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Antidepressants induce toxicity in human placental BeWo cells

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ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), and noradrenergic and specific serotonergic antidepressants (NaSSAs) are broadly used for the treatment of depression. Depression is one of the most common psychiatric disorders in pregnant women and SSRIs are commonly prescribed for depression during pregnancy. The placenta regulates the transport of nutrients and oxygen between the maternal and fetal circulation, and is essential for the survival and growth of the fetus. The present study investigated the effects of antidepressants on human placental BeWo cells.

BeWo cell viability was significantly decreased following exposure to sertraline (SSRI), paroxetine (SSRI), fluvoxamine (SSRI), and duloxetine (SNRI), whereas escitalopram (SSRI), venlafaxine (SNRI), and mirtazapine (NaSSA) showed little or no effects. Extracellular lactate dehydrogenase activity was increased by sertraline, paroxetine, fluvoxamine, and duloxetine, indicating toxicity to the cells. Sertraline increased the production of cellular reactive oxygen species (ROS) and decreased the mitochondrial membrane potential. Sertraline decreased the cellular ATP content in a time and concentration-dependent manner. Caspase-3/7 activity and apoptotic cells, detected using the phosphatidylserine-specific fluorescent probe Apotracker Green, were increased by sertraline.

Our findings suggest that antidepressants, such as sertraline, paroxetine, fluvoxamine, and duloxetine, induce toxicity in human placental BeWo cells. Sertraline may induce ROS-dependent apoptosis in human placental cells. These results are useful for further studies to determine the optimal dosage of antidepressants for pregnant women.

1. Introduction

Newer antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), and noradrenergic and specific serotonergic antidepressants (NaSSAs), are widely used for the treatment of depression. The clinical guidelines recommend these newer antidepressants as the first-choice treatment for patients with mild depressive disorders (American Psychiatric Association, 2010; Japanese Society of Mood Disorders, 2016).

Depression is one of the most common psychiatric disorders seen in women of childbearing age. Many pregnant women experience depressive symptoms during pregnancy and up to 20% and 7%–13% of pregnant women are exposed to antidepressants (Cooper et al., 2007; Andrade et al., 2008; Alwan et al., 2011). SSRIs are the most widely prescribed antidepressants during pregnancy.

While mental disorders and antidepressants may influence the development of the fetus, the associations and mechanisms involved are poorly understood. Recent studies have indicated an increased risk of adverse events, including persistent pulmonary hypertension, psychiatric disorders, and cardiac and craniofacial defects, in children born to women who used antidepressants during pregnancy (Chambers et al., 2006; Jimenez-Solem et al., 2012; Bérard et al., 2015; Liu et al., 2017; Shen et al., 2017). However, other studies have shown no correlation between the risk of malformation and use of antidepressants (Huybrechts et al., 2014; Furu et al., 2015).

The placenta plays a critical role in the survival and growth of the fetus, including delivery of nutrients and oxygen and removal of waste compounds from the fetal compartment. The placenta forms an interface between the maternal and fetal circulation. It is important to

Abbreviations: ATP, adenosine triphosphate; DCF, 2′,7′-dichlorofluorescin; DCFDA, 2′,7′-dichlorofluorescin diacetate; LDH, lactate dehydrogenase; NaSSA, noradrenergic and specific serotonergic antidepressant; PS, phosphatidylserine; ROS, reactive oxygen species; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and noradrenaline reuptake inhibitor.

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understand the effects of antidepressants on the placenta for the selection of antidepressants and improved therapy for pregnant women.

Human placental BeWo cells, which are human origin placental choriocarcinoma epithelial cells that are used as a model of syncytiotrophoblasts (Liu et al., 1997). BeWo cells are often used to study the role of transporters in the transplacental transfer of drugs (Nabekura et al., 2015; Cerveny et al., 2018). BeWo cells are also used to study the placental cell toxicity of drugs. Antiepiletic drug valproic acid decreased the proliferation and increased the caspase-3 activity in BeWo cells (Kwiecińska et al., 2011). BeWo cells are used to investigate the effects of diabetes on the placenta. Inadera et al. (2010) showed that BeWo cells respond to high glucose conditions and produce significant changes in the expression levels of cell cycle- and metabolism-related genes.

