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Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: informahealthcare.com/journals/irnf20

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To cite this article: Valeria Cernaro, Maria Antonietta Medici, Giuseppa Leonello, Antoine Buemi, Franz Heinrich Kohnke, Antonino Villari, Domenico Santoro & Michele Buemi (2015) Auxin induces cell proliferation in an experimental model of mammalian renal tubular epithelial cells, Renal Failure, 37:5, 911-913, DOI: 10.3109/0886022X.2015.1015683

To link to this article: https://doi.org/10.3109/0886022X.2015.1015683

Published online: 24 Feb 2015.

RENAL FAILURE

http://informahealthcare.com/rnf
ISSN: 0886-022X (print), 1525-6049 (electronic)

Ren Fail, 2015; 37(5): 911–913
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LABORATORY STUDY

Auxin induces cell proliferation in an experimental model of mammalian renal tubular epithelial cells

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Abstract

Indole-3-acetic acid is the main auxin produced by plants and plays a key role in the plant growth and development. This hormone is also present in humans where it is considered as a uremic toxin deriving from tryptophan metabolism. However, beyond this peculiar aspect, the involvement of auxin in human pathophysiology has not been further investigated. Since it is a growth hormone, we evaluated its proliferative properties in an *in vitro* model of mammalian renal tubular epithelial cells. We employed an experimental model of renal tubular epithelial cells belonging to the LLC-PK1 cell line that is derived from the kidney of healthy male pig. Growth effects of auxin against LLC-PK1 cell lines were determined by a rapid colorimetric assay. Increasing concentrations of auxin (to give a final concentration from 1 to 1000 ng/mL) were added and microplates were incubated for 72 h. Each auxin concentration was assayed in four wells and repeated four times. Cell proliferation significantly increased, compared to control cells, 72 h after addition of auxin to cultured LLC-PK1 cells. Statistically significant values were observed when 100 ng/mL ($p < 0.01$) and 1000 ng/mL ($p < 0.05$) were used. In conclusion, auxin influences cell growth not only in plants, where its role is well documented, but also in mammalian cell lines. This observation opens new scenarios in the field of tissue regeneration and may stimulate a novel line of research aiming at investigating whether this hormone really influences human physiology and pathophysiology and in particular, kidney regeneration.

Keywords

Auxin, growth hormone, indole-3-acetic acid, kidney, regeneration

History

Received 17 December 2014
Accepted 16 January 2015
Published online 24 February 2015

Introduction

The term ''auxin'' refers to a class of phytohormones that are responsible for plant growth and development. The main auxin produced by the plants is indole-3-acetic acid that modulates cell division, extension and differentiation and is crucial in several processes including tropisms, root initiation, apical dominance and senescence.[1]

This hormone is also present in mammals where it is able to influence cell plasticity under experimental conditions. A recent work has pointed out that auxin (henceforth this term will be used to indicate indole-3-acetic acid) and another plant hormone, the cytokinin isopentenyl adenosine, accelerate the cell cycle and regulate pluripotency genes so facilitating the reprogramming of mouse somatic cells into induced pluripotent stem cells. This results to be interesting because both the hormones are present in human cells as well.[2]

In humans, auxin is regarded as one of the uremic toxins deriving from tryptophan metabolism[3] and its serum levels increase as renal function declines;[4] furthermore, some authors observed that probably auxin is not only a biomarker of kidney function but also a factor that, together with other uremic solutes, contributes to thrombotic risk in patients with severe chronic kidney disease.[5,6]

However, beyond this peculiar aspect, the involvement of auxin in human pathophysiology has not been further investigated and currently, we do not know yet where and why it is produced and what is its function under normal conditions.[7]

Since auxin is a plant growth hormone but it is found even in humans for unknown reasons, we evaluated its effects on mammalian cell proliferation.

Materials and methods

We employed an *in vitro* model of renal tubular epithelial cells belonging to the LLC-PK1 cell line that is derived from the kidney of healthy male pig.

