Expression and characterization of vitamin C transporter in the human trophoblast cell line HTR-8/SVneo: effect of steroids, flavonoids and NSAIDs

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Vitamin C plays an important role in embryogenesis and fetal growth as well as in the progression of pregnancy and delivery. Therefore, it is important to understand the mechanism that mediates its transport to the fetus as well as the possible influences by endogenous and exogenous substances on its placental uptake. The aim of this study was to investigate placental sodium-dependent vitamin C transporters (SVCT) 1 and 2. By means of RT–PCR, we found that SVCT2, but not SVCT1, mRNA is expressed in human trophoblast cell line HTR-8/SVneo. Our method was able to confirm SVCT2 mRNA expression in human first-trimester chorionic villi but not in term placental tissue. Cell line kinetic studies of [14C] ascorbic acid (AA) uptake indicated a one-site model and a saturable process. Fetal bovine serum (FBS) and epidermal growth factor (EGF) do not influence the transport properties, although they significantly increase the expression of SVCT2. Steroid hormones (17β-estradiol, progesterone and cortisol), flavonoids (genistein and quercetin) and non-steroidal anti-inflammatory drugs (NSAIDs) (indomethacin and diclofenac) inhibit [14C]AA uptake in a dose-dependent and non-competitive manner. On the contrary, the process is not influenced by aspirin. Our study suggests the use of HTR-8/SVneo cells as a suitable model for trophoblast vitamin C transport investigation.

Key words: ascorbate/NSAIDs/flavonoids/steroids/trophoblasts

Introduction

Vitamin C, when present in its reduced form, ascorbic acid (AA), by acting as an anti-oxidant and as a co-factor in the synthesis of extracellular matrix proteins, regulates trophoblastic function, embryogenesis, fetal growth and the progression of pregnancy and delivery (Prasad et al., 1998; Jauniaux et al., 2004; Biondi et al., 2005). The first-trimester human placenta has limited antioxidant enzyme capacity and therefore, at this time, the embryo is most vulnerable to oxidative stress. The fetus receives antioxidant molecules, among which vitamin C, from the uterine glands and the secondary yolk sac (Jauniaux et al., 2004). The indispensable need for AA during prenatal life has been shown in mice deficient in the AA transporter Slc23a1 (Sotiriou et al., 2002), orthologue of the rat AA transporter sodium-dependent vitamin C transporters (SVCT2) (Tsukaguchi et al., 1999). Indeed, these newborn mice, Slc23a1−/−, die of respiratory failure and intra-parenchymal brain haemorrhage within a few minutes of birth.

As yet, it is known that L-AA transport is mediated by two different sodium-dependent proteins, SVCT1, mainly expressed in the intestine, liver and kidney epithelia, and SVCT2 expressed in other tissues (Tsukaguchi et al., 1999). At the placental level, SVCT2-mediated transport seems to be operative, and SVCT2 cDNA has been isolated from a human placental choriocarcinoma cell line library, whose expression in human retinal pigment epithelial (HRPE) cells is able to induce Na⁺-gradient-dependent ascorbate uptake (Rajan et al., 1999). This induced transport activity exhibits similar features to those previously described in JAR human placental choriocarcinoma cell line (Prasad et al., 1998), and it has been suggested that such an active transport mechanism could account for the much higher concentration of AA in the fetus, as compared with maternal blood (Rice, 2000).

Taking into account the supposed role of vitamin C in feto-maternal homeostasis throughout pregnancy, as well as the influence exerted by several substances on its transport under various experimental conditions, we decided to investigate the features of trophoblastic AA uptake. In this study, we tested the suitability of the human trophoblast cell line HTR-8/SVneo as a model for the in vitro study of vitamin C placental transport. For this purpose, we searched for SVCT1 and SVCT2 mRNA in the above-mentioned cell line, as well as in human first-trimester chorionic villi and placental tissue at term. Furthermore, we investigated the effect of the following substances on SVCT2 expression and activity in the trophoblast cell line: fetal bovine serum (FBS), epidermal growth factor (EGF), 17β-estradiol, progesterone and cortisol. As regards to these steroid hormones, that are involved in the control of pregnancy, their eventual effect on vitamin C placental transport, to our knowledge, has never been investigated. We also tested competition for vitamin uptake by genistein and quercetin. These flavonoids, which are commonly ingested by the diet, are reported to influence SVCT1- and SVCT2-mediated AA transport in different cellular models (Kuo et al., 1997; Park and Levine, 2000; Song et al., 2002). Finally, we tested the effect of aspirin, indomethacin and diclofenac, anti-inflammatory drugs frequently administered for therapeutic purposes during pregnancy. Indeed, some of these compounds have already been shown to influence SVCT2-mediated AA transport in other cells (Manfredini et al., 2002; Dalpiaz et al., 2004).
Materials and methods