The present study investigated the effects of SSRIs, SNRIs, and NaSSA on human placental cells. The effects of these antidepressants on the viability and toxicity of human placental choriocarcinoma BeWo cells were measured and the mechanisms underlying cytotoxicity of antidepressants were evaluated.

2. Materials and methods

2.1. Materials

Ham's F-12K medium (F-12K), sertraline hydrochloride, paroxetine hydrochloride, duloxetine hydrochloride, fluvoxamine maleate, and mirtazapine were obtained from Fujifilm Wako Pure Chemical Industries Ltd. (Osaka, Japan). Escitalopram oxalate, venlafaxine hydrochloride, and 2′,7′-dichlorofluorescin diacetate (DCFDA) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Apotracker Green (Apo-15) and Calcein Red-acetoxymethyl ester (Calcein Red-AM) were purchased from BioLegend, Inc. (San Diego, CA, USA). All other chemicals used in the study were of the highest purity available. The 96-well plates were obtained from Corning (Corning, NY, USA).

2.2. Cell culture

BeWo cells (CCL-98, lot number 59368210) were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in F-12K supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air.

2.3. WST-8 assay

WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt], a water-soluble tetrazolium salt, assay was used to assess cell viability using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. BeWo cells were seeded in 96-well plates at a density of 5×10^3 cells/well and cultured for 24 h. Cells were then cultured in the absence or presence of various concentrations of antidepressants for 24 h. The absorbance of WST-8 formazan was measured at 450 nm using a Spark 10 M microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

2.4. Measurement of lactate dehydrogenase (LDH) release

Cell plasma membrane integrity was evaluated by measuring the release of LDH. BeWo cells were seeded in 96-well plates (1×10^3 cells/well) and cultured for 24 h. The cells were then cultured in the absence or presence of antidepressants for 2, 6, or 24 h. LDH activity was measured using the Cytotoxicity LDH Assay Kit-WST (Dojindo Laboratories). LDH release was calculated as the percentage of LDH released into the medium compared with the maximum LDH release (i.e., fully lysed cells using lysis buffer in the kit).

2.5. Measurement of cellular reactive oxygen species (ROS) generation

ROS generation was measured using DCFDA, which becomes fluorescent when oxidized by intracellular ROS. BeWo cells were harvested in black 96-well plates (5×10^3 cells/well) and cultured for 24 h. Cells were then cultured in the absence or presence of sertraline for 23.5 h. DCFDA (50 μM final concentration) was then added and incubated in the dark at 37 °C for 30 min. Fluorescence intensity was measured using a Spark 10 M microplate reader with excitation at 485 nm and emission at 535 nm. Cellular ROS levels were calculated by comparing the fluorescence of sertraline-treated cells with that of medium-only treated cells.

2.6. Measurement of mitochondrial membrane potential

Changes in the mitochondrial membrane potential were assessed using a Cell Meter JC-10 Mitochondrion Membrane Potential Assay Kit Optimized for Microplate Assays (AAT Bioquest, Inc., Sunnyvale, CA, USA) according to the manufacturer's instructions. BeWo cells were seeded in clear-bottom black 96-well plates (2.5×10^4 cells/well) and cultured for 24 h. Cells were then cultured in the absence or presence of sertraline for 24 h. JC-10 dye-working solution (50 μL) was added and incubated at 37 °C for 30 min and then Assay Buffer B (50 μL) was added. Fluorescence intensity was measured using a Spark 10 M microplate reader with excitation at 490 nm and emission at 535 nm (green) and excitation at 540 nm and emission at 590 nm (orange). The ratio of orange to green fluorescence was calculated and normalized to that of the control cells.

2.7. Measurement of cellular ATP content

The amount of ATP produced by metabolically active cells was quantified using a CellTiter-Glo 2.0 Assay (Promega Corporation, Madison, WI, USA). BeWo cells were harvested in white 96-well plates (5×10^3 cells/well) and cultured for 24 h. The cells were then cultured in the absence or presence of antidepressants for 6, 24, or 48 h. Luminescent signals were measured using a Spark 10 M microplate reader. Cellular ATP content was calculated by comparing the luminescence of antidepressant-treated cells with that of cells treated with medium-only.

