

Final Report

The Effect of Pioglitazone on the In Vitro Activities of Human and Mouse MAO-B

Prepared for:

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1.0 STUDY TITLE

The Effect of Pioglitazone on the In Vitro Activities of Human and Mouse MAO-B

3.0 INTRODUCTION AND OBJECTIVE

The objective of this study was to assess potential inhibition of human and mouse monoamine oxidase B (MAO-B) by pioglitazone in *in vitro* assay systems.

4.0 STUDY SCHEDULE

Experimental Start Date: March 29, 2010

Experimental End Date: April 5, 2010

5.0 TEST ARTICLE INFORMATION

5.1 Identification of Test Article

Name: Pioglitazone

Supplier: RTI MHF

CAS No.: 111025-46-8

Lot No.: 080801

Purity: 99.6% (Certificate of Analysis to be maintained in study file)

Storage Conditions: Room temperature

Disposition: Returned to RTI MHF following study completion

6.0 POSITIVE CONTROL INFORMATION

6.1 Identification of Positive Control Article

Deprenyl (selegiline hydrochloride) is a potent, irreversible, and selective MAO-B inhibitor that was used as a positive control.

Name: Selegiline hydrochloride

Supplier: Sigma-Aldrich

CAS No.: 14611-52-0

Lot No.: 099K4707

Purity: >98% (HPLC)

Storage Conditions: Room temperature

7.0 TEST SYSTEMS

7.1 Recombinant Human MAO-B (rhMAO-B), 5.0 mg protein/mL

7.1.1 Biological Origin

Membrane preparation of heterologously expressed human MAO-B isolated from virally transfected BT1 insect embryonic cells.

7.1.2 Source

BD Biosciences, cat. #456284, lot #36152

7.2 Mouse Brain MAO-B Homogenate

7.2.1 Species and Strain

C57BL/6 male mice, untreated, approximately 30 gram body weight.

7.2.2 Source

Charles River Laboratories, Inc., Raleigh, NC

8.0 EXPERIMENTAL PROCEDURES

MAO-B assay methods are described in RTI laboratory method DLM-012.01, and in RTI laboratory method DLM-013.01 (Jensen, 2010a; Jensen, 2010b). Validation of the enzyme inhibition kinetics is presented in these laboratory methods. The methods are modified from the MAO-Glo™ Assay kit (Promega Inc.), which is described in Valley et al. (2006).

8.1 Recombinant Human MAO-B (rhMAO-B) Assay

8.1.1 Pioglitazone Incubations

Pioglitazone was incubated in 96-well opaque black plates with rhMAO-B (0.2 mg protein/mL final concentration) and 4.0 μ M luminogenic substrate (aminopropylether analog of methyl ester luciferin; APMLuc) in assay buffer consisting of 100 mM HEPES, pH 7.4, 5% glycerol, and 10% DMSO. Concentrations of pioglitazone tested were 0 (DMSO), 0.01, 0.1, 1.0, and 10 μ M. Incubations were conducted under initial rate reaction conditions, and reactions were started by addition of rhMAO-B. Samples were incubated for 1 hour at room temperature. Reactions were terminated by addition of luciferin detection reagent, and samples were incubated an additional 20 minutes to allow development of luciferase- and ATP-dependent luminescence. Relative luminescence was determined with a plate luminometer and was

corrected for background using no-protein controls. Results are presented as percent of vehicle controls.

8.1.2 Selegiline Incubations

Selegiline was incubated as described above for pioglitazone, except that the concentrations of selegiline tested were 0 (DMSO), 0.01, 0.1, 1.0, 10, and 100 μM .

8.2 Mouse Brain MAO-B Assay

8.2.1 Preparation of Mouse Brain Homogenate

Whole brains from untreated male C57BL/6 mice were rinsed in ice-cold phosphate-buffered saline, and were homogenized in 10 volumes of 100 mM HEPES, pH 7.5. Homogenates were centrifuged at 2000 \times g for 20 minutes in a 4 °C centrifuge. The supernatant was collected, the protein content was determined, and was adjusted to a final protein concentration of 0.4 mg/mL. Homogenates were stored at -80 °C prior to use.

