EXPRESSION OF A STABLE, BIOLOGICALLY ACTIVE FORM OF HUMAN RECOMBINANT PARAOXONASE-1, A POWERFUL ANTIOXIDANT AND PROTECTOR FROM ORGANOPHOSPHATE-INDUCED TOXICITY.

INTRODUCTION

Paraoxonase-1 (PON-1) is a high density lipoprotein (HDL)-associated calcium-dependent esterase with antioxidant and anti-atherogenic properties. The activity of paraoxonase-1 is essential in the pathogenesis of many disease states, including metabolic syndrome, cardiovascular and Alzheimer’s disease. Paraoxonase-1 also plays a crucial role in the metabolism and detoxification of insecticides and pesticides (1).

Serum paraoxonase-1 (PON-1) is a high-density lipoprotein (HDL)-associated calcium-dependent esterase with antioxidant and anti-atherogenic properties. The activity of paraoxonase-1 is essential in the pathogenesis of many disease states, including metabolic syndrome, cardiovascular and Alzheimer’s disease. Paraoxonase-1 also plays a crucial role in the metabolism and detoxification of insecticides and pesticides (1).

METHODS

Construction of the paraoxonase-1 cloning vector

The coding region for human paraoxonase-1 was amplified from human liver cDNA using a set of gene specific primers and ligated into a plasmid vector under the control of a CMV promoter. The construct of paraoxonase-1 gene expression vector was confirmed by restriction enzyme digestion and DNA sequencing. The recombinant plasmid was propagated in E. coli and purified using the Qiagen plasmid mini-prep kit.

Stability of human recombinant PON-1

The expressed, soluble, full length paraoxonase-1 protein had a molecular weight of 42.9 kD –as determined by western blotting using an anti-human paraoxonase-1 antibody.

RESULTS

Immunoreactivity of the human recombinant PON-1

The expressed, soluble, full length paraoxonase-1 protein had a molecular weight of 42.9 kD –as determined by western blotting using an anti-human paraoxonase-1 antibody.

Stability of the human recombinant PON-1

The bioactivity of paraoxonase-1 present in the cell lysate was demonstrated by its ability to hydrolyse 4 mM phenyl acetate in 20 mM Tris HCl buffer pH 8.0, containing 1 mM CaCl₂. As a control 10 mM EDTA was added. As expected the control was not hydrolysed and the rate of phenol formation was significantly reduced in the presence of 1 mM CaCl₂.

CONCLUSIONS

• These data demonstrate the expression of a biologically active human recombinant paraoxonase-1 protein in the mammalian cell line CHO as shown by the capacity to dephosphorylate para-nitrophenyl phosphate.

• The protein is immuno-reactive as demonstrated by western blot analysis

• This human recombinant paraoxonase-1 may be used as a control on western blots or as a tool to investigate paraoxonase-1 activity in vivo.

REFERENCES

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