2.8. Measurement of cellular caspase-3/7 activities

Caspase-3/7 activity was evaluated in BeWo cells using the Caspase-Glo 3/7 Assay System (Promega) according to the manufacturer's instructions. BeWo cells were harvested in white 96-well plates (5×10^3 cells/well) and cultured for 24 h. The cells were then cultured in the absence or presence of antidepressants for 24 h. Luminescent signals were measured using a Spark 10 M microplate reader. Cellular caspase-3/7 activity was calculated by comparing the luminescence of the antidepressant-treated cells with that of cells treated with medium-only.

2.9. Detection of apoptotic cells

Fluorogenic peptide Apotracker Green (BioLegend), which specifically binds to phosphatidylserine (PS) in the plasma membranes of apoptotic cells, was used to measure apoptotic cells according to the manufacturer's instructions. BeWo cells were seeded at a density of 9×10^4 cells/well into 8-well chambered cover slips (μ-slide 8 well, Cat. No. 80826, ibidi GmbH, Gräfelfing, Germany). At 24 h after seeding, cells were cultured in the absence or presence of sertraline or

camptothecin (positive control) for 6 h. Cells were then washed, and phosphate-buffered saline containing Apotracker Green (800 nM) and Calcein Red-AM (1 μM) was added and further incubated at 37 °C for 20 min in the dark. The staining solution was then removed and phosphate-buffered saline was added. Apoptotic cells (green, GFP filter, excitation at 470 ± 20 nm and emission at 525 ± 25 nm) and live cells (red, TRITC filter, excitation at 545 ± 12.5 nm and emission at 605 ± 35 nm) were visualized using a fluorescence microscope (BZ-X800, Keyence Corporation, Osaka, Japan). The number of apoptotic or live cells were counted using the Hybrid Cell Count software (Keyence).

2.10. Statistical analysis

Data were expressed as mean ± standard deviation (SD). The statistical significance of differences was determined using one-way analysis of variance followed by Dunnett's test using the KaleidaGraph software (Synergy Software, Reading, PA, USA), and *P*-values < 0.01 were considered statistically significant.

3. Results

3.1. Effects of antidepressants on human placental BeWo cell viability and toxicity

We first examined the effects of SSRIs, SNRIs, and NaSSA on the viability and toxicity of human placental BeWo cells, which are human origin placental choriocarcinoma epithelial cells that are used as a model of syncytiotrophoblasts (Liu et al., 1997). Exposure of BeWo cells to sertraline (SSRI) for 24 h decreased the cell viability, as determined by WST-8 assay. Significant effects were observed with sertraline at concentrations > 25 μM, whereas escitalopram showed no effects (Fig. 1).

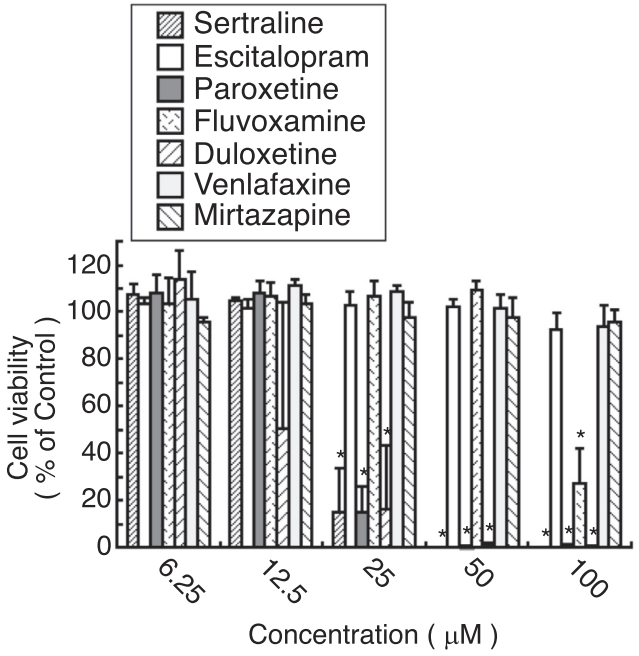

Fig. 1. Effects of sertraline, escitalopram, paroxetine, fluvoxamine, duloxetine, venlafaxine, or mirtazapine on human placental BeWo cell viability. BeWo cells were incubated with 6.25, 12.5, 25, 50, or 100 μM antidepressants for 24 h, and cell viability was analyzed by WST-8 assay. Data represent the mean ± SD of three independent experiments that were performed in triplicates. *$P < 0.01$ compared with the control.

