Comparison of the Anti-tumor Effects of Selective Serotonin Reuptake Inhibitors as Well as Serotonin and Norepinephrine Reuptake Inhibitors in Human Hepatocellular Carcinoma Cells

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The anti-tumor effects of selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) on several types of cancer cells have been reported. However, comparison of the anti-tumor effects of these drugs on human hepatocellular carcinoma (HepG2) cells has not been studied. We compared the anti-tumor effects of four SSRIs and two SNRIs on HepG2 cells. SSRIs and duloxetine dose-dependently decreased cell viability. Milnacipran had no effect on cell viability. The half-maximal inhibitory concentration was lower in the order of: sertraline, paroxetine, duloxetine, fluvoxamine, escitalopram, and milnacipran. Exposure to sertraline (2µM) significantly increased caspase-3/7 activity. These results suggest that, of the agents tested here, sertraline had the highest sensitivity to HepG2 cells, and activation of the caspase pathway is involved in the anti-tumor effects of sertraline in HepG2 cells.

Key words selective serotonin reuptake inhibitor; anti-tumor effect; sertraline; serotonin and norepinephrine reuptake inhibitor; human hepatocellular carcinoma cell

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the third most common cause of cancer-based death. Globally, >560000 people develop liver cancer each year and 55000 die from it. Chemotherapeutic agents against HCC have been studied extensively but they have never demonstrated improvement in overall survival compared with best supportive care.

Selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs) are used for the treatment of depression, anxiety, and certain behavioral disorders. They are prescribed frequently for the treatment of depression in cancer patients. Recently, the anti-tumor effects of SSRIs have been reported against several types of cancer cells. Paroxetine is thought to evoke cell death via apoptosis on human osteosarcoma cells by activation of p38 mitogen-activated protein kinase (MAPK) and caspase-3 pathways. Fluoxetine has been shown to inhibit proliferation of prostate cancer cells in vitro and in vivo, and induction of apoptosis has been observed in glioma cells. Exposure to sertraline and paroxetine has been shown to yield considerable reduction in the viability of malignant T cells by increasing the activity of caspase-3 and decreasing expression of Bel-2.

Anti-tumor effects of SSRIs and SNRIs on human HCC cells are not known. Recent reports have suggested that sertraline induces apoptosis in the human HCC cell line HepG2 through the MAPK pathway. Mun et al. showed the apoptotic effect of fluoxetine against the HCC cell line Hep3B.

However, comparison of anti-tumor effects of SSRIs and SNRIs on HepG2 cells has not been studied. Here, we compared the anti-tumor effects of four SSRIs (escitalopram, fluvoxamine, paroxetine, sertraline) and two SNRIs (duloxetine, milnacipran) on HepG2 cells.

MATERIALS AND METHODS

Chemicals Fluvoxamine and sertraline were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Paroxetine was generously provided by GlaxoSmithKline (West Sussex, U.K.). Escitalopram was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Milnacipran was purchased from Pierre Fable (Tokyo, Japan). Duloxetine was obtained from Tokyo Chemical Industry (Tokyo, Japan).

Cell Culture HepG2 cells were obtained from the cell bank of the Japanese Collection of Research Bioresources (Osaka, Japan). Cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) (MP Biomedicals, Irvine, CA, U.S.A.) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B (Gibco, Grand Island, NY, U.S.A.) and grown to subconfluence.

Cell Viability Cell viability was assessed by measuring the mitochondrial activity that reduced 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8) to formazan, as described previously. Briefly, after treatment with SSRIs and SNRIs for 24h in serum-free medium, cells were washed with phosphate-buffered saline and incubated with WST-8 Assay Solution (Cell Counting Kit-8; Dojindo Laboratories, Kumamoto, Japan) for 1h at 37°C in humidified air supplemented with 5% CO2. The incubation medium was withdrawn carefully and
transferred to 96-well, flat-bottomed plastic plates (Corning, NY, U.S.A.). The amount of formazan formed was measured from the absorbance at 450 nm with a reference wavelength of 620 nm using a Microplate Reader (Rainbow Sunrise; Tecan Austria, Salzburg, Austria).

