Effects of phenothiazines on low density lipoprotein metabolism in cultured human fibroblasts

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Treatment of cultured human fibroblasts with trifluoperazine or chlorpromazine resulted in a biphasic effect on low density lipoprotein (LDL) catabolism, depending upon the dose. At up to 10^{-5} M, a marked increase in LDL binding, internalization and degradation was observed. This phenomenon took place within the first hours of incubation with the drugs, suggesting a direct effect on cell membrane physical characteristics, probably related to the lipophilic properties of phenothiazines. Concentrations above 2×10^{-5} M resulted in a relative decrease in LDL binding and internalization, and in a dramatic decrease in LDL degradation, which may be related to an inhibition of calmodulin-dependent processes.

Trifluoperazine *Chlorpromazine* *Low density lipoprotein catabolism* *Human fibroblast*

1. INTRODUCTION

In [1-3], low density lipoprotein (LDL) catabolism is mainly achieved by receptor-mediated endocytosis, which results in lysosomal degradation and decrease in both endogenous sterol synthesis and LDL receptor expression. Calcium is generally required for LDL processing by cells, and it has been demonstrated that calmodulin is probably involved in receptor-mediated endocytosis [4]. More recently, authors in [5] found an inhibitory effect of the calmodulin effector trifluoperazine (TFP) on the acetylated-LDL degradation by liver cells, suggesting a role of calmodulin in LDL metabolism.

We here investigated the effects of two phenothiazines, chlorpromazine (CPZ) and TFP, on LDL binding, internalization and degradation by cultured human fibroblasts. For the two drugs studied, we report a 'biphasic' phenomenon, depending upon the dose: at low concentrations (10^{-6}-10^{-5} M), strong stimulation of both LDL internalization and degradation was observed, whereas a decrease of these processes was found for concentrations over $2\text{-}3 \times 10^{-5}$ M. The LDL binding to their specific receptors was less affected. These results are discussed in relation to the effects of CPZ and TFP on calmodulin-dependent processes, and to the lipophilic properties of these drugs.

2. MATERIALS AND METHODS

2.1. *Cell culture*

MRC5 (human fetal lung) fibroblasts purchased from Biomérieux were cultivated in 60-mm Petri dishes containing Ham F10 medium supplemented with 10% fetal calf serum (Gibco), at 37°C, in a humidified 5% CO_2 atmosphere. Experiments were performed on confluent cells.

2.2. *Effects of drugs on LDL binding, internalization and degradation*

Prior to experiments, cells were incubated 24 h in a medium containing 10% lipoprotein-deficient serum, for maximal expression of LDL receptors.

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CPZ and TFP (chlorhydrates) were introduced in saline solution, at final concentrations varying from 10^{-6} to 5×10^{-5}M. CPZ and TFP were gifts from Rhone-Poulenc and Theraplix Laboratories, respectively. In preliminary experiments using R 24571 (calmidazolium, Boehringer Mannheim) the drug (final concentration 10^{-6}–10^{-5}M) was introduced in ethanolic solution (final concentration of ethanol 0.5%). In this case, results were compared to controls with ethanol 0.5%. Incubations were carried out for 1–24 h at 37°C. Cells were then extensively washed with a phosphate-buffered solution, and LDL binding, internalization and degradation studied as in [1,2], using [^{125}I]LDL (10 μ/ml). LDL was prepared in [6] and iodinated as in [7]. The specific radioactivity of the [^{125}I]LDL was about 200–250 cpm/ng protein. Protein determination was done as in [8]. Results are expressed in cpm $\cdot 10^{-3} \cdot$ mg cellular protein^{-1}. Experiments were performed at least 4-times, except for short-term incubations (1–4 h), which were in triplicate, and experiments with R 24571, which were in duplicate.

