Cytoprotective effect of a synthetic triterpenoid against oxidative Stress in human umbilical vein endothelial cells (HUVEC)

[2]Phillip Bowman, [1,2]Xinyu Wang, [2]James Bynum, [1,2]John Yang, and [1]Salomon Stavchansky
[1]Pharmaceutics Division, College of Pharmacy, University of Texas, Austin TX and
[2]United State Army Institute of Surgical Research, San Antonio, TX 78234.

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Abstract

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Induction of phase II enzymes, in particular the 32 kd stress protein heme oxygenase-1 (HO-1), is cytoprotective in human endothelial cells. We previously demonstrated that caffeic acid phenethyl ester (CAPE) was cytoprotective against menadione-induced oxidative stress in HUVEC largely via the induction of HO-1.* Here, we tested the cytoprotective activity of 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), a new synthetic triterpenoid (Dr. Michael Sporn, Dartmouth University) against oxidative stress. Dose response studies indicated that CDDO-Im at 200 nM was more cytoprotective against menadione toxicity than an optimal dose of CAPE (20 µM), resulting in endothelial cell survival of 80% compared to 60% for CAPE. Messenger RNA for HO-1 was increased 90-fold in the presence of CDDO-Im, while only 13-fold by CAPE. Western blot analysis of HO-1 protein product indicated that by 6 h, CDDO-Im induced an 8-fold higher level of HO-1 while CAPE induced a 2-fold increase. The results indicate that CDDO-Im is a much more potent cytoprotectant than CAPE, and this beneficial effect correlates well with the induction of HO-1.
*Wang X, et al.. Eur J Pharmacol. 2008 Sep 4;591(1-3):28-35.

Introduction

The induction of phase II gene products has been proposed to protect cells from oxidative stress (1). We have previously shown that heme oxygenase-1 (HO-1), a phase II enzyme, protected HUVEC from menadione (MD)-induced oxidative injury following treatment by caffeic acid phenethyl ester (CAPE), a polyphenolic antioxidant (2). To further improve cytoprotection, we investigated 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), a new synthetic triterpenoid (from Dr. Michael Sporn, Dartmouth University) and potent inducer of phase II enzymes (3).

Methods

Cell culture:

HUVEC (Lifeline Cell Technology, Walkersville, MD) were cultivated on 1% gelatin-coated 48-well multiplates (Corning Incorporated, Corning, NY) in Vasculife® Medium. Only the second through fifth population doublings of cells were used.

Methods

***In vitro* assay:**

Cell viability was assessed at 24 hours after initiation of treatment using Alamar Blue™ (Biosource International, Camarillo, CA). CAPE and CDDO-IM were assayed for cytotoxic effects in HUVEC. Doses of CAPE and CDDO-IM causing less than 90% cell viability (compare to control group) were considered toxic and not applied in cytoprotection assay. Confluent HUVEC were pretreated with either various concentrations of CDDO-IM and CAPE or 0.1% DMSO (control) for 6 hrs, then exposed to a toxic dose of MD for additional 24 hrs. Cell viability was assessed compared to the vehicle controls. At least three independent experiments were performed and each was done in triplicate.

HO-1 induction confirmation:

HUVEC were pretreated with 100 nM CDDO-IM and 20 µM CAPE or 0.1% DMSO (control) for 6 hrs (RNA) and 24 hrs (Protein), respectively. HO-1 induction was confirmed using RT-PCR and western blot. For RT-PCR, the cDNA was obtained by reverse transcription RNA obtained directly from the treated cells using the Cells-to cDNA™ II kit (Applied Biosystems/Ambion, Austin, TX) and Real-time PCR was performed on a LightCycler™ thermal cycler (Idaho Technology, Salt Lake City, UT). HO-1 gene was normalized to the expression level of 18S for each sample. Relative quantification was performed with the comparative C_T method. For western blot, the protein was obtained by direct lysis of the treated cells and directly run on Invitrogen E-page gels, which were transferred to nitrocellulose membrane using the iBlot system (Invitrogen Corporation, Carlsbad, CA). Prior to HO-1 antibody application, the blots were stained with SyPro Ruby blot stain (Invitrogen) to normalize the amount of protein.

Results

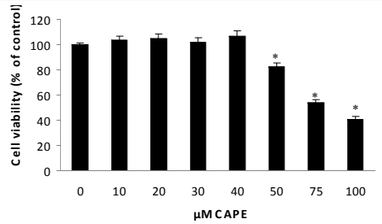

Figure 1. Cytotoxicity of CAPE in HUVEC. *: p< 0.05 versus control (0 µM CAPE). CAPE at doses of 50, 75, and 100 µM were cytotoxic and less than 40 µM were used for cytoprotection assay.