The effects of paroxetine, fluvoxamine, duloxetine, venlafaxine, and mirtazapine on BeWo cell viability were measured by WST-8 assay. Cell viability was decreased by the exposure to >25 μM paroxetine, >25 μM duloxetine, and 100 μM fluvoxamine. Venlafaxine and mirtazapine showed no effects at the concentrations of 100 μM (Fig. 1).

The membrane integrity of BeWo cells was evaluated as an indicator of cell toxicity by measuring the release of the cytosolic LDH after exposure to antidepressants. Exposure to sertraline at concentrations >25 μM increased LDH release, indicating that sertraline is toxic to BeWo cells (Fig. 2A). LDH release was also increased by paroxetine, fluvoxamine, and duloxetine. In contrast, escitalopram, venlafaxine, and mirtazapine showed no effects.

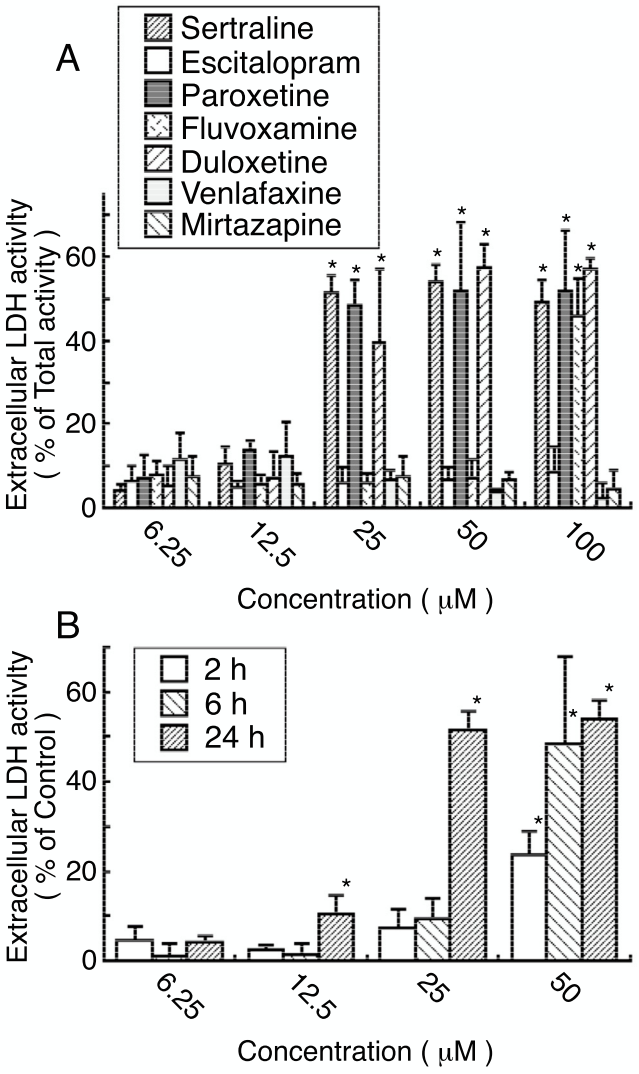

Fig. 2. (A) Effects of sertraline, escitalopram, paroxetine, fluvoxamine, duloxetine, venlafaxine, or mirtazapine on lactate dehydrogenase (LDH) release from BeWo cells. BeWo cells were incubated with 6.25, 12.5, 25, 50, or 100 μM antidepressants for 24 h and extracellular LDH activity was measured. (B) Effect of sertraline on the LDH release from BeWo cells. BeWo cells were incubated with 6.25, 12.5, 25, or 50 μM sertraline for 2, 6, or 24 h, and the extracellular LDH activity was subsequently measured. LDH release was calculated as the percentage of LDH released into the medium compared with the maximum LDH release. The data represent the mean ± SD of three independent experiments that were performed in triplicates. *$P < 0.01$ compared with the control.

We then measured the LDH release from BeWo cells exposed to 6.25–50 μM sertraline for 2, 6, or 24 h. Sertraline increased LDH release in a time and concentration-dependent manner (Fig. 2B).

3.2. Effects of antidepressants on ROS production, mitochondrial membrane potential, and ATP content of BeWo cells

ROS generation was examined using a DCFDA probe to evaluate the intracellular oxidative stress during sertraline-induced cell death. Nonfluorescent DCFDA was converted to fluorescent DCF by intracellular ROS. Exposure of BeWo cells to sertraline at concentrations of 25 and 50 μM for 24 h increased the intensity of DCF fluorescence (Table). This result indicates that sertraline induces ROS generation in BeWo cells.

