Elucidating the Pharmacodynamics and Disposition of Oxyresveratrol

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INTRODUCTION

- Oxyresveratrol is a stilbene found in mulberry, grapes, red wine, and peanuts [1].
- Oxyresveratrol is structurally analogous to resveratrol and comes in two distinct physicochemical forms: an anhydrous base and a dihydrate.[Fig.1]
- It has been suggested that oxyresveratrol and other hydroxystilbenes have anti-oxidant, anti-cancer, and COX inhibitory activity [2].

PURPOSE

- To develop and validate an analytical assay to reliably and accurately quantify oxyresveratrol in biological fluids.
- To evaluate the oral pharmacokinetics of oxyresveratrol anhydrous and oxyresveratrol dihydrate in a rodent model.
- To examine the pharmacological activity and mechanism of action of oxyresveratrol: including its cytotoxicity, histone deacetylase (HDAC), anti-oxidant, and sirutin-1 (SIRT-1) capacity, as well as cyclooxygenase (COX) inhibitory activity.

METHODS

Assay Development

- A method for quantifying oxyresveratrol was developed using high performance liquid chromatography (HPLC).
- Phenomenex C-18(2) column (250 x 4.60 mm) Flow rate: 0.60 mL/min
- Absorbance detection: 320 nm Internal standard: daidzein
- Mobile phase: acetonitrile : water : formic acid (30: 70: 0.04 v/v/v)

Sirtuin-1 activity

- A commercially available kit (Cayman Chemical) was used to measure the ability of oxyresveratrol to activate SIRT1. Activation of SIRT1 has been shown to prolong the lifespan of several species, and resveratrol has previously been identified as a potent SIRT1 activator [3].

Anti-Cancer Activity

- Cancer cell lines used: HCT-116 (Colon cancer), MDA-MB-231 (Estrogen receptor negative breast cancer), and PC-3 (Prostrate cancer).
- Oxyresveratrol (0-250 µg/mL) was incubated with all cancer cell lines.
- Alamar Blue Assay used to measure cell viability.

Pharmacokinetics

- Male Sprague-Dawley rats (N = 3, 250 g) were cannulated and dosed either intravenously with oxyresveratrol in PEG 600 (10 mg/kg) or orally with oxyresveratrol in methyl cellulose (200 mg/kg).
- Urine samples taken at 2, 6, 12, 24, 48, 72, 96, and 120 hours post dose.
- Serum samples taken at 1 min., 0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, 72, 96, and 120 hours post dose.

Antioxidant activity

- Measured ability of oxyresveratrol (0-250 µg/mL) to inhibit oxidation of ABTS to $ABTS^{\cdot+}$ by metmyoglobin.
- Amount of $ABTS^{\cdot+}$ produced measured by spectrophotometry at 750 nm.
- Antioxidant capacity compared to an equivalent amount of Trolox.

Histone Deactylase activity

- Commercially available kit (Cayman Chemical) was used to measure modulation of histone deacetylase activity when incubated with oxyresveratrol at 1, 10, 50, and 100 µg/ml.

Differential Scanning Calorimetry

- A Thermal Analyzer (STA 409PC Luxx®) Differential Scanning Calorimeter-Thermogravimetric Analyzer (NETZSCH, Inc., Selb, Germany) was used to monitor the thermal events as a function of temperature increase. Samples (2-4 mg) in closed aluminum pans were heated from 10 to 300 C at a heating rate of 10 C/min, with an oxygen purge of 100 mL/min.

Melting Point Determination

- Melting points measured using a Thomas Hoover Capillary Melting Point Apparatus®. The heat setting was set at 3.9 and the temperature was ramped at 1.0 ºC/ minute.

COX Inhibition

- The COX Inhibitor Screening Assay (Cayman Chemical) was used to measure $PGF_2\alpha$ produced by $SnCl_2$ reduction of COX-derived PGH_2.

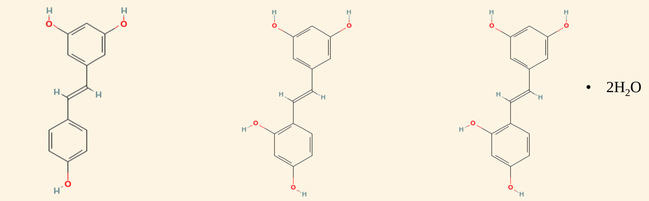
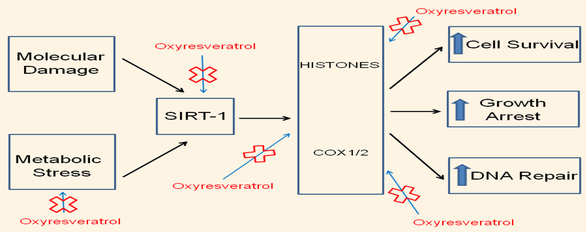