3. RESULTS AND DISCUSSION

It can be seen in table 1 that 24 h incubation of cells with CPZ or TFP, at concentrations up to 2×10^{-5}M, resulted in a sharp rise in LDL internalization. The maximal effect was obtained with 10^{-5}M, for which concentration an approximate 3-fold increase was observed with CPZ, and a 4–5-fold increase with TFP. Concentrations above 2×10^{-5}M resulted in relative decrease in internalization (about 50–60% of controls for TFP 3×10^{-5} M or CPZ 5×10^{-5} M). It can be noted

Table 1

Effects of trifluoperazine (TFP), chlorpromazine (CPZ) and R 24571 on LDL binding, internalization and degradation by cultured human fibroblasts, in relation to drug concentration

Drugs	LDL Binding (4°C)	LDL Binding + internalization (37°C)	LDL Degradation
None	26.5 ± 3.8 (100)	195 ± 30 (100)	490 ± 78 (100)
TFP			
10^{-6}M	29.7 ± 4.3 (112)	224 ± 29 (115)	-
5×10^{-6}M	36.0 ± 4.0 (136)	565 ± 96 (290)	1264 ± 227 (258)
10^{-5}M	41.9 ± 6.2 (158)	920 ± 156 (470)	2400 ± 384 (410)
1.5×10^{-5}M	40.3 ± 5.3 (152)	795 ± 127 (408)	1666 ± 283 (340)
2×10^{-5}M	33.9 ± 4.1 (128)	624 ± 106 (320)	1063 ± 191 (217)
3×10^{-5}M	22.5 ± 2.8 (85)	111 ± 16 (57)	137 ± 18 (28)
CPZ			
10^{-6}M	28.1 ± 4.6 (106)	218 ± 26 (112)	-
5×10^{-6}M	31.2 ± 5.2 (118)	331 ± 50 (170)	1127 ± 191 (230)
10^{-5}M	33.4 ± 4.8 (126)	499 ± 60 (256)	1617 ± 255 (330)
2×10^{-5}M	29.7 ± 3.9 (112)	526 ± 73 (270)	1029 ± 164 (210)
5×10^{-5}M	23.8 ± 4.1 (90)	127 ± 23 (66)	171 ± 26 (35)
Ethanol 0.5%	-	234 ± 37 (100)	587 ± 96 (100)
R 24571 in ethanol 0.5%			
10^{-6}M	-	178 ± 22 (76)	322 ± 48 (55)
5×10^{-6}M	-	154 ± 26 (66)	99 ± 17 (17)
10^{-5}M	-	98 ± 16 (42)	53 ± 9 (9)

Cells were incubated 24 h with the drugs in a medium containing 10% of lipoprotein-deficient serum. The LDL binding, internalization and degradation were studied as in [1] using [^{125}I]LDL (10 μg/ml, 200–250 cpm/ng). Results are expressed in cpm $\cdot 10^{-3}$/mg cell protein $^{-1}$. Experiments were performed at least 4-times for TFP and CPZ, and in duplicate for R 24571. Numbers in parentheses are percentages

that the LDL-specific binding was also increased by CPZ or TFP up to 2×10^{-5} M, but less than internalization (~1.3-fold increase with CPZ, and 1.5–1.7-fold increase with TFP).

As also shown in table 1, the lysosomal degradation of LDL was clearly increased for concentrations up to 2×10^{-5}M. Increasing the concentrations over 2×10^{-5}M for TFP, or $3\text{–}4.10^{-5}$M for CPZ resulted in a dramatic decrease in LDL degradation, as observed in [5] for acetylated-LDL in liver cells. It must be noted that the LDL lysosomal degradation appears to be more inhibited than the internalization, which is consistent with the results in [5], but these authors did not study the effects of TFP on the high-affinity internalization of native LDL.

The short-term effects (1–4 h incubation) were studied with TFP, which was the more effective in our system. As shown in table 2, significant increase in LDL internalization and degradation took place within the first hours (~1.5-fold increase for 4 h incubation with TFP 10^{-5}M), whereas no effect on the LDL-specific binding was found.

This rapid increase in LDL internalization and degradation and the discrepancy between the effects of TFP on the LDL binding and internalization suggest that the short-term effects observed could be related to a direct action of the drug on the cell membrane, which resulted in a change in its physical characteristics. Independently of their interaction with calmodulin, which is probably not very selective [9,10], phenothiazines are known to bind to phospholipids [11]. Thus, we assume that these lipophilic drugs could also interact with the membrane phospholipids, inducing changes in membrane organization. Preliminary studies performed by fluorescence polarization (using diphenylhexatriene or β-parinaric acid as probes) demonstrated a rapid and significant decrease in the cellular membranes' microviscosity by in vitro treatment of cell suspensions with CPZ or TFP (not shown). Such decrease in membrane microviscosity has been previously reported in synaptosomes treated with CPZ [12]. We think that the decrease in membrane microviscosity could account for the enhancement of the LDL internalization by low concentrations of phenothiazines, especially in short-term, experiments. This phenomenon may be compared to the increase in LDL internalization observed in fibroblasts treated with polyunsaturated fatty acids [13], which are also known to increase the membrane 'fluidity' [14].

