Hypertrophied Rat Kidney after Treatment with Hydroxyurea and Thalidomide

INTRODUCTION

It is known that plasminogen is an inactive form of plasmin that occurs in plasma and is converted to plasmin by organic solvents. The transformation of plasminogen to plasmin is crucial in many physiological and pathological processes, required for extracellular proteolysis regulation. It is referred that plasminogen activation system is involved in tissue regeneration (rat liver regeneration), cancer (human breast cancer) and inflammation. Plasminogen is abundant in human plasma and extracellular fluids (~0.15 mg ml⁻¹). It is converted to the proteolytic form plasmin, by eukaryotic activators, such as tissue-type plasminogen activator (tPA) and urokinase. Plasminogen is inhibited by PIs (plasminogen inhibitors). Following injury, uPA and

ABSTRACT: Hydroxyurea (HU) and Thalidomide (Th), apart from their properties, are discussed that can induce apoptosis in several cell types and tissues. The aim of this study is to analyse the effect of HU and Th in plasmin(ogen) activation system on proteolytic activity of normal and hypertrophied rat kidney, in the absence or presence of euglobulin as a source of plasminogen. Adult male Wistar rats 230-260 g of weight and aged of six months were divided into two main groups A and B. Animals belonging to the second group were operated to unilaterally nephrectomy. Both groups were divided in subgroups, i.e.: Group A to subgroups A1, A2, A3 and A4 and Group B to subgroups B1, B2, B3 and B4. The animal subgroups were injected with HU and Th as following: Group A1 and B1 were injected with NaCl 9% (Controls), groups A2 and B2 were injected with HU (4.5 mg/k.b.w), groups A3 and B3 were injected with Th (15.45 mg/k.b.w.) and groups A4 and B4 were injected with HU and Th in combination as above. The proteolytic activity of hypertrophied and no hypertrophied rat kidneys was determined in 0.1 ml of homogenated kidney tissue according to Goodwin’s method in the presence or absence of euglobulin, as a source of plasminogen, and estimated from the amino acids released in a fresh casein buffer. Euglobulin was prepared according to the method of Hougie. Our results showed increased proteolytic activity in normal rat kidney tissue under the effect of HU and Th alone or in combination of treatment, which was much more evident in the absence of euglobulin. This increase may be due to plasminogen activators release. In contrast, the proteolytic activity of hypertrophied rat kidney was found decreased at 72 hours, statistically significant in the presence of euglobulin. Our results suggest that these findings will contribute to elucidate the mechanisms of HU and Th action in extracellular proteolysis of renal cells, concerning activation of the plasmin(ogen) system, and will probably demonstrate new strategies to regulate renal cell proteolysis at renal pathology.

Key Words: Hydroxyurea, Thalidomide, Cytotoxic drugs, Plasminogen, Euglobulin, Hypertrophied kidney, Hyperplasia, Proteolysis, Apoptosis.

INTRODUCTION

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Hydroxyurea (HU, H₂NCONHOH, Hydrea) is representative of a group of compounds that has as primary site of action the enzyme ribonucleoside diphosphate reductase. This enzyme, which catalyzes the reductive conversion of ribonucleotides to deoxyribonucleotides, is a crucial and probably rate-limiting step in the biosynthesis of DNA. It is also specific for the S phase of the cell cycle, in which concentrations of the target reductase are maximal, and it causes cells to arrest at the G₁-S interface. HU also has been shown to be converted in vivo to nitric oxide, to induce the expression of a number of genes [for TN, interleukin-6 (IL-6), β-globin, etc.], and to accelerate the loss of extrachromosomally amplified genes present in double-minute chromosomes. The clinical relevance of these actions is unknown.

Thalidomide (Th, C₁₅H₂₁N₂O₄) is well known several years before for severe, life-threatening birth defects which has caused when administered to pregnant women in the past. This drug has been discussed as a potent angiogenesis inhibitor in vivo. Although the exact mechanism by which Th inhibits angiogenesis is unknown, Th has been shown to affect levels of tumor necrosis factor-alpha and interferon-gamma and has shown antitumor activity in patients with refractory multiple myeloma, a disease that is characterized by prominent bone vascularization as well as in RCC (Renal Cell Carcinoma).