[^14]CAA (specific activity 6 mCi/mmol) was obtained from NEN Life Science, Boston, MA, USA. Tissue culture media, sera, RNA extraction and RT–PCR reagents were purchased from Invitrogen (Paisley, Scotland, UK). Steroids, flavonoids, NSAIDs and dithiothreitol (DTT) were from Sigma, St. Louis, MO, USA. qPCR human reference cDNA, random-primmed, was purchased from Clontech Laboratories, Mountain View, CA, USA.

All other chemicals were the highest reagent grades commercially available.

HTR-8/SVneo cell culture and treatment

The HTR-8/SVneo trophoblast cell line was kindly provided by Dr CH Graham, Queen’s University, Kingston, Ontario, Canada. Cells were cultured at 37°C in an atmosphere of 5% CO₂/95% air in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% FBS and 2% penicillin/streptomycin. For SVCT2 mRNA expression and SVCT2 interaction studies, cells were seeded in 6-multwell and 24-multwell plates, respectively, grown to 100% cell density at confluence and pre-exposed 24 h before the experiments to RPMI 1640 and supplemented with 10% FBS, or serum-free, or serum-free plus 25 ng/ml EGF.

Tissue preparation

For RNA extraction studies, first-trimester human chorionic villi were obtained from four patients undergoing chorionic villus biopsy for prenatal diagnosis at the 11th week of gestation. In these cases, the small amount necessary for the assay derived from the tissue discarded by the geneticist. Term placental tissue was obtained from physiological pregnancies with appropriately grown singleton fetuses, by elective caesarean section in patients, which had been delivered by caesarean section in the previous pregnancy. All the patients gave verbal informed consent to the study. Approval for the experimental protocol was obtained from the University of Ferrara institutional review board. Tissue samples were immediately frozen in liquid nitrogen and stored at -80°C.

RT–PCR

Total RNA was isolated from HTR-8/SVneo cells, human chorionic villi and placental tissue at term using Trizol reagent (Invitrogen) as manufacturer’s suggested procedure. RT–PCR was employed to search for the expression of SVCT1 and SVCT2 transporter isomers. First-strand cDNA was synthesized by incubating 3 µg RNA with 0.5 µg oligo(dT)12–18, at 70°C for 10 min and subsequently a reaction mix of avian myeloblastosis virus reverse-transcriptase (AMV RT) buffer, 500 nM of dNTPs, 2.5 units of AMV RT was added. The samples were then incubated at 45°C for 50 min. cDNA was kept at -20°C until used for PCR.

The synthesized cDNA was amplified in a standard PCR reaction mix containing 200 µM dNTP, 1.5 mM MgCl₂, 0.25 µg of each sense and antisense primer and 0.25 units of Taq polymerase. Primer sequences were examined for specificity by comparison with known gene sequences in GenBank using the blastn program at the BLAST website (http://www.ncbi.nlm.nih.gov/BLAST/). PCR amplification from reverse transcribed cDNA was carried out using specific PCR primers for SVCT1 and SVCT2. SVCT1 sense: 5′-GCCCCCTGAAACACCTCTCAT-3′; SVCT1 antisense: 5′-ATGGCCGACAT-GATAAGGAAC-3′; SVCT2 sense: 5′-TTCTGTGTGGAATCTACAT-3′; SVCT2 antisense: 5′-ACCAAGAGGGCCCAATTAGG-3′ (Garcia et al., 2005). qPCR human reference cDNA (Clontech) was used as positive control template for validation of gene primer design. PCR amplification for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA was performed on the same samples as a parallel control. GAPDH sense: 5′-CCACCATGGGCAAATTCATGGCA-3′; GAPDH antisense: 5′-TCTAGACGGCAGTCTAGGCACC-3′ (Xue et al., 1996). A 1/20 volume of the generated cDNA reaction was used in the amplification reaction. PCR was performed in a 25 µl volume using 1.5 mM magnesium chloride, 0.2 mM dNTPs, 0.25 µg of each sense and antisense primer and 0.25 units of Taq polymerase. PCR for both SVCT1 and SVCT2 was performed for 20–40 cycles of amplification using the following parameters: denaturation at 94°C for 20 s, annealing at 55°C for 30 s and extension at 72°C for 40 s. The curve for SVCT1 and SVCT2 amplified sequences for 26–30 cycles, and 28 cycles were used in the semi-quantitative analysis. PCR amplification for GAPDH was performed on the same samples as a parallel control, with a linear range of amplification between 20 and 26 cycles, and 23 cycles were used in the semi-quantitative analysis under the following PCR conditions: denaturation at 90°C for 20 s, annealing at 55°C for 30 s and extension at 72°C for 40 s. The amplified products, of length 339 bp for SVCT2 and 598 bp for GAPDH, were separated on a 1.5% agarose gel containing ethidium bromide, using a DNA ladder as size marker. After gel electrophoresis, scanning densitometry of the areas of interest was performed using the ‘Gel Doc 2000’ video imaging system (Bio-Rad Laboratories, Hercules, CA, USA) to obtain the arbitrary density units. Band densities corresponding to SVCT2 expression following several treatments were quantified using Quantity One software (Bio-Rad Laboratories), and the ratio of SVCT2 to GAPDH band intensities was used as an indication of the relative abundance of the two templates in the samples.