8.2.2 Pioglitazone Incubations

Pioglitazone was incubated in 96-well opaque white plates with mouse brain homogenate (0.2 mg protein/mL final concentration) and 4.0 μM APMLuc in assay buffer consisting of 100 mM HEPES, pH 7.4, 5% glycerol, and 10% DMSO. Concentrations of pioglitazone tested were 0 (DMSO), 0.01, 0.1, 1.0, and 10 μM . Incubations were conducted under initial rate reaction conditions, and reactions were started by addition of mouse brain homogenate. Samples were incubated for 2 hours at room temperature. Reactions were terminated by addition of luciferin detection reagent, and samples were incubated an additional 20 minutes to allow development of luciferase- and ATP-dependent luminescence. Relative luminescence was determined with a plate luminometer and was corrected for background using no-protein controls. Results are presented as percent of vehicle controls.

8.2.3 Selegiline Incubations

Selegiline was incubated as described above for pioglitazone, except that the concentrations of selegiline tested were 0 (DMSO), 0.01, 0.1, 1.0, 10, and 100 μM .

9.0 RESULTS

Pioglitazone produced concentration-dependent inhibition of human MAO-B ([Figure 1](#)). The inhibition curve was sigmoidal and the concentration of pioglitazone giving half-maximal inhibition, IC_{50} , was estimated to be 0.804 μM . Greater than 75% inhibition of MAO-B activity

was seen at 10 μM , the highest concentration of pioglitazone tested due to limited solubility. A similar inhibition by pioglitazone was seen for mouse MAO-B in mouse brain homogenates (Figure 2). The IC_{50} value for inhibition of mouse MAO-B was 0.412 μM , and greater than 95% inhibition of MAO-B activity was seen at 10 μM pioglitazone. Selegiline, a known selective inhibitor of MAO-B, inhibited human (Figure 3) and mouse (Figure 4) MAO-B as expected, with IC_{50} values of 0.403 μM and 0.106 μM for the human and mouse enzymes, respectively. Selegiline at 100 μM produced greater than 95% inhibition of MAO-B activities in both human and rat systems. These results indicate that pioglitazone is a potent inhibitor of human and mouse MAO-B, with potency comparable to selegiline in both systems.

