeks of amiodarone treatment sufficed for induction of

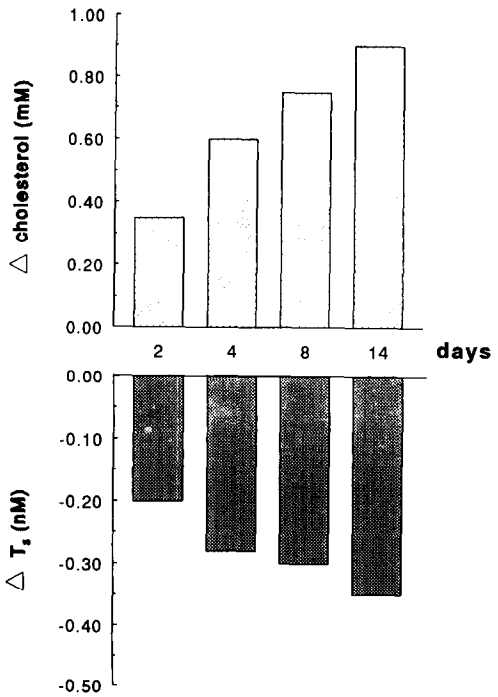

Fig. 1. Changes in plasma T_3 and cholesterol values after amiodarone treatment. Rats were treated for 2, 4, 8 and 14 days with amiodarone. Bars represent difference between day 0 and day 2, 4, 8 and 14, respectively (mean of two rats per time-point).

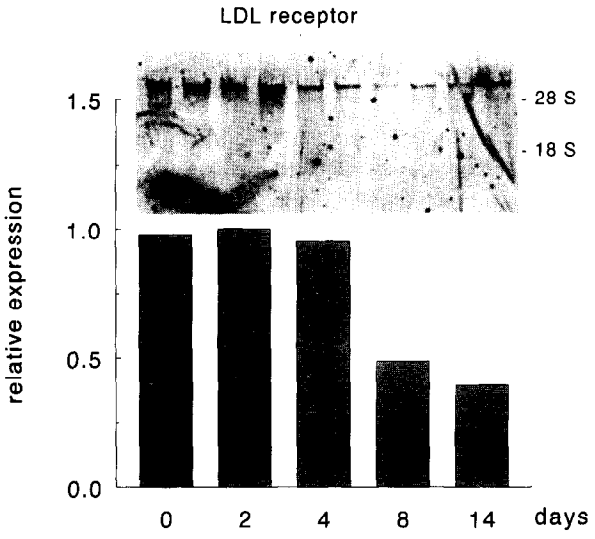

Fig. 2. Liver LDL receptor mRNA in rats treated with amiodarone. The figure shows the relative concentrations of mRNA for the LDL receptor, corrected for C/EBP. Results are given as mean of a duplicate experiment for 2 rats per time-point. In the insert a typical Northern blot obtained with 2 μg poly A$^+$ RNA from livers treated with amiodarone is shown.

the hypercholesterolemic effect. Consequently, amiodarone was administered for 2 weeks in experiment II. Propylthiouracil effectively induced hypothyroidism, as we could demonstrated by low T_4 and T_3 and high TSH plasma concentrations and a decrease in liver 5′-deiodinase mRNA. Hypothyroidism is associated with hypercholesterolemia. In our experiments however, we found no significant increase in plasma cholesterol levels ($P = 0.07$). This may be due to the mild hypothyroid condition of the animals or the relatively short propylthiouracil treatment in our experiments. On the other hand, liver LDL receptor mRNA was decreased by ≈50% in the hypothyroid animals, which was also found in other studies [11,12]. It is possible that, in time, the decrease in LDL receptor mRNA/protein may lead to a further increase in plasma cholesterol levels. The level of hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase mRNA were not affected by propylthiouracil treatment. Day et al. [13] also found no decrease of the HMG-CoA reductase mRNA using methimazole for the induction of hypothyroidism.

Amiodarone medication resulted in an increase of plasma T_4 and a decrease in plasma T_3, as described previously [3]. A new finding is that the level of liver 5′-deiodinase mRNA during amiodarone medication is not altered. This is in line with kinetic studies showing inhibition of T_4 transport into peripheral tissues by the drug [14]. The observed reduction in liver 5′-deiodination is caused by a reduced availability of the substrate T_4, instead of a reduction of 5′-deiodinase protein [15,16].

The amiodarone-induced hypercholesterolemia cannot be explained by the rise in TSH because the higher TSH levels in the mildly hypothyroid animals did not result in a substantial increase of plasma cholesterol. The ≈60% increase of plasma cholesterol during amiodarone medication was associated with a ≈50% decrease of liver LDL-receptor mRNA, but not with changes in liver HMG-CoA reductase or cholesterol 7α-hydroxylase mRNAs. These findings suggest a diminished liver uptake of cholesterol via the LDL receptor as a major mechanism for the amiodarone-induced hypercholesterolemia. The exact mechanism in which amiodarone causes a downregulation of the gene expression of the LDL receptor remains to be elucidated. Gene