[^14]CAAascorbate uptake and SVCT2 transporter interactions

Transport assays were performed following the method described by Rajan et al. (1999). In brief, the uptake buffer was prepared fresh each time, and the composition was 25 mM Hepes/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄ and 5 mM glucose. DTT (1 mM) was also added to the uptake buffer to prevent the oxidation of AA. At this concentration, DTT had no effect on the transport process. The incubation time for the transport measurements was 60 min, within the time range of linear uptake (data not shown), at 37°C, after this the time uptake buffer containing the radioactive substrate was aspirated off, and cells were washed with 2 × 2 ml of ice-cold uptake buffer. Cells were then dissolved in 250 µl of 0.2 M NaOH solution containing 0.5% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS) (Sigma), transferred to vials, and radioactivity associated with the cells was evaluated by liquid scintillation spectrometry. The kinetics of SVCT2-mediated[^14]CAA uptake were analysed, performing a homologous competition experiment. It consisted of a concentration-response curve where unlabelled AA ranged from 2.5 to 1000 µM, and the concentration of[^14]CAA ranged from 2.5 to 50 µM, being kept constant at 50 µM. Data were analysed by nonlinear regression of the Michaelis–Menten equation and confirmed by Eadie–Hofstee linear regression.

Inhibition of AA transport was determined by adding the indicated concentrations of unlabelled compounds to plated cells along with either[^14]CAA at a fixed concentration of 50 µM or[^14]CAA ranging from 2.5 to 100 µM. The unlabelled inhibitor concentrations displacing 50% of[^14]CAA (IC50 values) were obtained by computer analysis of displacement curves. Inhibitory binding constants (K_i values) were derived from the IC50 values according to the Cheng and Prusoff equation K_i = IC50(1+[C]/K_t), where [C] is the concentration of[^14]CAA and K_t its Michaelis–Menten constant (Cheng and Prusoff, 1973). Competition studies were performed according to Lineweaver–Burt analysis. All calculations were performed using the Graph Pad Prism (GraphPad) computer programme.

Data analysis

Statistical analysis was performed by ANOVA followed by Dunnett’s t-test. The difference was considered statistically significant at P < 0.05.

Results

Transporter expression

We analysed the expression of AA transporter mRNA in HTR-8/SVneo cells, human chorionic villi and placental tissue at term by RT–PCR, using the set primers previously reported by Garcia et al. (2005) for both SVCT1 and SVCT2. We found that the amplified DNA band corresponding to 339 bp was the expected size for the amplification product of SVCT2, whereas no amplification product was detected with the specific primers for SVCT1, corresponding to 360 bp. This result was obtained for HTR-8/SVneo cells and chorionic villi, whereas no PCR products were detected in placental tissue at term (Figure 1A). The commercially available human reference cDNA, the positive control, showed two bands of 339 and 360 bp as amplification products of SVCT2 and SVCT1, under each PCR conditions, respectively (Figure 1B).
We studied the influence of AA, 17β-estradiol, progesterone and cortisol on [14C]AA uptake. Furthermore, the Eadie–Hofstee linear regression of data was linearly related to its concentration, thus indicating a saturable transport process. The rate of [14C]AA uptake by HTR-8/SVneo cells is hyperbolically described by the Michaelis–Menten constant (K_m) = 23.0 ± 3.0 μM; the V_max = 2.8 ± 0.2 nmol/10^6 cells/60 min (Figure 2).