For concentrations above $2\text{–}3 \times 10^{-5}$M, the decrease in LDL internalization and degradation is probably related to the inhibitory effect of phenothiazines on calmodulin-dependent processes [15,16]. In this range (10^{-5}–10^{-4}M), TFP has been shown to inhibit the receptor-mediated endocytosis [4] and the degradation of acetylated-LDL by liver cells [5]. Such effects also occurred in our experimental system for TFP concentrations over 2×10^{-5}M or CPZ concentrations over $3\text{–}4 \times 10^{-5}$ M. In preliminary experiments with R 24571, the

Table 2

Short-term effects of trifluoperazine (TFP) 10^{-5} M on LDL binding, internalization and degradation by cultured human fibroblasts

Drug	LDL Binding (4°C)	LDL Binding + internalization (37°C)	LDL Degradation
None	24.3 ± 3.2 (100)	182 ± 25 (100)	465 ± 74 (100)
1 h	23.8 ± 3.4 (98)	203 ± 28 (112)	502 ± 80 (108)
2 h	22.5 ± 3.2 (93)	218 ± 33 (120)	530 ± 68 (114)
3 h	24.7 ± 3.6 (102)	247 ± 40 (136)	669 ± 98 (144)
4 h	26.8 ± 4.2 (110)	281 ± 37 (154)	757 ± 120 (163)

Prior to experiments, cells were incubated 24 h in a medium containing 10% lipoprotein-deficient serum. After incubation for 1–4 h with TFP 10^{-5} M, cells were washed and LDL binding, internalization and degradation studied as in [1] using [^{125}I]LDL (10 μg/ml, 200–250 cpm/ng). Results are expressed in cpm · 10^{-3}/mg cell protein^{-1}. Experiments were performed in triplicate. Numbers in parentheses are percentages

most potent and selective calmodulin inhibitor [17], concentrations of 10^{-6} to 10^{-5}M, only caused a decrease (about 50–60% of controls) in LDL internalization, and a strong inhibition of the LDL lysosomal degradation (about 10% of controls for R 24571 10^{-5}M, see table 1). Thus, we assume that the stimulation of LDL internalization observed with low concentrations of phenothiazines is mainly related to the decrease in membrane microviscosity, whereas the inhibitory effect found for concentrations over 2×10^{-5}M is especially related to the inhibition of calmodulin-dependent processes.

The moderate increase in LDL binding which took place in 24 h incubations, mainly with TFP, is probably related to late perturbations in cell metabolism. In [18] we described alterations in LDL binding and internalization by cyclic-AMP and drugs which affect its intracellular concentration in cultured human fibroblasts. Adenylate cyclase has been shown to be related to calmodulin in various tissues: brain [19], pancreas islets [20], adrenal medulla [21], or even in procaryotic cells [22], and thus affected by calmodulin effectors such as phenothiazines. There is still no report concerning the effects of phenothiazines on the intracellular cAMP level in fibroblasts, but in some experiments we found that cAMP partially counteracted the increase in LDL binding and internalization induced by TFP (24 h incubation with dibutyryl cAMP 10^{-3}M + TFP 10^{-5}M, 2 experiments: a 4.5-fold increase was observed with TFP alone, whereas only a 2-fold increase was found with TFP + cAMP). Thus, some of the late effects of phenothiazines on the LDL metabolism could be at least partially related to alterations in the cAMP intracellular level.

To our knowledge, the stimulation of LDL internalization and degradation by low concentrations of phenothiazines (10^{-6}–10^{-5}M) has not been reported. We assume that it is probably related to the lipophilic properties of these drugs, and that a similar phenomenon could be involved, at least partially, in the hypocholesterolemic effect of other lipophilic drugs such as suloctidil, which has also been reported to decrease membrane microviscosity [12]. This concept could be of use for synthesis of new hypocholesterolemic agents which enhance the LDL peripheral catabolism by means of a decrease in cell membrane microviscosity.

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