Striking Growth-inhibitory Effects of Minocycline on Human Prostate Cancer Cell Lines

Francesca Regen, Isabella Heuser, Irmelin Herzog, and Julian Hellmann-Regen

OBJECTIVE To elucidate a hypothetical link between retinoic acid (RA) signaling and minocycline for targeting prostate carcinoma (PCA). RA signaling has been implicated in growth-inhibition of malignant PCA, and intracellular RA homeostasis has been investigated as a potential therapeutic target. Minocycline is a tetracycline antibiotic with pleiotropic actions in many tissues and reaches comparably high levels in human prostate tissue. Interestingly, minocycline exhibits the rare side effect of a pseudotumor cerebri, which is otherwise known to occur from vitamin A intoxication or in retinoid therapy. Therefore, we hypothesized minocycline to putatively interact with intracellular RA homeostasis in PCA.

METHODS Using LN-CAP, DU-145, and PC-3 cell lines, effects of minocycline on microsomal RA metabolism and on cell growth were assessed in vitro.

RESULTS Minocycline was identified to potently inhibit cell growth, at concentrations within the range of tissue levels readily reached under standard therapeutic conditions. In vitro inhibition experiments revealed inhibition of RA breakdown, yet only at comparably high concentrations of minocycline. Using all trans-RA, RA metabolism inhibitor liarozole, and different retinoid receptor antagonists, the putative RA-dependent effects of minocycline were further evaluated and confirmed to be independent of RA signaling.

CONCLUSION Our findings add to the growing body of evidence for the many pleiotropic actions of minocycline. In view of the striking effects of minocycline on cell growth in PCA cell lines in vitro and its relatively safe side effect profile, the use of minocycline for targeting PCA should be timely clinically evaluated. UROLOGY 83: 509.e1–509.e6, 2014. © 2014 Elsevier Inc.

Minocycline is a widely used and well-established tetracycline antibiotic that is used in a variety of infectious conditions, exhibiting excellent penetration to the brain,[1] the bone, and to the prostate.[2-4] Pleiotropic actions far beyond its antibiotic properties are increasingly being recognized, especially in the field of cancer research[5] and neuroscience,[6] in which minocycline is known to act as a potent inhibitor of microglial activation,[7] and also in the field of dermatology, in which potentent anti-inflammatory effects of minocyclin closely approximate therapeutic efficacy of retinoic acid (RA), the most potent antiacne treatment available. Interestingly, minocycline has repeatedly been demonstrated to be associated with the development of the rare neurologic condition of a pseudotumor cerebri, a condition that is well known to be otherwise associated with systemic retinoid therapy or hypervitaminosis A.[8] This coincidence of a similar, yet rare side effect caused by 2 at first sight fairly divergent compounds led us to the hypothesis of minocycline potentially interacting with intracellular RA homeostasis.

RA homeostasis has been intensely investigated in the context of prostate carcinoma (PCA) in animal models since the 1970s and is recognized as a mechanism relevant to PCA carcinogenesis.[9,10] In fact, RA levels have previously been demonstrated to be pathologically lowered in human PCA vs healthy prostate tissue,[11] and RA metabolism-inhibitors are effective in inhibiting PCA growth, possibly via elevating intracellular RA levels.[12,13] Likewise, tetracyclines, especially doxycycline,[14-17] and other nonantibiotic "chemically modified" tetracyclines[18] have been demonstrated to show cytostatic and even immediate cytotoxic effects toward human cancer cell lines. Furthermore, tetracyclines have been revealed to act as inhibitors of matrix metalloproteinases, exhibiting antimetastatic effects in bone metastasis models, including PCA cell lines.[14,15,19] The exact molecular actions underlying the antimetastatic, cytostatic, and cytotoxic effects of tetracyclines, however, are still not

Financial Disclosure: *The authors declare that they have no relevant financial interests.*
Funding Support: *This work was solely supported by an independent research grant from the Charité, University Medicine Berlin.*

From the Department of Psychiatry, Clinical Neurobiology, Charité – Campus Benjamin Franklin, Berlin, Germany

Reprint requests: Julian Hellmann-Regen, M.D., Department of Psychiatry, Clinical Neurobiology, Charité – Campus Benjamin Franklin, Eschenallee 3, 14050 Berlin, Germany. E-mail: julian.hellmann@charite.de

Submitted: July 12, 2013, accepted (with revisions): October 21, 2013

© 2014 Elsevier Inc.
All Rights Reserved

http://dx.doi.org/10.1016/j.urology.2013.10.029

fully understood.[19] Likewise, local pharmacokinetic interactions for the tetracycline minocycline with endogenous RA metabolism are unknown to date.

Therefore, we aimed at systematically investigating a putative novel mechanism of action of minocycline by determining whether and to which extent minocycline might target RA turnover in human PCA models. Furthermore, we quantified direct effects of minocycline, RA, and select retinoid antagonists on cell growth of PCA cell lines in vitro.

MATERIALS AND METHODS

Cell Culture and Tissue Preparation

Three well-established human PCA cell lines PC-3, DU-145, and LN-CAP were generously provided by Hans Krause, Ph.D., Department of Urology, Charité, University Medicine Berlin. All 3 cell lines were cultured in a humidified atmosphere at 37°C and 5% CO_2 using Dulbecco's Modified Eagle Medium with 10% heat-inactivated fetal calf serum. All substances were from Sigma-Aldrich (Taufkirchen, Germany) unless otherwise stated.

