Species Differences in the Effects of Bezafibrate as a Potential Treatment of Mitochondrial Disorders

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Dear Editor,

Genetic defects of mitochondrial respiratory chain form an expanding family of rare diseases, whose number and global incidence increases constantly whereas treatment options remain extremely limited. In line with recent literature data (Bastin et al., 2008) suggesting a potential of bezafibrate for correction of respiratory chain (RC) defects in human fibroblasts, Viscomi et al recently published in *Cell Metabolism* the results of *in vivo* experiments aimed at evaluating the effects of bezafibrate in RC deficient knockout mice (Viscomi et al., 2011). The conclusions from this study appeared in marked contrast with those drawn from *in vitro* studies in patient cells, and apparently casts doubt on therapeutic properties of bezafibrate in RC deficiencies. However, we consider that limitations in the study design could explain the apparent inefficacy and toxic effects of bezafibrate reported by the authors. Furthermore, based on clinical data obtained in individuals treated with bezafibrate, we present data showing that this drug can stimulate the respiratory chain function in the human skeletal muscle.

One of the most questionable points in the study of Viscomi et al is the bezafibrate dosage tested in the knockout mice (0.5% drug added to standard diet for one month), for several reasons. At first, when using this diet, it is easy to calculate that the daily drug supply is in considerable excess compared to the pharmacological dose used in humans. Indeed, assuming a mouse body weight of 25-30 grams and a 4 grams/day food intake, 0.5% bezafibrate in chow is equivalent to 666-800 mg/kg/day bezafibrate, i.e. represents up to 80-fold the dose used for the treatment of dyslipidemia (10 mg/kg/day). The second and main concern is the known toxicity and carcinogenic potential of such high doses of fibrate in rodents, established in the early 1980's. Indeed, it is known that a two-fold increase in liver weight is already observed in mice after 1-week on a diet containing 0.5% bezafibrate, likely due to PPAR-alpha mediated induction of genes involved in hepatocyte proliferation (cyclin D1, CDK4, and c-Myc), whereas mice kept on this regimen will develop hepatocarcinoma in the long term (Hays et al., 2005). Importantly, recent studies also show that clinically-relevant doses of bezafibrate elicit triglyceride-lowering effects in mice, and no toxic effects (Nakajima et al., 2009).

Taking into account these literature data, there is no rationale to use 0.5% bezafibrate in diet when investigating pharmacological properties of this drug. Furthermore, it appears likely that liver hepatomegaly reported both in treated Surf 1-/- and wild-type animals reflect a classical toxic response to high doses of bezafibrate. PPAR agonists at high doses can also induce muscle damages (myofibril degeneration and inflammatory cell infiltration). Accordingly, worsening of muscle damages in ACTA-Cox15-/- mice treated by bezafibrate, could also be ascribed to toxic effects of bezafibrate overdosage. Under these conditions, conclusions on the therapeutic potential of bezafibrate in RC-deficient mouse models cannot be drawn, and extrapolation to the treatment of RC-deficient patients appears irrelevant .

Importantly, the hepatotoxicity and carcinogenic activity of fibrates are clearly rodent-specific. Indeed, it has long been known that humans are resistant to the development of hepatocarcinoma after chronic exposure to fibrates, and, large-scale studies performed since the 1980's consistently established that bezafibrate is a safe drug, with limited side effects (Tenenbaum, A et al, 2005 cited in Bonnefont et al).

Regarding the possible use of this drug in patients with inborn metabolic myopathies, we tested bezafibrate in patients with the myopathic form of Carnitine Palmitoyl Transferase 2 (CPT2) deficiency, one of the commonest inborn mitochondrial fatty acid ß-oxidation defects. In contrast with the assumption made by Viscomi et al on the basis of their study in mice, this pilot trial did not reveal contraindications in the use of bezafibrate in myopathic patients. On the contrary, CPT2-deficient patients treated by bezafibrate for 6 months at 10 mg/kg/day generally experienced a clear decline in muscular pain and rhabdomyolysis episodes, and less limitation in physical activity. Furthermore, follow-up of these patients for three years indicated stable beneficial effects of the treatment in the long-term, without adverse effects (Bonnefont et al., 2010).