Figure 1: Physicochemical Properties of Oxyresveratrol and Resveratrol



Schematic: Possible Mechanistic Action of Oxyresveratrol

RESULTS

HPLC Analytical Method

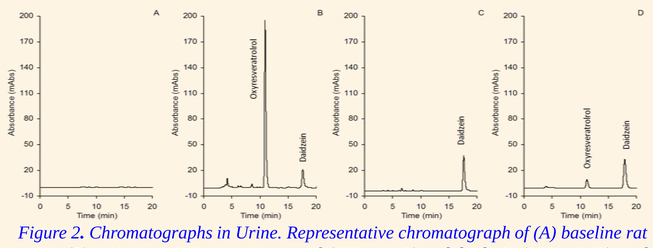


Figure 2. Chromatographs in Urine. Representative chromatograph of (A) baseline rat urine, (B) urine containing oxyresveratrol (100 µg/mL) and diadezin (100 µg/mL), and (C) Rat urine time zero urine sample (D) Rat urine 12 h post oral dose (230 mg/kg).

Differential Scanning Calorimetry

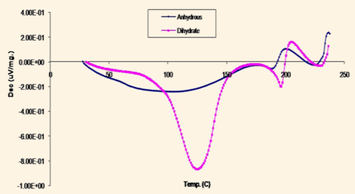


Figure 3: Differential Scanning Calorimetry of Oxyresveratrol forms

SIRT-1 Activity

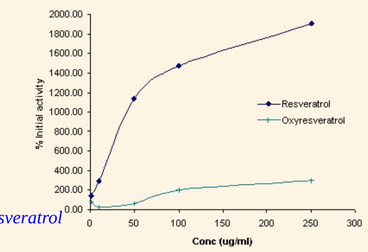


Figure 5: SIRT-1 activation of both resveratrol and oxyresveratrol in terms of % initial activity. Sirtinol is a known SIRT-1 inhibitor.

HDAC Activity

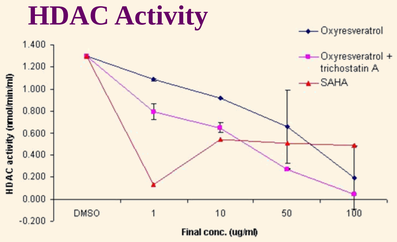
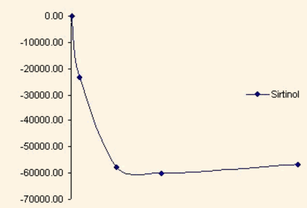


Figure 4: Concentration dependent HDAC activity of oxyresveratrol compared to positive controls.



Antioxidant capacity

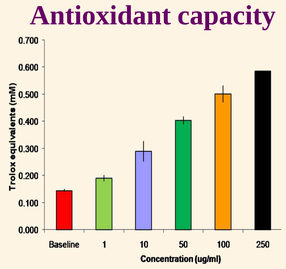


Figure 6: Concentration dependent antioxidant activity of oxyresveratrol in Trolox equivalents.

COX 1 Inhibition

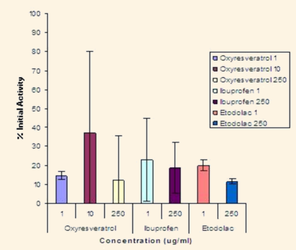


Figure 7: Inhibition of COX 1in terms of % initial activity.

Cell Culture

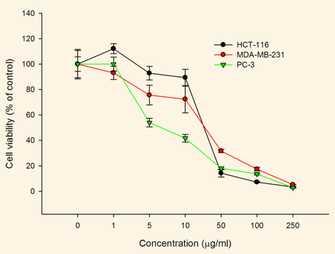


Figure 8: Oxyresveratrol anti-cancer activity in various cancer cell lines

COX 2 Inhibition

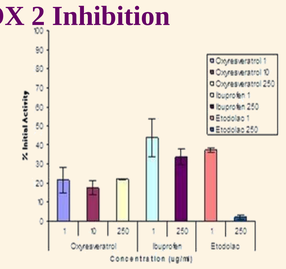


Figure 9: Inhibition of COX 2 in terms of % initial activity.

Pharmacokinetics

Table 1: Pharmacokinetic disposition of Oxyresveratrol in rats (N=3-6) +/- SEM.