Assessment of Cell Growth and Viability

Cell growth curves were obtained using the well-established 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide-based (MTT) colorimetric method in a 96-well format. Cells of all the 3 cell lines were seeded at an initial low density of 1*10^3 cells/well in 100-µL culture medium. After allowing cells to attach for 24 hours, cells were simultaneously treated with the active compounds or solvents only (sham). All compounds were dissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich, Taufkirchen, Germany) unless otherwise stated with final DMSO concentrations never exceeding 0.1% (v/v). At each time point, an equal proportion of each 96-well plate was treated with 20 µL of sterile MTT working solution (prepared as 5 mg/mL in phosphate buffered saline). MTT-treated samples were allowed to incubate in the incubator. After exactly 4 hours of incubation, the supernatants of MTT-treated samples were carefully but completely removed by vacuum, and 100 µL of PBS was added. At the final time point, the plate was treated with 100 µL of MTT-solvent (4 mM hydrochloric acid and 0.1% Nonidet NP-40, all in isopropanol) per well subsequent to MTT incubation, and absorbance was read in a microplate reader (Biorad 500-model, Biorad, Germany) at 550 nm.

RA Quantification by High Performance Liquid Chromatography

RA quantification was performed in principle as previously described[20,21] with minor modifications. In brief, retinoid-containing methanolic extracts of in vitro RA metabolism assay-reactions were subjected to reversed-phase high performance liquid chromatography (HPLC) analysis on an Agilent 1100 model liquid chromatography system (Agilent Technologies, Santa Clara, CA) equipped with a Supelco Suplex pkb-100 solid phase (Sigma-Aldrich), isocratically eluted using a mobile phase composed of acetonitrile, 2% (w/v) ammoniumacetate, methanol, glacial acetic acid, and n-butanol (69:16:10:3:2; v/v) and detected using a high sensitivity 1290 Infinity diode array detector (Agilent Technologies, Böblingen, Germany). Peak purity was monitored using online spectral analysis and compared with authetic RA standards (Santa Cruz Biotechnology, Santa Cruz, CA).

In Vitro RA Metabolism Assays

In vitro pharmacokinetic assays, including the preparation of microsomes from cultured cells were performed in principle according to previously published protocols.[22,23] In brief, metabolically active microsomal preparations from 3 different human PCA cell lines LN-CAP, PC-3, and DU-145 were diluted in phosphate buffer (pH = 7.4, 100 mM) to yield a final protein concentration of 500 µg/mL in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH; 800 µg/mL), all-trans RA (1 µM), and various concentrations of minocycline (0, 1, 20, 200 µM) in a total volume of 60 µL. Each assay contained vehicle controls (VEH; DMSO only) and appropriate inactive controls (inactive), which were gained after heat inactivation (5 minutes at 95°C) of microsomes before the assay. RA stock solution was prepared at 30 mM in DMSO, minocycline was prepared as a 100 mM stock solution. Assays were prepared in 1.5 mL microcentrifuge polypropylene reaction tubes, initiated by the addition of RA and NADPH and allowed to incubate for 1 hour at 37°C in a shaking water bath in the dark. Assays were stopped by the addition of 240 µL of ice-cold methanol. After the addition of methanol, reaction tubes were vortexed thoroughly, placed at −20°C for 30 minutes and centrifuged at 14,000 ×*g* at 4°C. Retinoid-containing supernatants were subsequently subjected to HPLC analysis as outlined previously.

Statistical Analyses

Values are always presented as means ± S.E.M. from at least 3 samples per group. All experiments have been repeated at least twice with similar results. HPLC measurements were always performed in duplicate for each sample. All numerical analyses were performed using the statistical software GraphPad Prism (Ver. 5.04, GraphPad Software, Inc., LaJolla). Differences between group means were analyzed by one-way analysis of variance followed by Tukey's post hoc where appropriate. $P < .05$ was considered statistically significant.

RESULTS

Interaction of Minocycline With RA Metabolism

To quantify putative effects of minocycline on RA metabolism in PCA cell lines, equal amounts of microsomal preparations of all 3 human PCA cell lines (LN-CAP, PC-3, and DU-145) were subjected to in vitro RA metabolism assays. Figure 1A-C compares the dose-dependent effects of minocycline on RA metabolism, indicating the presence of strong RA metabolism in all 3 cell lines compared with heat-inactivated controls. Minocycline results in a dose-dependent inhibition, for all 3 cell lines equally, only at the highest concentration of 200 µM.

Retinoid Signaling and PCA Cell Growth

To estimate the relevance of retinoid signaling in significantly modifying in vitro growth characteristics, cells were seeded at a very low initial density of 5*10^2 cells per well and grown for 7 days in the presence of widely used RA receptor antagonists, including the pan-RAR antagonist RO-41-5253 (RO; 10 µM), RAR-γ

Download English Version:

https://daneshyari.com/en/article/3899441

Download Persian Version:

https://daneshyari.com/article/3899441

Daneshyari.com