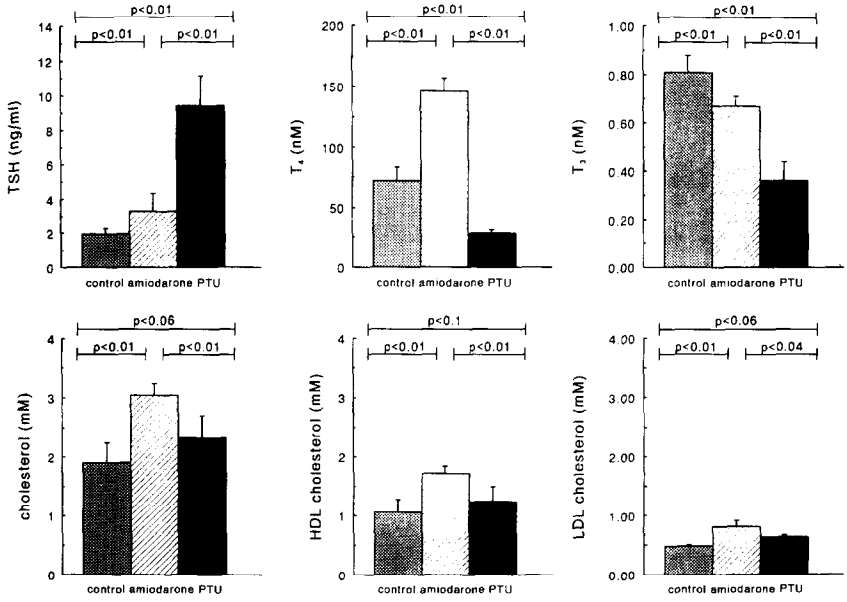

Fig. 3. Effect of amiodarone and propylthiouracil (PTU) treatment of rats for 14 days on thyroid function tests and plasma cholesterol (mean ± S.E.M., $n = 6$).

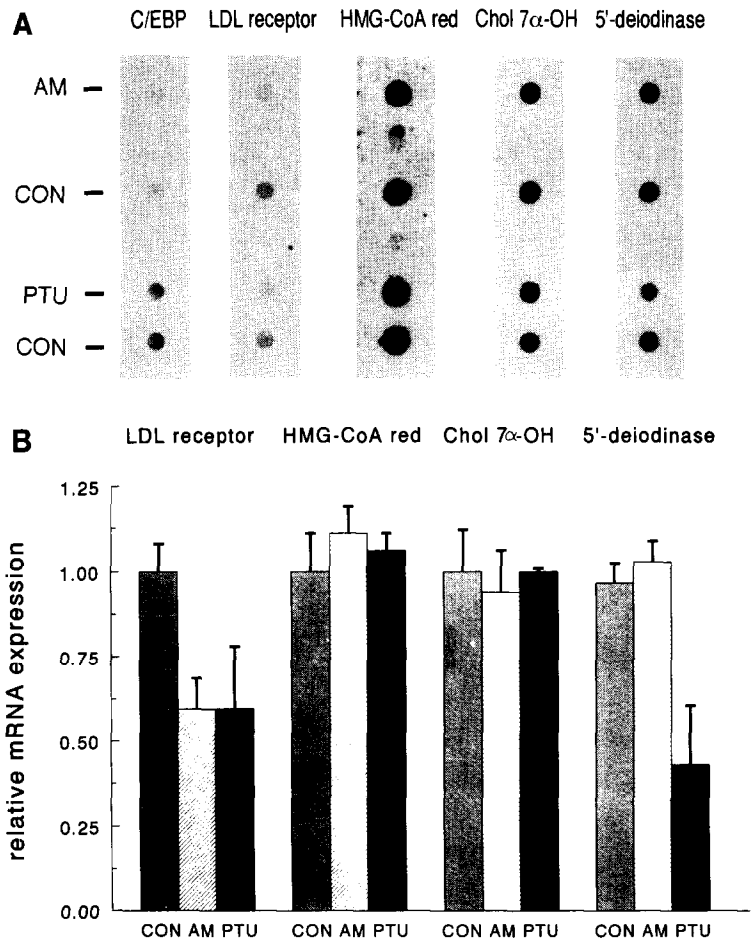

Fig. 4. Dot-blot analysis of hepatic mRNA for C/EBP, LDL receptor, HMG-CoA reductase, cholesterol 7α-hydroxylase and 5′-deiodinase in control, amiodarone treated rats, and propylthiouracil treated rats. (A) The figure shows typical dot-blots obtained with 2 μg poly A^+ RNA from livers for the LDL receptor (LDLr), HMG-CoA reductase (RED), cholesterol 7α-hydroxylase (7α-OH) and 5′-deiodinase (DEI)from the 3 experimental groups: amiodarone (AM), control (CON) and propylthiouracil (PTU). (B) The figure shows the relative contents (mean ± S.E., $n = 3$) of liver mRNA corrected for the internal control C/EBP.

expression of the LDL receptor is T_3-dependent and possible binding sites for the T_3-receptor are present in the promotor region of the LDL receptor gene [17]. We have recently demonstrated that desethylamiodarone, the major metabolite of amiodarone, inhibits the binding of T_3 to the T_3-receptor in a noncompetitive manner [18] and may therefore interfere with T_3 action. This could explain the modulation of the LDL receptor gene expression by amiodarone, but not the absence of an effect of the drug on HMG-CoA reductase and cholesterol 7α-hydrolyse gene expression. An effect of amiodarone on other transcription factors, on mRNA stability, or on the interaction between the T_3-receptor and its responsive element cannot be excluded.

Acknowledgements: The authors like to thank their colleagues of the laboratory of Endocrinology for the measurement of thyroid hormones. Thanks are also due to biotechnicians Adrie Maas and Jan de Haan for their assistance during the animal experiments.

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