Thalidomide is an immunomodulatory agent that blocks angiogenesis inhibits cytokines (tumor necrosis factor-[alpha], basic fibroblast growth factor and vascular endothelial growth factor) and modifies cell adhesion molecule expression. Based on this activity, thalidomide has successfully been applied in the treatment of various malignancies including multiple myeloma and Waldenstrom's macroglobulinemia, glioma, Kaposi's sarcoma, and malignant melanoma, as well as erythema nodosum leprosum. Recently, several studies indicated activity of thalidomide-based regimens also in metastatic RCC with response rates ranging from 0 to 22% and disease stabilization in 13-64% of patients. Nevertheless, several authors reported considerable thalidomide-related, dose-dependent toxicity, especially somnolence, constipation, lethargy, venous thromboembolism and, increasing with prolonged therapy, neurotoxicity.

In a previous experimental work, we have described that HU and Th influence the proteolysis in rat spleen and also at the intestinal epithelial cells. In the present study we analyse the proteolytic mechanisms of plasmin(ogen) activation system under the influence of HU and Th, alone or in combination of treatment, in normal as well as in hypertrophied rat kidney (in the presence or absence of euglobulin as a source of plasminogen).

**MATERIALS AND METHODS**

In this study we used male rats (Wistar) of 230-260 g of weight. At the half of them (group B) we have operated the animals by unilateral nephrectomy according to the method of Waynforth (1980). In addition, nephrectomized (Group B) and no nephrectomized rats (Group A) were divided in four groups. Groups A₁, B₁ (controls) were injected intramuscularly with NaCl 9‰. Groups A₂, B₂ were injected with HU (Fluka) 4.56 mg/k.b. weight. Groups A₃, B₃ were injected with Th (Sigma) 15.45 mg/k.b. weight, and groups A₄, B₄ with HU and Th were injected in combination, as above. Animals remained in starvation after treatment for 24 hours and were sacrificed 24, 48 and 72 hours after treatment. After percutaneous laparotomy the kidneys were rapidly removed, washed in an isotonic solution of KCl, weighted and then homogenized with a Potter Elvehjem homogenizer in a sucrose solution 8.5% (final tissue concentration of kidney: 1 g tissue/20 ml sucrose solution 8.5%). For our analyses we have used 0.1 ml of homogenated tissue for proteolytic activity determination, in the presence or absence of euglobulin, as a source of plasminogen. 0.1 ml of euglobulin was added in each sample for proteolytic activity determination only in the case with the presence of euglobulin. Euglobulin is a source of plasminogen and it was prepared from plasma following the Hougie’s method (1972). Proteolytic activity was determined according to Goodwines method (1974), and estimated from the aminoacids (mg per %) released in a fresh casein buffer pH 7.4, after 0, 2 and 4 hours of incubation, at 37°C. The numbers in the figures correspond to mean values of 5 animals ± S.D. Statistically significant
RESULTS

Normal rat kidney

Our results have shown increased proteolytic activity in normal tissue under the effect of HU and Th alone or in combination (Figure 1), which was much more evident in the absence of euglobulin (Figure 1. A, B, C).

Hypertrophied rat kidney

Proteolytic activity of rat hypertrophied kidney in the absence of euglobulin

We have found decreased proteolytic activity of rat hypertrophied kidney after 24 and 72 hours of HU (Figure 2.A, E) and Th administration (Figure 2.E). In contrast, this activity was identified increased when both of the above drugs (HU and Th) were injected to the animals (Figure 2.E).