Importantly, as reported here (Supplemental Figure 1), we established in the course of this trial that bezafibrate treatment led to increase the RC capacity in the human skeletal muscle. Indeed, stimulation of RC capacities was reflected by the rise in maximal O2 consumption observed in muscle mitochondria of treated patients. Thus, as shown in Figure 1A, the oxidation rates of pyruvate+malate (a RC complex I substrate) or of succinate (a RC complex II substrate), markedly increased ($p=0.028$, two-sided Wilcoxon signed-rank test) after 6 months of bezafibrate treatment, in patient muscle mitochondria. Consistent with this, the levels of key respiratory chain proteins i.e. NDUFV1 (Complex I) and COX4 (Complex IV), encoded by nuclear genes, or COX2, a mitochondrial DNA encoded gene, were found strongly increased in the muscle of bezafibrate-treated patients (Figure 1B). Finally, Cytochrome c oxidase (Complex IV, COX) and Citrate Synthase (mitochondrial matrix protein) enzyme activities measured in muscle homogenates significantly increase after bezafibrate treatment (Figures 1C and 1D).

Altogether, our *in vivo* data, as well as *in vitro* studies performed in patients' cells, reinforce the notion that activation of the PPAR-PGC1 signaling pathway by bezafibrate could be a promising approach for pharmacological correction of partial FAO or RC deficiencies. Clinical trials will be needed to assess the possible beneficial effects of bezafibrate in various RC disorders and the absence of adverse effects.

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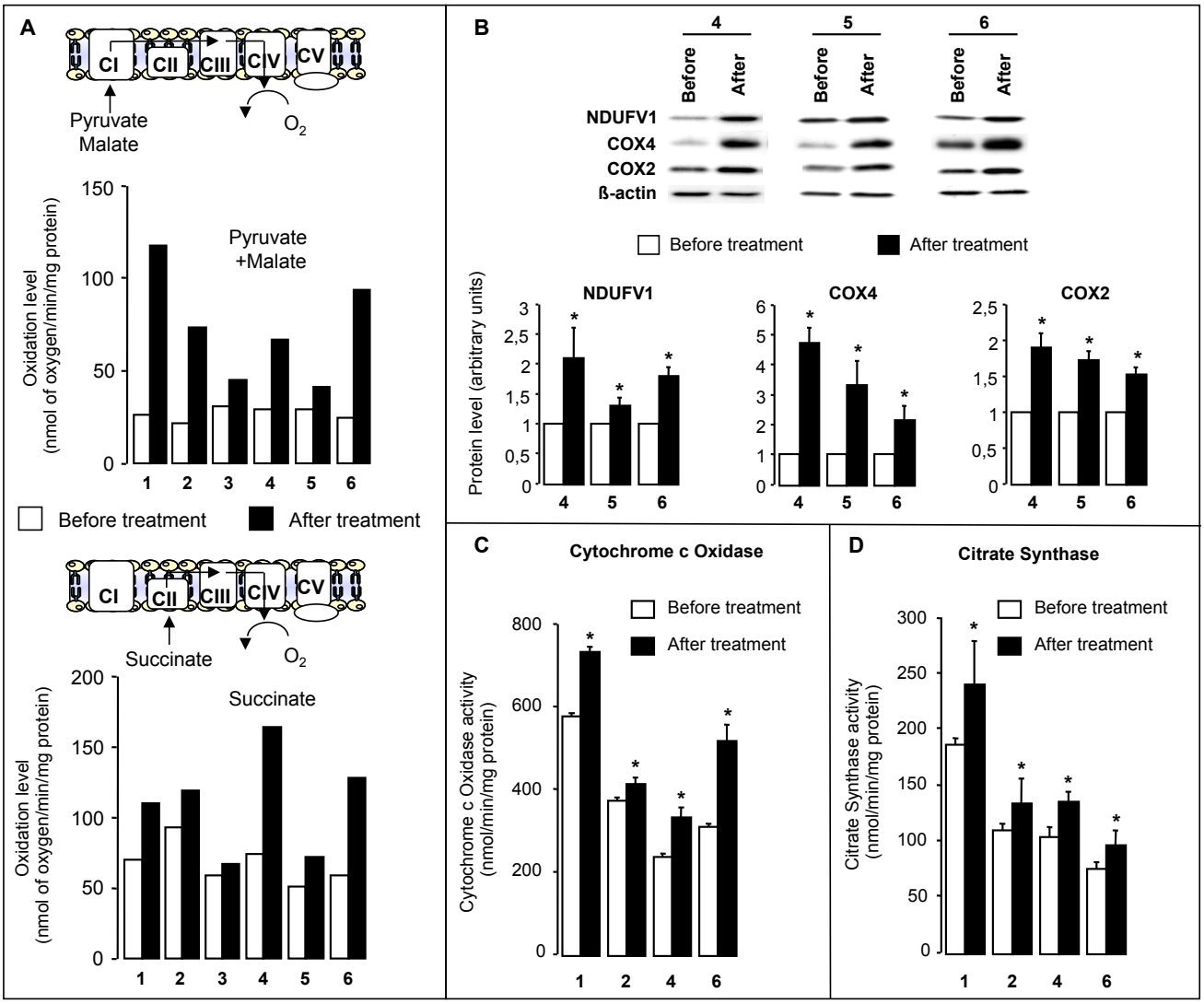
A
CI
CII
CIII
CIV
CV
Pyruvate
Malate
O2
Oxidation level
(nmol of oxygen/min/mg protein)
Pyruvate
+Malate
Before treatment
After treatment
Succinate
Succinate
B
4
5
6
Before
After
NDUFV1
COX4
COX2
ß-actin
Before treatment
After treatment
Protein level (arbitrary units)
NDUFV1
COX4
COX2
C
Cytochrome c Oxidase
Cytochrome c Oxidase activity
(nmol/min/mg protein)
D
Citrate Synthase
Citrate Synthase activity
(nmol/min/mg protein)