Results

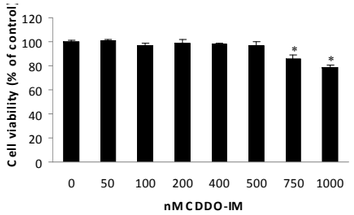

Figure 2. Cytotoxicity of CDDO-IM in HUVEC. *: p< 0.05 versus control (0 nM CDDO-IM). CDDO-IM at doses of 750 and 1000 nM were cytotoxic and less than 500 nM were used for cytoprotection assay.

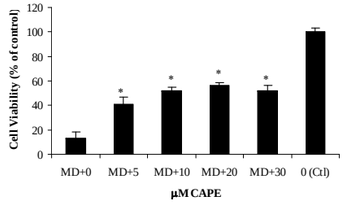

Figure 3. Cytoprotection of CAPE against 70 µM MD toxicity in HUVEC. *: p< 0.05 versus MD alone (MD+0 µM CAPE). The cytoprotective effect of CAPE was dose dependent. CAPE at 20 µM protected HUVEC against MD-induced toxicity (~10% cell survival) resulting in around 60% cell survival.

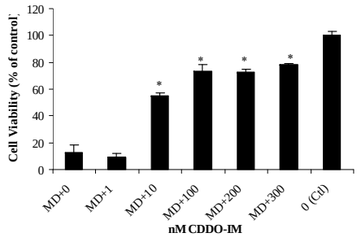

Figure 4. Cytoprotection of CDDO-IM against 70 µM MD toxicity in HUVEC. *: p< 0.05 versus MD alone (MD+0 nM CDDO-IM). The cytoprotective effect of CDDO-IM was dose dependent. CDDO-IM at 100 nM protected HUVEC against MD-induced toxicity (~10% cell survival) resulting in around 80% cell survival, which is much more potent than CAPE in cytoprotection.

Results

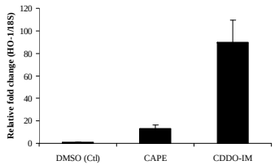

Figure 5. HO-1 mRNA induction in HUVEC by 6 hr treatment of 20 µM CAPE and 100 nM CDDO-IM. HO-1 RNA was induced up to 90 fold by CDDO-IM compared to a 13-fold increase following CAPE treatment.

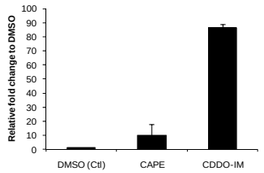

Figure 6. HO-1 protein expression in HUVEC by 24 hr treatment of 20 µM CAPE and 100 nM CDDO-IM. HO-1 protein was induced up to 87 fold by CDDO-IM compared to a 10-fold increase following CAPE treatment.

Conclusions

1. Cytotoxicity profiles of CAPE and CDDO-IM were established in HUVEC. CAPE above 40 µM and CDDO-IM over 500 nM were cytotoxic.
2. The cytoprotective effect of CAPE and CDDO-IM were dose dependent. The cytoprotection of CDDO-IM are much more potent than that of CAPE.
3. The induction of HO-1 by CAPE and CDDO-IM correlated well with their cytoprotection.
4. Since CDDO-IM appears to provide improved cytoprotection against oxidative stress it is a good candidate for testing in animal models of ischemia reperfusion injury.

References

1. Holtzclaw WD, Dinkova-Kostova AT, Talalay P. Protection against electrophile and oxidative stress by induction of phase 2 genes: the quest for the elusive sensor that responds to inducers. Adv Enzyme Regul. 2004;44:335-67. Review.
2. Wang X, Stavchansky S, Zhao B, Bynum JA, Kerwin SM, Bowman PD. Cytoprotection of human endothelial cells from menadione cytotoxicity by caffeic acid phenethyl ester: the role of heme oxygenase-1. Eur J Pharmacol. 2008 Sep 4;591(1-3):28-35.
3. Liby K, Hock T, Yore MM, Suh N, Place AE, Risingsong R, Williams CR, Royce DB, Honda T, Honda Y, Gribble GW, Hill-Kapturczak N, Agarwal A, Sporn MB. The synthetic triterpenoids, CDDO and CDDO-imidazolide, are potent inducers of heme oxygenase-1 and Nrf2/ARE signaling. Cancer Res. 2005 Jun 1;65(11):4789-98.