Auxin was purchased from Sigma-Aldrich® (Milano, Italy). Growth effects of auxin against LLC-PK1 cell lines were determined by a rapid colorimetric assay, using MTT. In this assay soluble MTT was metabolized by mitochondrial

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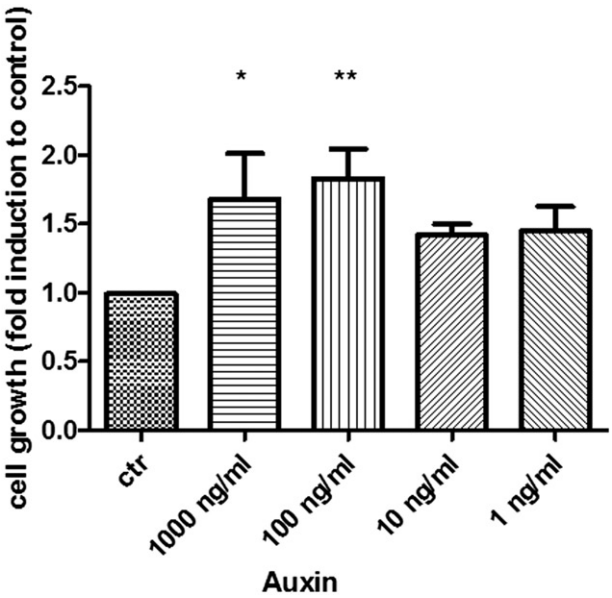

Figure 1. Cell growth expressed as fold induction with respect to control cells after addition of different amounts of auxin to cultured LLC-PK1 cells. *$p < 0.05$; **$p < 0.01$.

enzyme activity of viable cells into an insoluble colored formazan product. Subsequently, formazan was dissolved in DMSO and measured spectrophotometrically at 540 nm. Briefly, 100 µL of cell suspension (4×10^4 cells per mL of RPMI without phenol red, with 10% FCS) were seeded in 96-well microplates. Increasing concentrations of auxin (to give a final concentration from 1 to 1000 ng/mL) were added and microplates were incubated (37 °C, 5% CO_2 air humidified) for 72 h. For the cell control (ctr), wells contained only the cells in the media without auxin. To evaluate cell growth, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h. Then, gently, the medium containing MTT was replaced by DMSO and pipetted to dissolve any formed formazan crystals. Absorbance was then determined at 540 nm by enzyme-linked immunosorbent assay (ELISA) plate reader. Each auxin concentration was assayed in four wells and repeated four times.

Statistical analyses and data plotting were performed using GraphPad Prism software. Results were expressed as mean ± SEM of four biological replicates. One-way analysis of variance (ANOVA), followed by Dunnet comparison post-test, was used to analyze the differences in cell growth assays. p-Values <0.05 were considered significant for all analyses.

Results

Cell proliferation significantly increased, compared to control cells, 72 h after addition of auxin to cultured LLC-PK1 cells. Statistically significant values were observed when 100 ng/mL ($p < 0.01$) and 1000 ng/mL ($p < 0.05$) were used (Figure 1).

Discussion

Our study demonstrates that auxin influences cell growth not only in plants, where its role is well documented, but also in mammalian cell lines. The observation that auxin can be involved in the regenerative process also in mammals is challenging and opens new scenarios in the field of tissue fibrosis and regeneration. Indeed, studying molecular mechanisms that trigger and/or downregulate auxin activity could allow us to understand why it is present in humans too and elucidate the reasons why the effects of this highly conserved growth factor on human cell development and proliferation are apparently less evident as compared to how they are in plants. Probably, auxin regenerative abilities have been negatively modulated in humans in the course of evolution. One might speculate that this has happened in order to limit tissue regeneration potential and then avoid or reduce the risk of cancer development that could result from the stimulation of cell proliferation.[8]

This would be in line with the noteworthy differences in the regenerative properties shown by other growth hormones, such as erythropoietin, between simpler and more complex organisms.[9] Moreover, it has been demonstrated that auxin is implicated in plant tumors[10] and that the plant receptor for auxin, called TIR1, is similar to ubiquitin ligases that are known to influence cell division in different human cancers; in particular, auxin is believed to improve the binding of TIR1 to its specific peptide target.[11,12]

In conclusion, the preliminary results we report about the proliferative effects of the plant growth factor auxin on mammalian renal tubular cells may stimulate a new line of research aiming at investigating whether this hormone really influences human physiology and pathophysiology and in particular, kidney regeneration. Experimental models such as that employed in the present study can be helpful to elucidate the molecular pathways involved in the mechanism of action of auxin. Furthermore, it could be interesting to evaluate whether there is some release of auxin by tumour cells. This might confirm our hypothesis and provide new perspectives in the knowledge of tissue regeneration and in cancer therapy.

Declaration of interest

The authors report no conflicts of interest.

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