Mitochondria are the major source of oxidants and target of their harmful effects. We examined the mitochondrial membrane potential using JC-10, which is a mitochondrial membrane potential-sensitive dual-emission probe, to determine mitochondrial function in the toxic effects of sertraline in BeWo cells. JC-10 forms aggregates that display orange fluorescence in healthy mitochondria. When the mitochondrial membrane potential decreases, JC-10 exists as a monomer and displays green fluorescence. The fluorescence ratio of JC-10 aggregate (orange) to monomer (green) is an indication of mitochondrial membrane potential. Exposure to 50 μM sertraline decreased the ratio of JC-10 aggregate to monomer fluorescence intensity, suggesting that exposure to sertraline led to depolarization of the mitochondrial membrane (Table).

BeWo cells were exposed to 6.25–100 μM sertraline for 6, 24, or 48 h, and the cellular ATP content was subsequently measured. Sertraline decreased the cellular ATP content in a time and concentration-dependent manner (Fig. 3). We also measured the cellular ATP content in BeWo cells after 24 h exposure to escitalopram. Escitalopram showed no significant effects on ATP levels [6.25 μM, 100.9 ± 4.3; 12.5 μM, 101.8 ± 3.7; 25 μM, 104.2 ± 5.3; 50 μM, 100.6 ± 5.2; 100 μM, 86.1 ± 8.9, (% of control, mean ± SD of three independent experiments)]. Our data revealed that sertraline, but not escitalopram, impaired mitochondrial function in BeWo cells.

3.3. Sertraline induces apoptosis in BeWo cells

Activation of caspase-3/7 is a key step in cell apoptosis (Green and Llambi, 2015; Nagata, 2018; Bock and Tait, 2020). BeWo cells were exposed to 3.125–50 μM sertraline for 24 h, and caspase-3/7 activity was then measured. Exposure to 25 or 50 μM sertraline caused significant increases in caspase-3/7 activity, suggesting that sertraline induces apoptosis in BeWo cells (Table).

The anionic phospholipid, PS, is localized in the inner leaflet of the plasma membrane in healthy cells. When cells undergo apoptosis, activated caspase-3 cleaves and activates XKR8 scramblase, leading to translocation of PS to the outer leaflet of the plasma membrane. Exposure of PS on plasma membrane is widely regarded as an early biomarker of apoptosis (Green and Llambi, 2015; Nagata, 2018; Bock and Tait, 2020). Apotracker Green (Apo-15) is a newly developed fluorogenic heptapeptide that specifically binds to PS in the outer leaflet of the plasma membrane (Barth et al., 2020). Apotracker Green was used to detect apoptotic cells showing PS on the cell surface to confirm that sertraline-induced apoptosis.

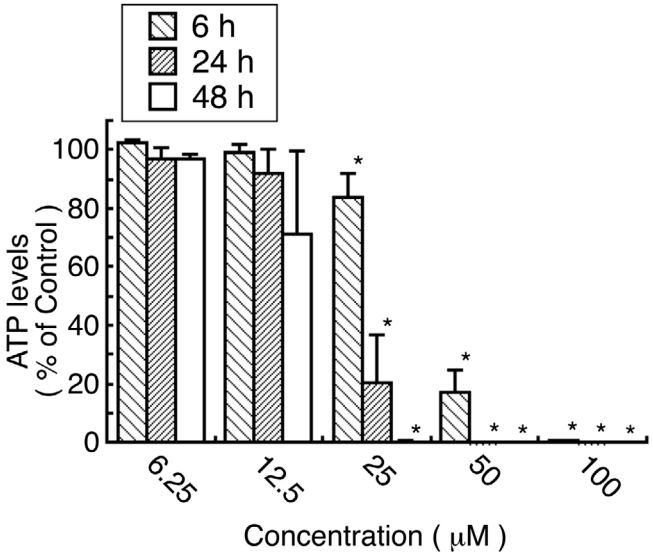

Fig. 3. Effect of sertraline on cellular ATP contents in BeWo cells. BeWo cells were incubated with 6.25, 12.5, 25, 50, or 100 μM sertraline for 6, 24, or 48 h and the cellular ATP content was determined using a CellTiter-Glo 2.0 Cell Viability Assay. Data represent the mean ± SD of three independent experiments that were performed in triplicates. *$P < 0.01$ compared with the control.

