Prevention by L-carnitine of oxidative DNA damage in 3-hydroxy-3-methylglutaric aciduria.

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INTRODUCTION

3-hydroxy-3-methylglutaric aciduria (HMGA) is an inherited disorder of the leucine catabolic pathway in which occurs a deficiency of the 3-hydroxy-3-methylglutaryl-CoA lyase enzyme. Therefore, the organic acids 3-hydroxy-3-methylglutaric (HMG) and 3-methylglutaric (MGA), mainly, accumulate in tissues of affected patients.

The treatment of this disease is based in a restricted protein ingest and supplementation with L-carnitine (LC), an antioxidant and detoxifying agent.

In the present work, we investigated the in vitro and in vivo oxidative damage to DNA induced by the accumulation of organic acids, as well as the effect of the recommended therapy.

MATERIALS AND METHODS

In vitro study

Venous blood specimens were collected under sterile conditions from four healthy volunteers. Leukocytes were isolated and incubated with HMG (1 mM, 2.5 mM or 5 mM) and MGA (1 mM, 2.5 mM or 5 mM) at 37°C for 6 hours. The samples were also co-incubated with LC (90 µM or 150 µM).

The alkaline comet assay was performed as described by Singh et al. and followed the general guidelines for use of the comet assay. The damage classes were classified from 0 (no damage) to 4 (maximal migration) according to tail intensity.
**MATERIALS AND METHODS**

**In vivo study**

Urine samples were obtained at diagnosis from 7 patients (median age 4.5 years, range 1 month-9 years) and during treatment from 7 patients (median age 6.4 years, range 0.3-13 years). The median of treatment duration was 3 years (range 1 month-6 years). The urine samples from the control group were obtained from 9 age-matched healthy children (median age 3 years, range 0.2-6.2 years).

Urinary 8-hydroxy-2’-deoxyguanosine (from DNA), 8-hydroxyguanosine (RNA) and 8-hydroxyguanine (DNA and RNA) levels were defined by the DNA/RNA Oxidative Damage ELISA kit (Cayman Chemical, USA).

**RESULTS**

![Comet assay results](image)

**Figure 1.** In vitro effect of HMG 1 mM co-incubated with L-carnitine (LC 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; # p < 0.05 compared to HMG 1 mM group; ### p < 0.001 compared to HMG 1 mM group; ++ p < 0.01 compared to 90 µM group.

**Figure 2.** In vitro effect of HMG 2.5 mM co-incubated with L-carnitine (LC 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; # p < 0.05 compared to HMG 2.5 mM group; ### p < 0.001 compared to HMG 2.5 mM group; +++ p < 0.001 compared to 90 µM group.

**Figure 3.** In vitro effect of HMG 5 mM co-incubated with L-carnitine (LC 3 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; ### p < 0.001 compared to HMG 5 mM group; +++ p < 0.001 compared to 90 µM group.
RESULTS

Comet assay results

Figure 4. *In vitro* effect of MGA 1 mM co-incubated with L-carnitine (LC 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; ### p < 0.001 compared to MGA 1 mM group; +++ p < 0.01 compared to 90 µM group.

Figure 5. *In vitro* effect of MGA 2.5 mM co-incubated with L-carnitine (LC 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; ### p < 0.001 compared to MGA 1 mM group; + p < 0.05 compared to 90 µM group.

Figure 6. *In vitro* effect of MGA 5 mM co-incubated with L-carnitine (LC 90 µM, 150 µM) on DNA damage (comet assay) in leukocytes from whole blood. Data represent the mean ± SD. ***p < 0.001 compared to control group; ### p < 0.001 compared to MGA 5 mM group; +++ p < 0.01 compared to 90 µM group.

Urinary oxidized guanine species result

Figure 7. Urinary oxidized guanine species measurement in urine from patients with HMGA and control. Group A represent patients at diagnosis and Group B patients under treatment. Data represent the mean ± SD. ***p < 0.001 compared to control group; ## p < 0.01 compared to group A.
The present study evaluated the in vitro effect of LC on DNA damage induced by HMG and MGA in leukocytes from healthy individuals, using alkaline comet assay. We firstly verified a significantly greater level of DNA damage in leukocytes treated with both HMG and MGA concentrations compared to control groups.

In what concern to the in vitro effect of LC upon DNA damage induced by HMG and MGA, we observed that LC 90 µM and 150 µM reduced almost all organic acids tested concentrations, with 150 µM of LC the most effective since DNA damage index was reduced to control levels for all concentrations of both HMG and MGA. Our results demonstrate that the deleterious effects of HMG are more difficult to be reversed by LC than those caused by MGA, thus corroborating with the proposition that HMG is the most toxic metabolite of the disease.

We also verified a significant increase of guanine oxidized species levels in urine patients samples. The presence of guanine oxidized species (8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine and 8-hydroxyguanine) means oxidative DNA damage. It can occur as a result of the attack of reactive oxygen species to both nuclear and mitochondrial DNA. DNA is constantly attacked by endogenous and exogenous sources, which may affect its integrity, provoking lesions. The treatment with LC reestablished guanine oxidized species in this patients, demonstrating that this compound contribute to redox homeostasis in this disease.

In conclusion, we reported for the first time that HMGA patients have oxidative DNA damage. Moreover, our findings highlights the importance of the antioxidant therapy with LC in these patients, since it can prevent in vitro DNA damage induced by HMG and MGA by removing intracellular toxic metabolites and promoting antioxidant protection.

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REFERENCES