**Inhibition of SVCT2-mediated uptake**

We studied the influence of AA, 17β-estradiol, progesterone, cortisol, genistein, quercetin, aspirin, indomethacin and diclofenac on [14C]AA uptake (50 μM) by HTR-8/SVneo cells. In Figure 3A (left), the inhibition curves of [14C]AA uptake in the presence of increasing concentrations of AA, 17β-estradiol, progesterone and cortisol are reported. Calculation of the inhibition constants (K_i) demonstrates that, among steroids, the highest affinity for SVCT2 is expressed by 17β-estradiol (K_i = 6.2 ± 0.3 μM) and the lowest by progesterone (K_i = 64.0 ± 5.0 μM). The Ki for endogenous substrate AA was 23.0 ± 3.0 μM. On the right of the Figure 3A, the Lineweaver–Burk plots show that the most potent inhibitor, 17β-estradiol, inhibited SVCT2-mediated uptake in a non-competitive manner.

The inhibition curves of [14C]AA uptake obtained in the presence of flavonoids genistein and quercetin are reported. Calculations of the inhibition constants (K_i) shows the highest affinity for SVCT2 is expressed by quercetin (K_i = 7.4 ± 0.8 μM) with which a non-competitive inhibition was exhibited (Figure 3B, right).

As for the NSAID–SVCT2 interaction, both indomethacin and diclofenac were shown to inhibit [14C]AA uptake (Figure 3C, left). The highest affinity (K_i = 5.3 ± 0.6 μM) which behaved as a non-competitive inhibitor (Figure 3C, right). Furthermore, Figure 3C, on the left, highlights that aspirin did not interact with SVCT2.

The highest concentration of DMSO and ethanol (0.1% v/v) had no effect on [14C]AA uptake.

**Modulation of SVCT2 mRNA expression**

Twenty-four-hour cell culture in a medium containing FBS (10% v/v) induced a significant increase (2.3-fold; P < 0.001) of SVCT2 mRNA expression with respect to the serum-free condition (Figure 4). Treatments with EGF and FBS of cultures at complete confluence did not alter cell number compared with serum-free control cells. Cell viability remained at 98–100% in all experiments. Cell number, total cellular protein and total cellular RNA per well were also unaffected (data not shown). Taking into account the ubiquitous effect of serum on gene expression, the housekeeping GAPDH gene was used for normalization of target gene values throughout experiments, because its level did not vary significantly as a function of time or serum and EGF stimulation; indeed, ratios of SVCT2 mRNA to GAPDH mRNA remain virtually constant. A comparable increase (2.2-fold; P < 0.001) was elicited by adding EGF (10 ng/ml) to the serum-free medium (Figure 4). Twenty-four-hour cell treatment with 17β-estradiol,
progesterone and cortisol at 10⁻⁶M did not modify SVCT2 mRNA expression (data not shown).

Modulation of SVCT2-mediated uptake

Pre-treatment of HTR-8/SVneo cells for 24 h with FBS (10% v/v), as well as with EGF (10 ng/ml), increased the V_max, although to different extents (Figure 5A), without affecting the K_t value (Figure 5B). Figure 6 shows the statistical analysis of V_max and K_t values obtained in the presence and absence of serum and EGF. In the presence of FBS, V_max significantly increases (200%; P < 0.001) with respect to the serum-free condition; similarly, EGF significantly increases V_max (166%; P < 0.05) (Figure 6A). FBS and EGF treatment did not affect K_t values (Figure 6B). Pre-treatment with 10⁻⁶ M steroid hormones for 24 h did not change the SVCT2-mediated transport parameters (Figure 7).

Discussion

It is well known that oxidative processes play a fundamental role in pregnancy because of their influence upon the vascular changes in the maternal organism, as well as on the regulation of uterine and cervical tone during gestation and delivery (Biondi et al., 2005). Vitamin C represents a classical anti-oxidant component of the oxidative balance, exerting its regulatory influence throughout the entire course of gestation (Prasad et al., 1998; Jauniaux et al., 2004; Biondi et al., 2005), although its physiological role is not yet completely understood.