Nonfluorescent Calcein Red-AM penetrates the cell membrane then cytosolic nonspecific esterases present in live cells immediately convert it to the highly fluorescent compound Calcein Red. Apoptotic (green, Apotracker Green) and live (red, Calcein Red) cells were visualized using a fluorescence microscope (Fig. 4A) and the ratio of the number of apoptotic to live cells was calculated (Fig. 4B). Exposure to 4 μM camptothecin (positive control) and 50 μM sertraline significantly increased the number of apoptotic cells.

4. Discussion

In this study, we investigated the effects of SSRIs, SNRIs, and NaSSA on the viability and toxicity of human placental BeWo cells. BeWo cell viability was decreased due to the exposure to sertraline (SSRI), paroxetine (SSRI), fluvoxamine (SSRI), and duloxetine (SNRI). Escitalopram (SSRI), venlafaxine (SNRI) and mirtazapine (NaSSA) showed no effects (Fig. 1). BeWo cell toxicity, as determined by the LDH release, was induced by sertraline, paroxetine, fluvoxamine, and duloxetine, whereas escitalopram, venlafaxine, and mirtazapine showed no effects (Fig. 2A). Sertraline showed cell toxicity in a time and concentration-dependent manner (Fig. 2B).

Table
Effect of sertraline on reactive oxygen species (ROS) production of BeWo cells, mitochondrial membrane potential of BeWo cells, and cellular caspase-3/7 activity in BeWo cells.

Sertraline (μM)	3.125	6.25	12.5	25	50
DCF Fluorescence (% of Control)	103.4 ± 6.4	106.2 ± 10.3	108.7 ± 8.6	149.4 * ± 20.0	164.0 * ± 26.7
JC-10 Aggregate/Monomer (% of Control)	97.7 ± 12.4	98.9 ± 6.0	99.2 ± 5.2	75.2 ± 25.0	12.3 * ± 8.1
Caspase-3/7 activity (% of Control)	100.0 ± 10.9	117.9 ± 10.7	141.2 ± 28.9	291.7 * ± 62.2	240.4 * ± 53.6

Data represent the mean ± SD of three independent experiments that were performed in triplicates. *$P < 0.01$ compared with the control.