**Caspase-3/7 Assay**  Activity of caspase-3 and caspase-7 was determined using the Apo-One Homogenous Caspase-3/7 Assay (Promega, Madison, WI, U.S.A.). Briefly, HepG2 cells were cultured on 96-well, flat-bottomed plastic plates at $0.3 \times 10^4$ cells/well. Cells were exposed to sertraline (2 µM) and then incubated for 6–24 h in serum-free medium. After incubation, cells were mixed with the same volume of the Apo-One Homogeneous Caspase-3/7 Reagent and incubated at room temperature for 1 h. Caspase-3/7 activation was estimated from the fluorescence of samples at an excitation wavelength of 490 nm and emission wavelength of 530 nm using a Fluorescence Microplate Reader (MTP-601; Corona Electric, Ibaraki, Japan).

**Statistical Analysis**  Data are presented as mean±standard error of the mean (S.E.M.). Data were analyzed by one-way ANOVA followed by the Tukey–Kramer post hoc test. A $p<0.05$ was considered significant. Half-maximal inhibitory concentration (IC$_{50}$) was estimated by probit analyses.

**RESULTS**

**Anti-tumor Effects of SSRIs and SNRIs on HepG2 Cells**

All the SSRIs and SNRI examined except for milnacipran...
Table 1. IC_{50} Values of SSRIs and SNRIs in HepG2 Cells

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>IC_{50} (µM)</th>
<th>IC_{50} (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRI</td>
<td>Sertraline</td>
<td>1.24±0.0551</td>
<td>(0.425±0.0189)</td>
</tr>
<tr>
<td></td>
<td>Paroxetine</td>
<td>7.34±0.376</td>
<td>(2.75±0.141)</td>
</tr>
<tr>
<td></td>
<td>Fluvoxamine</td>
<td>31.0±3.33</td>
<td>(13.5±1.45)</td>
</tr>
<tr>
<td></td>
<td>Escitalopram</td>
<td>94.8±5.22</td>
<td>(39.3±2.16)</td>
</tr>
<tr>
<td>SNRI</td>
<td>Duloxetine</td>
<td>8.95±4.31</td>
<td>(2.99±1.44)</td>
</tr>
<tr>
<td></td>
<td>Milnacipran</td>
<td>&gt;100</td>
<td>(&gt;28.3)</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of Sertraline on Caspase-3/7 Activity in HepG2 Cells

Cells were exposed to sertraline (2 µM) and incubated for 6–24 h. Data are presented as mean±S.E. (N=4). **p<0.01 vs. control.

decreased the dose dependency of cell viability (Fig. 1). The SSRIs sertraline, paroxetine, fluvoxamine, and escitalopram showed significant decreases at concentration (in µM) of ≥0.3 (sertraline 0.3 µM: *p<0.05; sertraline 1 and 3 µM: **p<0.01), ≥5 (paroxetine 5, 10, and 20 µM: **p<0.01), ≥20 (fluvoxamine 20, 30, 40, 50, and 60 µM: **p<0.01), and ≥30 (escitalopram 30 µM: *p<0.05; escitalopram 100, 300, and 1000 µM: **p<0.01), respectively. The SNRI duloxetine showed significant decreases at ≥1 µM (1, 3, 10, 30, and 100 µM: **p<0.01), whereas another SNRI, milnacipran, did not decrease cell viability at <100 µM.

IC_{50} values of SSRIs sertraline, paroxetine, fluvoxamine, and escitalopram were (in µM) were 1.24, 7.34, 31.0, and 94.8, respectively (Table 1). IC_{50} value of the SNRI duloxetine was 8.95 µM, whereas that of milnacipran was >100 µM.

Sertraline-Induced Increase in Caspase-3/7 Activity in HepG2 Cells

To ascertain if sertraline induces apoptosis, we measured caspase-3/7 activity. Exposure to sertraline (2 µM) significantly increased caspase-3/7 activity 12 h and 24 h after treatment (p<0.01, Fig. 2).

DISCUSSION

In the present study, sertraline, paroxetine, fluvoxamine, escitalopram, and duloxetine induced marked injury to HepG2 cells, suggesting the anti-tumor effects of these drugs. Moreover, IC_{50} values of sertraline were the lowest among these drugs.

