Ketoconazole increases the endocrine disrupting potential of ibuprofen exposure in the H295R cells and Japanese medaka

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**Introduction**

- Pharmaceutical residues found in the aquatic environment occur as mixtures, while most of toxicity tests were made on individual chemicals.
- In the present study, we investigated whether exposure to low level ketoconazole would increase the estrogenicity of ibuprofen exposure.

**Materials & Methods**

- **In vitro H295R cell bioassay**
  - H295R cells were exposed to ibuprofen alone at concentrations ranging from 0.02 to 20 mg/mL, or in combination with ketoconazole (5 ng/mL) for 48 hr.
  - Hormone measurements
    - Culture medium was extracted twice with 2.5 mL diethyl ether, and hormones were measured by enzyme-linked immunosorbent assay.
  - Aromatase activity assay
    - Direct and indirect effects on aromatase activity were measured by the rate of conversion of 17β-androstenedione to estrone using liquid scintillation counter.
  - Quantitative PCR assay
    - Transcription of five steroidogenic genes (3β-HSD2, CYP11β2, CYP17, CYP19, and 17β-HSD) plus one housekeeping gene (β-actin) were measured using real-time PCR.

- **In vivo Japanese medaka exposure**
  - Male adult medaka (6 fishes/group) were exposed to ibuprofen alone at concentrations ranging from 0.02 to 0.2 mg/mL or with ketoconazole (10 µg/L) for 14 d.
  - On day 14, all surviving fish were euthanized for measurement of sex hormones and related mRNA expressions.

**Results & Discussion**

- **In vitro H295R cell bioassay**
  - 17β-estradiol (E2) and testosterone (T) measurements
    - Exposure to ibuprofen resulted in significant increase of E2.
    - In combination with ketoconazole, E2 production by ibuprofen exposure was more elevated.
  - Aromatase activity assay
    - Exposure to ibuprofen resulted in significant increase of direct aromatase activity.
    - When 5 ng/mL of ketoconazole was added, the extent of increase in aromatase activity became greater, i.e., up to 1.5-fold compared to ibuprofen only exposure.
    - However, direct aromatase activity did not change.
  - Mechanisms of ibuprofen and ketoconazole exposure
    - Expression of COX2 mRNA was down-regulated in both groups.
    - Expression of CYP11B2 mRNA was up-regulated in ibuprofen-exposed group, whereas CYP11B2 mRNA expression was down-regulated in ibuprofen-ketoconazole-exposed group.
    - CYP19 and 17β-HSD mRNA expression were observed.

<table>
<thead>
<tr>
<th>Gene</th>
<th>IBP 0.02 mg/L (µg/L)</th>
<th>IBP 0.2 mg/L (µg/L)</th>
<th>IBP+KCZ 0.02 mg/L (µg/L)</th>
<th>IBP+KCZ 0.2 mg/L (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP19</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
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<tr>
<td>17β-HSD</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
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<tr>
<td>CYP17</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
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<tr>
<td>CYP11B2</td>
<td>1.00 ± 0.33</td>
<td>1.00 ± 0.33</td>
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<tr>
<td>17β-HSD</td>
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- **Quantitative PCR assay**
  - In cells exposed to ibuprofen, the significant differences in CYP11B2 mRNA expression were observed.
  - However, with a combined exposure to ibuprofen and ketoconazole resulted in an elevated expression of CYP17, CYP19, and CYP11B2 mRNAs compared to ibuprofen exposure alone.

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<tr>
<td>CYP17</td>
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<tr>
<td>CYP11B2</td>
<td>1.00 ± 0.33</td>
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<tr>
<td>17β-HSD</td>
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<td>CYP19/17β-HSD</td>
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</table>

- **In vivo Japanese medaka exposure**
  - E2 and T measurements
    - Ibuprofen only did not cause any significant effects on E2 concentration compared to that of control.
    - In combination with ketoconazole, however, E2 production by ibuprofen exposure was more elevated.
    - Significantly lower concentration of T was observed in combination with ketoconazole.

**Conclusion**

- We have shown that non-effective concentrations of ketoconazole can increase the potential for endocrine disrupting effects of ibuprofen in both human adrenal cell line and the freshwater fish.
- Potential consequences of such mixture toxicity should warrant further investigation.

For questions or comments please email me at jkh526@snu.ac.kr