Figure 3. (A) Left: 50 μM [¹⁴C] ascorbic acid (AA) transport inhibition curves in the presence of AA and the hormones 17β-estradiol, progesterone and cortisol. [¹⁴C]AA transport in the presence of inhibitors was measured at 37°C. Right: 50 μM [¹⁴C]AA transport inhibition curves in the presence of 20 and 50 μM 17β-estradiol. Graph slopes indicate non-competitive inhibition of [¹⁴C]AA transport by 17β-estradiol. (B) Left: 50 μM [¹⁴C]AA transport inhibition curves in the presence of AA and the flavonoids quercetin and genistein. [¹⁴C]AA transport in the presence of inhibitors was measured at 37°C. Right: 50 μM [¹⁴C]AA transport inhibition curves in the presence of 20, 50 and 100 μM quercetin. Graph slopes indicate non-competitive inhibition of [¹⁴C]AA transport by quercetin. (C) Left: 50 μM [¹⁴C]AA transport inhibition curves in the presence of AA and the NSAIDs indomethacin, diclofenac and aspirin. [¹⁴C]AA transport in the presence of inhibitors was measured at 37°C. Right: 50 μM [¹⁴C]AA transport inhibition curves in the presence of 20, 60 and 120 μM diclofenac. Graph slopes indicate non-competitive inhibition of [¹⁴C]AA transport by diclofenac. All plots are representative of three experiments, each one run in duplicate.
Several early and late complications of pregnancy, from fetal malformation to pre-eclampsia, can be interpreted as a consequence of hypoperfusion of the product of conception (Vesco et al., 1997) and are related to the inflammatory changes following derangement of the oxidative balance (Biondi et al., 2005). Therefore, it is possible that AA exerts a protection against these conditions by reducing utero-placental vascular impairment induced by oxidative stress, thus preventing inflammation. Several experimental and clinical evidences enforce the concept of a protective role of vitamin C during pregnancy. AA influences trophoblastic apoptosis (Kamudhamas et al., 2004), and it appears to prevent diabetic (El-Bassiouni et al., 2005) as well as ethanol-induced embryopathy (Peng et al., 2005). Furthermore, respiratory failure and intra-parenchymal brain haemorrhage observed in newborn mice Slc23a1−/− (Sotiriou et al., 2002) represent features of human premature birth as well, the risk of which is significantly elevated in a variant in the SVCT SLC23A2 (Erichsen et al., 2004), which plays an important role in the pathogenesis of pre-eclampsia (Redman and Sargent, 2005) as well as ethanol-induced embryopathy (Peng et al., 2005). Furthermore, respiratory failure and intra-parenchymal brain haemorrhage observed in newborn mice Slc23a1−/− (Sotiriou et al., 2002) represent features of human premature birth as well, the risk of which is significantly elevated in a variant in the SVCT SLC23A2 (Erichsen et al., 2004), which plays an important role in the pathogenesis of pre-eclampsia (Redman and Sargent, 2005) as well as ethanol-induced embryopathy (Peng et al., 2005)

Our attempts to demonstrate the expression of placental SVCT2 mRNA throughout pregnancy were only in part successful. Indeed, we found that in the human trophoblast cell line HTR-8/SVneo and in human first-trimester chorionic villi, the AA transporter SVCT2, but not SVCT1, mRNA is expressed. Such a result is consistent with the isolation of SVCT2 cDNA from a human placental choriocarcinoma cell line library (Rajan et al., 1999), which represents by definition early pregnancy cancer tissue. As for advanced pregnancy, we could not obtain normal human second-trimester placental tissue. Furthermore, as regards normal placental tissue at term, although we strictly observed the technical rules aimed at avoiding RNA degradation (Haimov-Kochman et al., 2006), in our hands RT–PCR technique was unable to show the expression of the transporter mRNA. At the moment, we do not have any explanation for such a result. Because SVCT2 specific transcript was found by northern blot analysis in commercially available placental tissue of unknown gestational age by Rajan et al. (1999), the transporter mRNA expression in term placental tissue deserves further investigation. To our knowledge, the transporter has never been studied in primary cell cultures. However, due to our failure to demonstrate both SVCT1 and SVCT2 mRNA expression in term placental tissue, at the moment the interpretation of our results must be limited to early pregnancy.