Several studies have shown the cytotoxic effect of sertraline in other types of cancer cells. Gil-Ad et al. showed the IC_{50} values of sertraline in human multidrug-resistant human colon carcinoma (LS1034) cells and human colon carcinoma (HT-29) cells to be 13.1 µM and 15.8 µM, respectively.28 Amit et al. showed that sertraline induces injury to human T leukemia (Jurkat) cells and that the IC_{50} value is 9.5 µM.29 Lin et al. also showed the IC_{50} value of sertraline in a human breast cancer cell (MCF-7) to be about 25 µM.30 Moreover, we found that IC_{50} values of sertraline in porcine proximal tubular cell line LLC-PK1 cells and porcine aortic endothelial cells were 4.41 µM and 5.88 µM, respectively. Taken together, these reports and our results suggest that sertraline has high sensitivity to HepG2 as compared with other types of tumor cells and normal cells.

In clinical setting, the plasma concentrations (in µM) after the administration of the SSRIs sertraline, paroxetine, fluvoxamine and escitalopram have been reported to be 0.0992±0.0671, 0.190±0.114, 0.0235±0.00622, and 0.0531±0.0135, respectively.20–23 The plasma concentrations (in µM) after the administration of the SNRIs duloxetine and milnacipran have been reported to be 0.0704±0.0422 and 0.847±0.117, respectively.24,25 In the present study, the order of cytotoxicity in HepG2 cells was sertraline, paroxetine, and duloxetine, with IC_{50} values (in µM) of 1.24, 7.34, and 8.95, respectively. The plasma concentration of sertraline was about one-half that of paroxetine, and nearly equal to that of duloxetine. Taken together, these results suggest that, considering plasma concentration in clinical setting, sertraline might have more potent anti-tumor effects on HCC than the other SSRIs and SNRIs tested.

Recent research has suggested that the serotonin receptors 1B and 2B express human HCC, and are associated with an increased proliferation index or correlated with tumor size.26 Liang et al. showed that serotonin was involved in tumorigenesis and induced a pro-proliferative effect in HCC cells via upregulation of expression of FOXO3a (a member of class O of the forkhead box family of transcription factors).27 On the other hand, some reports indicated that serotonin reuptake transporter did not exist on liver tissue.28,29 Therefore, it seems unlikely that serotonin reuptake inhibition is implicated in the toxicity of HepG2 cells. It is shown that sertraline increases cytosolic-free Ca^{2+} levels ([Ca^{2+}]) in human prostate cancer cells.30 Paroxetine also induces [Ca^{2+}]\_rise and death in human oral cancer cells.31 Therefore, SSRIs and SNRIs may induce cell injury in HepG2 cells through the different biological activities that appear to be unrelated to its serotonin reuptake inhibition.

In the present study, exposure to sertraline significantly increased caspase-3/7 activity in HepG2 cells. A multitude of studies have reported that several drugs can induce anti-tumor effects via apoptosis of HepG2 cells. Aspirin induces apoptosis in HepG2 cells through inhibition of ATP production and decrease in expression of Bcl-2.32 Methylsulfonylmethane induces apoptosis in HepG2 cells by regulating the expression of cleaved caspase-3, cleaved caspase-8, and cleaved poly(ADP ribose) polymerase.33 A recent report suggested that sertraline induces apoptosis in HepG2 cells through the
MAPK pathway.\textsuperscript{15} Based on these reports, it is suggested that apoptosis through activation of the caspase pathway is involved in sertraline-induced toxicity in HepG2 cells.

In conclusion, the present study demonstrated (for the first time) that IC\textsubscript{50} values of SSRIs and a SNRI in HepG2 cells were lower in the order: sertraline, paroxetine, duloxetine, fluvoxamine, and escitalopram. Another SNRI, milnacipran, did not induce cell injury. These results suggest that the anti-tumor effects of sertraline are strongest among these antidepressant drugs. Moreover, apoptosis through activation of the caspase pathway may be involved in sertraline-induced cytotoxicity. Further study (including the cytotoxicity in other HCC cell lines and in vivo research) is needed to assess the anti-tumor effects of SSRIs and SNRIs on HCC.

Conflict of Interest The authors declare no conflict of interest.

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