

ORIGINAL ARTICLE

Caspase 3 Activity in Kidney Tissues From Mice (Balb/C) treated with Teratogenic Agents During Pregnancy

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ABSTRACT

Introduction

All-trans retinoic acid (All-Trans-R-A), 13-cis retinoic acid (13-Cis-R-A), thalidomide (Th) and hydroxyurea (HU) are considered to be teratogenic and toxic agents. Although the side effects of these agents have been documented in mammalian tissues years ago, they are recently used to treat certain types of blood cancer, viral infections, skin and mucosal diseases. According to current international literature the referred substances induce apoptosis of several cell types in mammalian organs. Caspase 3 is recognized as a molecular effector of apoptosis.

Aim of the study

We have studied the effect of the above substances on caspase 3 activity in kidney crude and subcellular fractions of pregnant mice.

Materials and methods

For this experimental study we have treated pregnant Balb/C mice ~30 gr during their midgestational period, for three days with: a) corn oil, b) All-Trans- R- A, 50 mg/kg c) 13-Cis R-A, 50 mg/kg d) NaCl 9%, e) Th 15,45 mg/kg f) HU 4.56 mg/kg. The animals were sacrificed on the 18th day of pregnancy, the kidneys were removed, washed, weighted and homogenated with a Potter Elvehjem homogenator. Following subcellular fractionation according to the method of Nordlie and Lardy, the caspase 3 activity was determined in nuclear, cytosolic + ribosomal fractions and crude, by a colorimetric assay (kit, Sigma), based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-Nitroaniline (pNA) moiety, according to the method of Nicholson. The caspase 3 activity was calculated in $\mu\text{mol pNA}$ released per min per ml.

Results

Our results show reduced caspase 3 activity in crude and subcellular fractions of pregnant mice kidneys, under the effect of the tested teratogenic agents, except of the cytosolic plus ribosomal kidney fraction after All-Trans-R-A administration.

Conclusion

Known teratogenic agents when administered in pregnant mice may influence apoptosis in kidneys by different mechanism than caspase pathway. These results are encouraging to continue the research on the apoptosis induced by teratogenic agents.

Keywords: *teratogenic drugs; caspase 3; apoptosis; mice kidney; pregnant mice kidney; thalidomide; hydroxyurea; All-Trans-R-A; 13-Cis-R-A*

INTRODUCTION

One of the cellular responses to DNA damaging events, for example cytotoxic agents, is the activation of programmed cell death, also known as apoptosis.¹

Caspase-3 works as an effector, cleaving various death substrates that ultimately cause morphological and biochemical changes in apoptotic cells.² In normal and healthy cells, caspases normally lie dormant. In response to diverse stimuli they become activated when cell death is required.³

Thalidomide (Th, $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_5$) is known for its teratogenic effects, especially phocomelia, related to its antiangiogenic properties. Th is clinically useful as a chemotherapeutic in numerous cancers. Antitumor activity is related to a number of known properties, including anti-tumor necrosis factor (TNF)-alpha and T-cell costimulatory and antiangiogenic activities. However, the drugs may also involve direct antitumor effects.⁴ Th influences the Bcl-2 expression, a gene which is also involved in apoptosis.⁵

Hydroxyurea (HU, $\text{H}_2\text{NCONHOH}$, Hydrea) is an analog of urea whose mechanism of action is through inhibition of DNA synthesis, exerting its lethal effect on cells in S phase by inhibiting the enzyme ribonucleotide reductase, resulting in the depletion of deoxynucleoside triphosphate pools. Its major uses as a chemotherapeutic drug are in melanoma and chronic myelogenous leukemia.⁶ However, it plays a secondary role in both these circumstances. The major adverse effect of the drug is bone marrow depression. HU acts as a chemotherapeutic agent useful in neoplastic diseases and as anti-retroviral drug as well.

All-Trans-R-A as well as 13-Cis-R-A are retinoids, nat-

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