Figure 1

Figure legend

Figure 1: The individuals considered in this study are patients diagnosed with the myopathic form of Carnitine Palmitoyl Transferase 2 (CPT2) deficiency, who were included in a clinical trial of bezafibrate. It was registered under n°NCT00336167 on the ClinicalTrial.gov web site. The patients and protocols have been described in detail in reference (Bonnefont et al., 2010). Briefly, all the patients underwent a first muscle biopsy before receiving bezafibrate and a second biopsy after 6-month of treatment by bezafibrate as three 200-mg tablets per day, which is the regular drug dosage for treatment of hyperlipidemia. These muscle biopsies were primarily used for the measurement of mitochondrial respiratory capacities. When the size of the tissue sample was not limiting, additional parameters were measured to further evaluate the respiratory chain functions.

(A) Pyruvate plus malate (complex I substrate) and succinate (complex II substrate) oxidation rates in isolated muscle mitochondria from 6 individuals, before (open bars) and after (dark bars) treatment by bezafibrate. O2 consumption rates measured by polarography, are expressed in nmol oxygen consumed per minute per milligram of mitochondrial protein, and represent the average of duplicate measurements. Values obtained in untreated patients were in the normal range

(B) Changes in NDUFV1, COX4 and COX2 protein levels in patient muscle before and after treatment by bezafibrate. The protein signals were quantified by computerized analysis. Results of western-blot analysis are expressed in arbitrary units as means ± SD of three different experiments. *$p<0.001$ versus before treatment (paired t test).

(C) Spectrophotometric assay of Cytochrome C oxidase activity in muscle homogenates before and after treatment by bezafibrate. Values are means ± SD of three to five determinations. *$p<0.001$ versus before treatment (paired t test).

(D) Spectrophotometric assay of Citrate Synthase activity in muscle homogenates before and after treatment by bezafibrate. Values are means ± SD of three to six determinations. *$p<0.001$ versus before treatment (paired t test).