Thioridazine: a selective inhibitor of peroxisomal β-oxidation in vivo

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In vivo administration of the phenothiazine drug, thioridazine, inhibits hepatic peroxisomal β-oxidation in mice. In starving animals, 3-hydroxybutyrate concentration is not decreased by thioridazine treatment. These results provide the first demonstration of thioridazine as a selective inhibitor of peroxisomal β-oxidation in intact animals. Thioridazine might supply a tool for simulation of pathological conditions in which peroxisomal β-oxidation is impaired.

Thioridazine *Peroxisome* *β-Oxidation* *Catalase* *In vivo inhibition*

1. INTRODUCTION

Fatty acid oxidation in liver occurs in 2 different cell compartments, mitochondria and peroxisomes [1,2]. The relative contribution to overall fatty acid degradation in the cell and the precise function of peroxisomal β-oxidation are not clearly established [3–7]. To gain more insight into peroxisomal function, specific inhibitors of peroxisomal β-oxidation would be of great value, especially when they can be used in vivo. Though attention has been paid to inhibitors of the mitochondrial system, there was no description of inhibitors of the peroxisomal system until recently. In 1984, Leighton et al. [8] demonstrated the selective inhibition of peroxisomal β-oxidation by 3 phenothiazine drugs, of which thioridazine proved to be the most effective. These results were obtained with an in vitro system (isolated rat hepatocytes). As points of divergence between in vitro and in vivo findings are often apparent [6], the purpose of this study is to investigate whether and how specifically thioridazine inhibits peroxisomal β-oxidation of endogenous substrates in intact animals. Due to the fact that H_2O_2 is produced during the first step of peroxisomal β-oxidation, changes in H_2O_2 generation can be used to estimate peroxisomal activity. In vivo H_2O_2 production in the liver of unanaesthetized mice can be evaluated by measuring the catalase activity which remains after inhibition of the catalase-H_2O_2 complex by 3-amino-1,2,4-triazole in combination with methanol [9]. In an earlier study we demonstrated that starvation causes an increased flux of fatty acids through the peroxisomal fatty acid oxidase [9]. We now report on the influence of thioridazine on enhanced peroxisomal β-oxidation in starving mice. 3-Hydroxybutyrate concentration in serum is used as a reflection of mitochondrial activity [3,4,10,11].

Abbreviations: aminotriazole, 3-amino-1,2,4-triazole; RCA, residual catalase activity; Th, thioridazine

2. MATERIALS AND METHODS

Male BALB/C mice (20–25 g) were used in our experiments. 3-Amino-1,2,4-triazole (1 g/kg in 0.9% NaCl), methanol (3.2 mmol/kg) and thioridazine (in 0.9% NaCl) were administered by intraperitoneal injection. Aminotriazole and methanol were administered simultaneously. Mice were killed by dislocation of the neck 1 h after administration of aminotriazole-methanol and 2 h

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received standard laboratory diet (AO3-UAR, France). Starved mice were deprived of food (water ad libitum) for 24 h.

Catalase activity was assayed at 0°C in total liver homogenate by the titanium oxysulphate method [12]. 1 U_B is the amount of catalase which breaks down 90% substrate (1.5 mmol H_2O_2/l) in a 50 ml volume at 0°C in 1 min; maximal reaction time is 10 min. For each animal the catalase activity calculated is the mean of 10 measurements after 1–10 min reaction time. Residual catalase activity (RCA) is the catalase activity that remains after inhibition by aminotriazole in combination with methanol. A lower RCA reflects a higher H_2O_2 production [9].

3-Hydroxybutyrate in serum was determined according to Williamson et al. [13].

Each experimental group consisted of at least 5 animals. All results are expressed as the mean ± SE. For statistical analysis, the Mann-Whitney test was used [14].

Thioridazine hydrochloride (Th) was a gift from Sandoz Pharmaceuticals; aminotriazole was obtained from Sigma.

3. RESULTS

Catalase activity in the liver of untreated, standard diet fed mice is 88.68 ± 2.48 U_B/g liver [9]. Sole administration of Th (250 μmol/kg) has no influence on this value.

The influence of Th administration on liver RCA is summarized in table 1.

Enhanced H_2O_2 production caused by starvation is completely undone by treatment with 50 μmol/kg Th; in fact the level of H_2O_2 production is reduced to a level seen in fed mice treated with Th. At low doses (0.5 and 5 μmol/kg) Th has no inhibitory effect on increased liver H_2O_2 production; the inhibitory effect is less pronounced at 250 and 500 μmol/kg of the drug.

In 24 h starved mice 3-hydroxybutyrate concentration in serum has risen significantly compared to fed animals. Treatment with different doses of Th does not decrease 3-hydroxybutyrate concentration in serum.

In fed mice also administration of 50 and 250 μmol/kg Th significantly increases RCA ($P < 0.05$).

Table 1

Influence of thioridazine (Th) treatment on RCA values in total mouse liver homogenate and on serum 3-hydroxybutyrate concentration

Conditions (Th in μmol/kg)	RCA (U_B/g liver)	3-Hydroxybutyrate (mM)
Fed		
no Th	50.78 ± 3.03 (a)	0.33 ± 0.03
Th 50	72.83 ± 1.90 (b)	0.42 ± 0.03
The 250	68.89 ± 5.70 (c)	0.49 ± 0.02
Starvation, 24 h		
no Th	18.85 ± 1.63 (d)	1.06 ± 0.04
Th 0.5	19.68 ± 3.11 (e)	1.17 ± 0.13
Th 5.0	27.07 ± 2.31 (f)	1.37 ± 0.25
Th 50	61.08 ± 5.25 (g)	1.28 ± 0.31
Th 250	49.17 ± 3.97 (h)	1.98 ± 0.11
Th 500	39.66 ± 5.44 (i)	1.84 ± 0.24

Statistical significance of differences. $P < 0.01$: a vs d, e and f; b and c vs d, e, f and i; all fed 3-hydroxybutyrate values vs fasted values. $P < 0.05$: a vs b, c and i; b and c vs h

4. DISCUSSION

The present results demonstrate that Th inhibits peroxisomal β-oxidation in vivo: enhanced flux of fatty acids through the peroxisomal oxidase is reversed by treatment with Th. Inhibition is concentration dependent.

When Th is administered to fed mice we also note a significant increase of liver RCA compared to fed, untreated animals. This means that there is a measurable basal peroxisomal β-oxidation present in fed mice. Administration of 50 μmol/kg Th to fasting mice inhibits both the basal peroxisomal β-oxidation and increased peroxisomal β-oxidation due to starvation.

In mouse serum 3-hydroxybutyrate concentration is not diminished after treatment with Th in a wide range of concentrations. In the living animal therefore Th is not inhibiting mitochondrial activity, but is selective for peroxisomes. Moreover this result proves that the contribution of peroxisomal β-oxidation to the production of ketone bodies is negligible.

The level of RCA reached after Th treatment is the same in fed and in fasted animals and equal to

the level reached after insulin administration to fasted mice [9]. This suggests that H_2O_2 production from β-oxidation has reached its minimum, and consequently that near therapeutic doses Th inhibits peroxisomal β-oxidation completely. This makes the drug, which can be administered orally, an interesting tool for the study of pathological conditions in which impairment of peroxisomal function related to fatty acid metabolism is suspected or proven, such as in adrenoleucodystrophy [15], in Zellweger syndrome [16] and in Refsum's disease [17]. Long term administration of Th should demonstrate to which extent inhibition of peroxisomal β-oxidation causes the development of a disease.

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