Pharmacokinetic Parameter	IV	Anhydrous	Dihydrate
AUC_{inf} (ug.h/mL)	9.44 (+/- 2.44)	26.10 (+/- 4.29)	5.25 (+/- 2.41)
V_d (L/kg)	77.73 (+/- 21.90)	-	-
CL_h (L/h/kg)	1.16 (+/- 0.25)	0.01 (+/- 0.00)	0.13 (+/- 0.07)
CL_r (L/h/kg)	0.033 (+/- 0.01)	2.0 x 10^{-5} (+/- 0.00)	2.4 x 10^{-5} (+/- 0.01)
CL_{tot} (L/h/kg)	1.19 (+/- 0.26)	8.17 x 10^{-3} (+/- 0.28)	0.12 (+/- 0.07)
Extraction ratio	0.67 (+/- 0.14)	-	-
$t_{1/2}$ (h) serum	45.92 (+/- 7.62)	6.36 (+/- 0.66)	23.43 (+/- 12.49)
Fe	2.49 (+/- 0.84)	0.23 (+/- 5.88)	0.058 (+/- 0.04)

IV Administration:

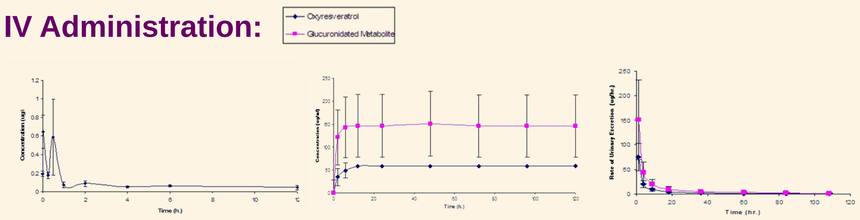


Figure 10: Oxyresveratrol disposition in serum.

Figure 11: Total amount of oxyresveratrol excreted in urine.

Figure 12: Rate of oxyresveratrol excretion in urine.

Oral Administration: Anhydrous

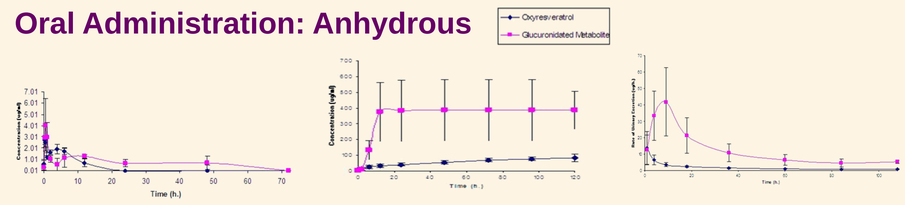


Figure 13: Oxyresveratrol disposition in serum.

Figure 14: Total amount of oxyresveratrol excreted in urine.

Figure 15: Rate of oxyresveratrol excretion in urine.

Oral Administration: Dihydrous

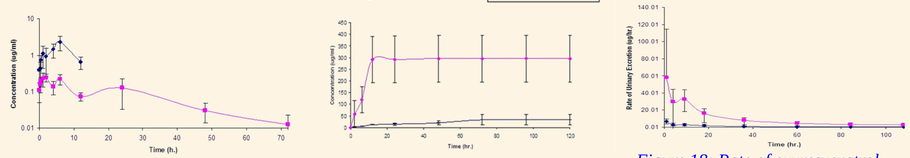


Figure 16: Oxyresveratrol disposition in serum.

Figure 17: Total amount of oxyresveratrol excreted in urine.

Figure 18: Rate of oxyresveratrol excretion in urine.

CONCLUSIONS

- Oxyresveratrol exists in two distinct crystalline forms.
- The HPLC assay is accurate, reproducible, reliable, and sensitive.
- Oxyresveratrol demonstrates dose-dependent anticancer activity.
- Oxyresveratrol is an anti-oxidant that activates SIRT-1 and inhibits HDAC, COX 1, and COX 2.
- Formation of a glucuronidated metabolite indicates Phase II metabolism.
- Pharmacokinetic data indicates that oxyresveratrol has formulation dependent pharmacokinetics and is orally bioavailable, rapidly glucuronidated, and excreted in urine and via non-renal routes.

ACKNOWLEDGEMENTS

- A Summer Undergraduate Research Fellowship (SURF) from WSUCOP to RMB.
- An Auvil Undergraduate Scholars Fellowship to RMB.
- An Unrestricted Grant from Sabinsa to NMD.
- Connie M. Remsberg, Jody K. Takemoto, Karina-Vega-Villa, Dr.Yusuke Ohgami, Dr. Vamsi Balla, Dr. Susmita Bose, Dr. Rob Ronald

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This work was supported by the National Science Foundation's REU program.