The kinetics of [14C]AA uptake by HTR-8/ SVneo cells show properties similar to SVCT2-mediated transport described in other human cells (Prasad et al., 1998; Rajan et al., 1999; Manfredini et al., 2002). Our study also shows that the expression of the AA transporter mRNA is under the control of growth factors, as revealed by its up-regulation by serum, as well as by EGF, addition to the culture medium. Accordingly, pre-treatment with FBS and EGF induces a significant increase in AA uptake Vmax without changing the affinity for the substrate, thereby suggesting an increase in transporter number.
in the control of pregnancy, flavonoids commonly ingested in the diet and NSAIDs, which are frequently used for therapeutic purposes. We found that all tested compounds, except for aspirin, inhibited $^{[14]}\text{C}AA$ uptake by HTR-8/SVneo cells in a non-competitive manner, namely binding to the transporter in a different site from that of endogenous ligand ascorbate, whose velocity of transport ($V_{\text{max}}$) was therefore reduced. Among the steroids, 17$\beta$-estradiol exhibited the strongest inhibitory action, as did quercetin among the flavonoids and diclofenac among the NSAIDs. All tested substances, except for progesterone, show a $K_i$ value lower than that of the endogenous substrate AA. As for progesterone, the tissue concentration of the hormone can reach up to $5000 \text{ ng/gm}$ in the placenta throughout pregnancy (Siiteri and Stites, 1982). This concentration, which corresponds to $\sim 10-20 \mu\text{M}$, i.e. lower than progesterone $K_i$, should not be able to significantly inhibit AA uptake by SVCT2. However, it is worth outlining that in some gestational complications such as threatened or recurrent abortion, progesterone can be prescribed at doses as high as $800 \text{ mg}$ daily for several weeks. In this condition, the hormone could possibly reach the tissue concentration able to inhibit AA uptake. The same consideration should be made for 17$\beta$-estradiol and for cortisol, which show a $K_i$ about 10 and 6 times lower, respectively, when compared with progesterone.

As regards to flavonoids, our results are consistent with the inhibitory action by different compounds on the human intestinal ascorbate transporter SVCT1 observed in transfected Chinese hamster ovary (CHO) cells and Xenopus laevis oocytes (Song et al., 2002) and in some intestinal cell lines (Kuo et al., 1997), as well as in other SVCT2-expressing human cell lines (Park and Levine, 2000). Indeed, we found that quercetin and genistein inhibited SVCT2-mediated ascorbate uptake by HTR-8/SVneo cells in a non-competitive manner. However, the concentration required for inhibitory action is much higher ($K_i = 7.4 \mu\text{M}$ for quercetin and $K_i = 28.0 \mu\text{M}$ for genistein) than that reached systemically by dietary ingestion. Instead, such a concentration is realistic in the intestine, and therefore, flavonoids may impair maternal AA absorption at this level, thus decreasing trophoblastic availability of the vitamin (Song et al., 2002).

Finally, it is important to note the behaviour of plasma therapeutic concentrations (Giagoudakis and Markantonis, 2005) of the NSAIDs in the light of their possible use for clinical purposes during pregnancy. As for diclofenac, our data show a non-competitive inhibitory action on $^{[14]}\text{C}AA$ uptake by HTR-8/SVneo cells similar to that exhibited in HRPE cells (Manfredini et al., 2002; Dalpiaz et al., 2004), with a $K_i$ about four times lower than that of indomethacin. Most interestingly, aspirin, which has been reported to slightly inhibit $^{[14]}\text{C}AA$ uptake in oocytes expressing SVCT-1 or SVCT-2 (Tsukaguchi et al., 1999), did not exert any influence on its uptake by HTR-8/SVneo cells. Although both diclofenac and indomethacin, along with other cyclo-oxygenase inhibitors, have been reported to induce several adverse effects on fetal development and well-being (Chan et al., 2001; Auer et al., 2004; Benini et al., 2004; Friedman et al., 2005), they are still in use as tocolytic agents (Vermillion and Robinson, 2005; Loe et al., 2005). In our opinion, the inhibitory action of diclofenac and indomethacin on SVCT2-mediated transport of AA should be added to their adverse effects, suggesting caution in their use during pregnancy. On the contrary, aspirin, which is widely prescribed for the prevention of several complications of pregnancy (Rumbold et al., 2005), appears to be the drug of choice as regards AA trophoblastic uptake in our data. Taken together, our results should suggest to preserve AA levels in the fetal compartment through maternal supplementation of the vitamin, carefully considering the impairment of its uptake by dietary components, as well as by compounds administered for therapeutic purposes.

The results of this study need to be confirmed using trophoblast primary cell cultures as well as models for in vivo investigation.
Nevertheless, our experiments show that HTR-8/SVneo cells represent a suitable model for the first trimester in vitro study of AA transport and SVCT2 protein.

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References

Cheng YC and Prusoff WH (1973) Relationships between the inhibition constant (kI) and the concentration of inhibitor which cause 50 per cent inhibition (IC50) of an enzymatic reaction. Biochem Pharmacol 22,3099–3108.