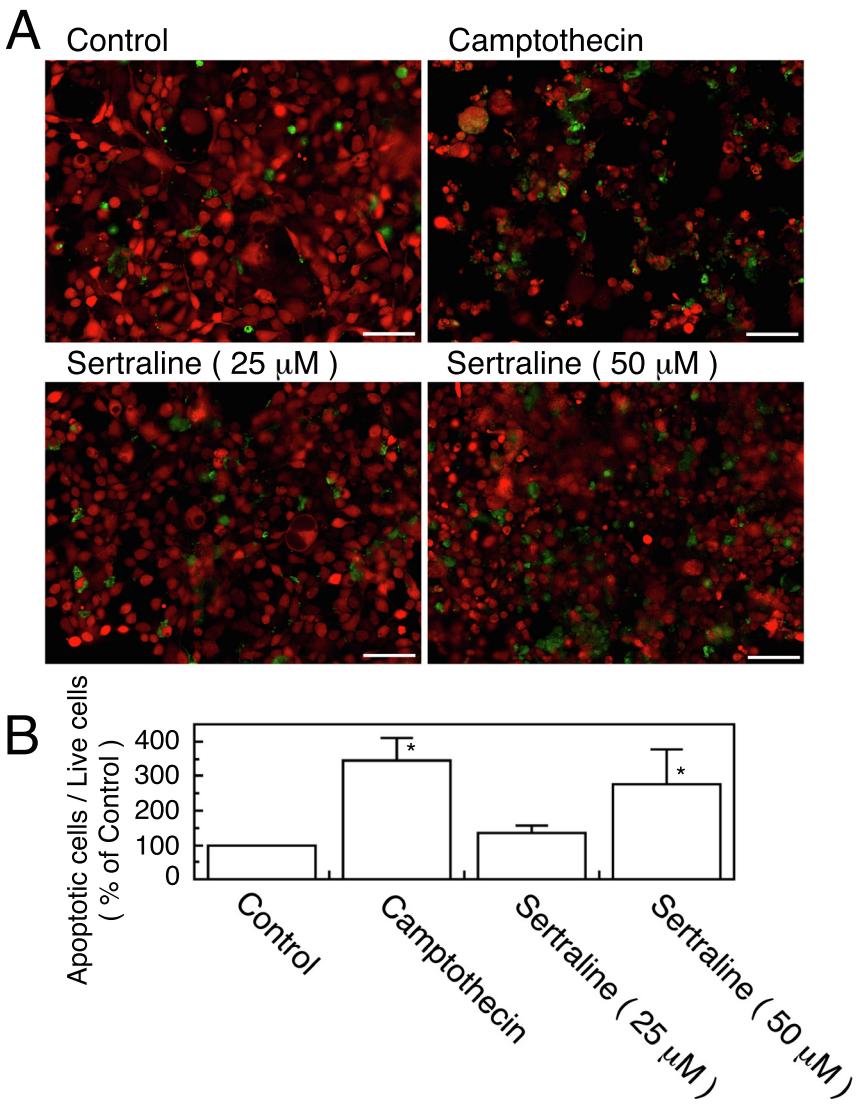

Fig. 4. Measurement of apoptotic and live cells. BeWo cells were incubated with 25 or 50 μM sertraline or 4 μM camptothecin for 6 h and live and apoptotic cells were visualized using Calcein Red-AM and Apotracker Green. (A) Representative images of apoptotic and live cells. Apoptotic cells are visualized as green and live cells are red. Scale bar represents 100 μm. (B) Quantitative analysis of the ratio of apoptotic to live cells. Data represent the mean ± SD of four independent experiments. *$P < 0.01$ compared with the control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Although SSRIs, SNRIs, and NaSSAs have different chemical structures, they primarily act by modulating the signaling of the neurotransmitter serotonin. All SSRIs share a similar mechanism of action and inhibit the serotonin transporter, leading to higher extracellular concentrations of serotonin. Our results showed that sertraline (SSRI), paroxetine (SSRI), fluvoxamine (SSRI), and duloxetine (SNRI) decreased the viability of BeWo cells and induced toxicity. In contrast, escitalopram (SSRI), venlafaxine (SNRI), and mirtazapine (NaSSA) showed no effects. Therefore, the psychological action of these antidepressants and their effects on placental cells appear unrelated.

Sertraline is one of the most prescribed antidepressants to pregnant women; however, its use in the first trimester is associated with an increased risk of congenital malformations (Chambers et al., 2006; Jimenez-Solem et al., 2012; Bérard et al., 2015; Liu et al., 2017; Shen et al., 2017). We further investigated the underlying mechanisms involved in the toxicity of sertraline in human placental BeWo cells. We further investigated the underlying mechanisms involved in the toxicity of sertraline in human placental BeWo cells. ROS generation in BeWo cells was induced by sertraline exposure (Table). Sertraline led to the depolarization of the mitochondrial membrane (Table). Mitochondrial function in BeWo cells was impaired by sertraline (Fig. 3).

Novel antidepressants, such as SSRIs, SNRIs, and NaSSAs, are considered to have a lower risk of adverse events than older tricyclic antidepressants. However, sertraline has been reported to cause toxicity in hepatocytes, prostate cancer cells, and astrocytes, in addition to the teratogenic toxicity (Li et al., 2012; Chen et al., 2014a, 2014b; Huang et al., 2011; Then et al., 2017). Therefore, we further examined the involvement of apoptosis in sertraline-induced toxicity. Sertraline increased the executioner caspase-3/7 activity (Table). Using the newly developed fluorogenic peptide Apotracker Green (apoptotic cells) and Calcein Red-AM (live cells), we examined the effects of sertraline on the number of apoptotic and live cells (Fig. 4B). Sertraline exposure increased the number of apoptotic cells.

Apoptosis is triggered via two main pathways: the mitochondria-mediated intrinsic pathway or the death receptor-mediated extrinsic pathway (Green and Llambi, 2015; Nagata, 2018; Bock and Tait, 2020). Sertraline decreased the mitochondrial membrane potential

(Table), suggesting that the mitochondria-mediated intrinsic pathway may be involved in sertraline-induced apoptosis.