Proteolytic activity of rat hypertrophied kidney in the presence of euglobulin

Enhanced proteolytic activity was also found at 48 hours from nephrectomy when both cytotoxic drugs were injected (Figure 2.D). In contrast, this activity in rat hypertrophied kidney (72 hours) was found significantly diminished when treated with HU and Th alone or in combination, in the presence of euglobulin (Figure 2.F).
**DISCUSSION**

In the international literature, scarcely can be found experimental studies concerning the correlation between hypertrophied kidney and the activation system of plasminogen, therefore it is a pioneer study explaining our results in this.

In our experiments we found increased proteolytic activity under the influence of HU and Th (Figure 1.A, G). The prementioned increase was much more evident without the presence of euglobulin. These findings indicate that after administration of the above drugs, plasminogen activators may be released, in the untreated as well as in hypertrophied rat kidney (Figure 2.E, F). Urokinase (u-PA) dissolves and removes fibrin deposits in the renal secretory pathways in various renal diseases. The reduced proteolytic activity we have found in hypertrophied kidney after treatment with Th (Figure 2.E, B) may due to release of plasminogen activator inhibitor (PAI). It is referred that PAI-1 is the major physiologic inhibitor of plasminogen activators (tissue-type plasminogen activator and urokinase-type plasminogen activator) in vivo and has been implicated in extracellular matrix accumulation by its effects to inhibit matrix degradation. PAI-1 synthesis is stimulated by TGF-β in normal and nephritic glomeruli. Angiotensin II and aldosterone stimulate expression of PAI-1 in vitro in a number of cell types and organs as in kidney.

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**Figure 2.** Proteolytic activity of rat hypertrophied kidney (24, 48 and 72 hours after HU and Th administration) in the absence (A, C, E) or in the presence (B, D, F) of euglobulin. The numbers in the figures represent the mean values of 5 animals ± S.D. Statistically significant according to «t» test of significance: ***p ≤ 0.001, **p ≤ 0.002, *p ≤ 0.01.
Th has been recently discussed as an anti-angiogenic agent on Renal Cell Cancer (RCC)\textsuperscript{37}.

Our findings proved reduced proteolytic activity in hypertrophied rat kidney in the absence of euglobulin (Figure 2.A) and are in agreement with international literature. It is referred that induced expression of plasminogen activator inhibitor type-1 (PAI-1), a major negative regulator of pericellular plasmin generation, accompanies wound healing and repair \textit{in vitro} and \textit{in vivo}. In addition it is referred that transcriptional control of the PAI-1 gene is superimposed on a growth state-dependent program of cell activation\textsuperscript{38}. In addition, PAI-1 induction is an early event in creation of the wound-activated phenotype and it appears to participate in the regulation of renal epithelial cell motility during \textit{in vitro} injury resolution\textsuperscript{39}. \textit{In vitro} studies have indicated that some uremic toxins induce oxidative stress and activate NF-kappaB to upregulate plasminogen activator inhibitor-1 in tubular cells\textsuperscript{40}.

The increased proteolytic activity we have found in hypertrophied rat kidney after 24 hours of HU administration (Figure 2.F) was appeared much later than 24 hours, time depended of HU action in pharmacokinetics. PAI-1 mRNA expression is rapidly up-regulated in response to wounding (or healing) with inductive kinetics approximating that of serum-stimulated cultures. PAI-1 synthesis in cells that locomote into the wound field is continued until injury closure\textsuperscript{39}.

The diminished proteolytic activity which we found after Th administration indicate that Th at 72 hours in hypertrophied rat kidney, in the presence of euglobulin may be explained by its antiangiogenetic action. Indeed, antiangiogenesis agents like thalidomide may be able to achieve disease stabilization by cytostatic inhibition of further tumor growth\textsuperscript{41}. We suggest that these findings will help to elucidate the mechanisms of HU and Th action in extracellular proteolysis of renal cells, concerning the plasmin(ogen) system, and will contribute probably to demonstrate and design new strategies to regulate renal cell proteolysis at non normal and pathological situations also. Our study will be continued in directions of new applications of HU and Th in modern therapies of renal disease\textsuperscript{42}.
REFERENCES