Figure 1: Inhibition of rhMAO-B by Pioglitazone

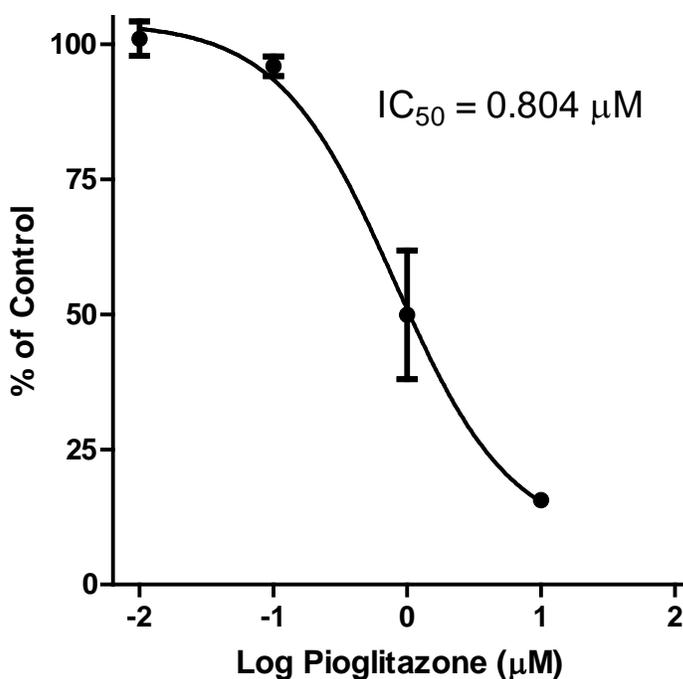


Figure 2: Inhibition of MAO-B in Mouse Brain Homogenates by Pioglitazone

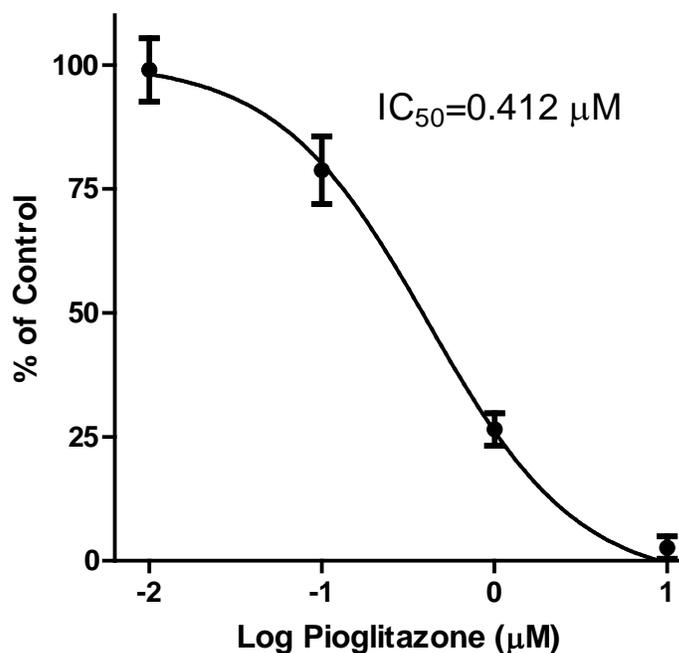


Figure 3: Inhibition of rhMAO-B by Selegiline

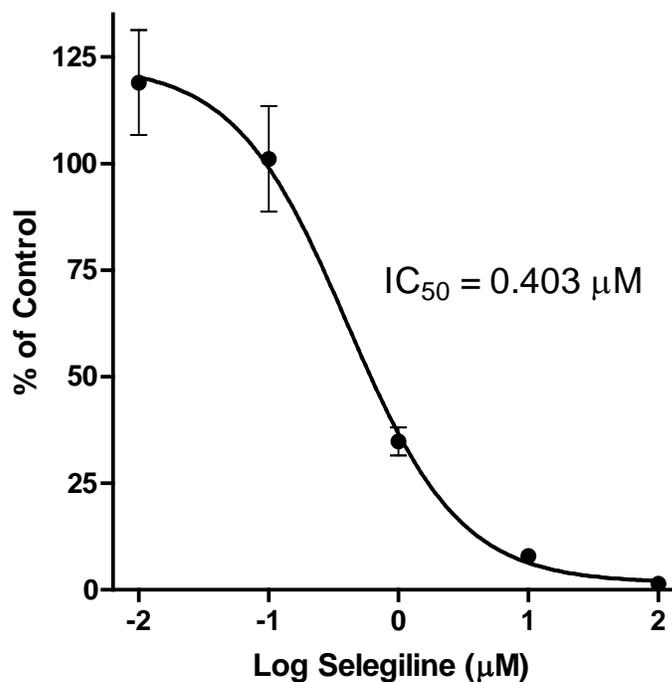
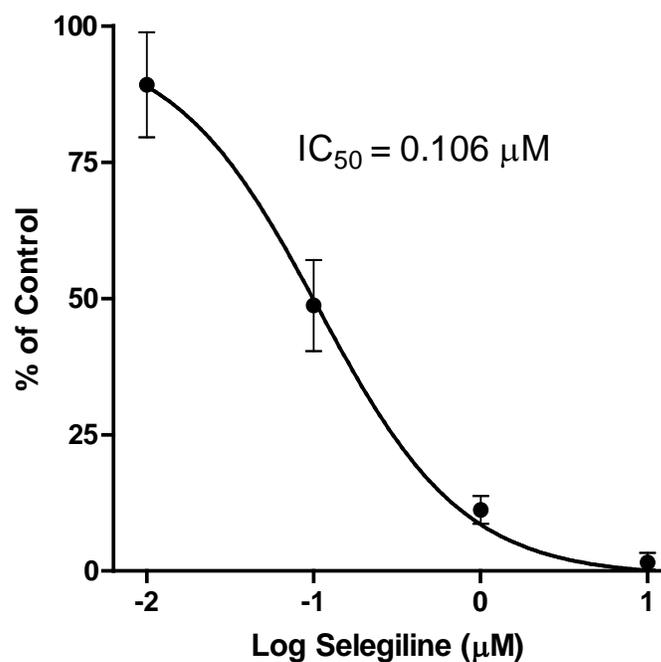


Figure 4: Inhibition of MAO-B in Mouse Brain Homogenates by Selegiline



10.0 REFERENCES

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- Jensen, C. B. (2010a). Recombinant human MAO-B inhibition assay. RTI technical document DLM-012.01.
- Jensen, C. B. (2010b). Mouse Brain MAO-B Inhibition Assay. RTI technical document DLM-013.01.