BeWo cells are often used to study the chemical-induced toxicity on placenta. Kim et al. (2020) investigated the effects of parabens, which are preservatives for foods and drugs. Ethylparaben increased the caspase-3 activity and induced apoptosis in BeWo cells. Clabault et al. (2018) showed that sertraline affects the villous trophoblasts syncytialization. Further, they showed that sertraline (10 μM) slightly decreased the proliferation of BeWo cells. These results are comparable to our study results of sertraline-induced apoptotic cell death in the BeWo cells. Olivier et al. (2015) also investigated the gene expression differences in the placentas of healthy, depressed, and SSRI-treated women. They showed that genes important for the development of cardiovascular system, such as *ROCK2* and *NEXN*, were down-regulated in antidepressants-treated women and to a lesser extent in depressed women.

The placental tissue barrier is mainly composed of the syncytiotrophoblasts and the fetal enodothelial cells. In this study, BeWo cells were used as a model of syncytiotrophoblasts. To improve the *in vivo* relevance, new *in vitro* co-culture model with BeWo cells and placental microvascular endothelial cells has been developed (Aengenheister et al., 2018). Further studies, such as using this co-culture model, should be conducted to explore detailed *in vitro* to *in vivo* extrapolations.

Sertraline is predominantly eliminated by the hepatic drug-metabolizing enzymes cytochrome P450 (CYP) 2B6, CYP2C9, CYP2C19, and CYP2D6 (Hiemke et al., 2011). There is large interindividual variability in the plasma concentrations of sertraline, which may be partially due to genetic variation in drug-metabolizing enzymes. Drug–drug interactions, such as CYP2D6 inhibiting drugs, and hepatic impairment may also increase the plasma concentration of sertraline.

In the present study, we selected the concentration of antidepressants based on the concentrations used in previous studies (Li et al., 2012; Chen et al., 2014a, 2014b; Huang et al., 2011; Then et al., 2017). Although the concentrations at which effects have been observed are widely used for *in vitro* assays, these levels are not typically achieved in plasma for general dosage. However, genetic variation, drug–drug interactions, and decreased liver function may alter the plasma concentrations. In addition, antidepressants are often used long-term (for months or years) rather than as single 24-h administration. It can be possible that long-term use of lower concentrations of antidepressants affects placenta functions. In this study, we have shown that there are differences in the acute toxicity of antidepressants. These data are useful for determining optimal *in vivo* concentrations for safer use of antidepressants.

The present study was designed to determine the toxicity of various antidepressants in human placental cells, as well as the mechanisms of toxicity. Further studies are required to investigate the relationship between the toxicity and chemical structures of antidepressants and the detailed mechanisms of apoptotic pathways induced by sertraline. Chen et al. (2014a) reported that sertraline-induced apoptosis in hepatic cells is mediated by the mitogen-activated protein kinase (MAPK) pathway. We are currently investigating the involvement of the anti-apoptotic Bcl-2 family proteins and MAPK pathways in sertraline-induced apoptosis in human placental cells.

To our knowledge, this is the first study to show the difference in antidepressants toxicity in human placental BeWo cells. Further studies, such as *in vivo* effects of long-term exposure of antidepressants to placenta, are required for investigation of correlation between the risk of malformation of fetus and use of antidepressants during pregnancy.

5. Conclusions

We evaluated the effects of SSRIs (sertraline, escitalopram, paroxetine fluvoxamine), SNRIs (duloxetine, venlafaxine), and NaSSA (mirtazapine) on the viability and toxicity of human placental BeWo cells. Sertraline, paroxetine, fluvoxamine, and duloxetine decreased viability and induced toxicity in placental cells. Escitalopram, venlafaxine, and mirtazapine showed little or no effects. Sertraline increased the LDH release and decrease in cellular ATP contents of BeWo cells in a time and concentration-dependent manner. Sertraline promoted ROS production and decreased the mitochondrial membrane potential. Caspase-3/7 activity and the number of apoptotic cells were increased following exposure to sertraline. Sertraline may induce ROS-dependent apoptosis in BeWo cells. These results are useful for further studies to determine the optimal dosage of antidepressants for pregnant women.

CRediT authorship contribution statement

Tomohiro Nabekura: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Shinya Ishikawa:** Investigation. **Makoto Tanase:** Investigation. **Taichi Okumura:** Investigation. **Tatsuya Kawasaki:** Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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