Selective Impairment of Taurine Transport by Cyclosporin A in a Human Placental Cell Line

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ABSTRACT. We investigated, using a human placental choriocarcinoma cell line as a model, the effects of the immunosuppressive drug cyclosporin A on several placental transport systems mediating the transfer of glucose and amino acids from mother to fetus. The results of the investigation show that the transport system responsible for the transfer of taurine is selectively impaired by the drug, whereas the other transport systems are either stimulated or not affected. The inhibitory effect of the drug on taurine transport appears to be due to interference with calmodulin-dependent processes because calmodulin antagonists such as W-7, calmidazolium, and CGS 9343B mimic the effects of cyclosporin A. FK506, another immunosuppressive drug that is currently undergoing clinical trials, does not have this inhibitory effect. (*Pediatr Res* 32: 125-127, 1992)

[2,3-³H]aspartic acid; 13.5 Ci/mmol; L-[¹⁴C(U)]alanine, 174 mCi/mmol; L-[5-³H]proline, 9.9 Ci/mmol; L-[¹⁴C(U)]lysine, 312 mCi/mmol; and 3-O-[methyl-³H]methyl-D-glucose, 79.0 Ci/mmol. Cyclosporin A and FK506 were gifts from Sandoz AG (Basel, Switzerland) and Fujisawa Pharmaceutical Co. (Deerfield, IL), respectively. CGS 9343B was a gift from CIBA-GEIGY Corp. (Summit, NJ). DMSO, cremophor, and calmidazolium (compound R24571) were purchased from Sigma Chemical Co. (St. Louis, MO). W-7 was from Research Biochemicals, Inc. (Natick, MA). The JAR human placental choriocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). RPMI 1640 medium, penicillin-streptomycin, Fungizone (amphotericin B), and fetal bovine serum were purchased from GIBCO (Grand Island, NY). All other chemicals were of analytical grade.

Cell culture and uptake measurement. The JAR cells were cultured as described previously (5, 6). Exactly 24 h after subculturing, fresh culture medium containing the test compounds was added to the cells. The following solvents were used to make stock solutions of the compounds: cremophor for cyclosporin A; DMSO for FK506, W-7, calmidazolium, and CGS 9343B. An equal amount of respective solvent was added to the cells to serve as control. After overnight incubation (12 h) at 37°C, the cultures were used for uptake measurements. The composition of the uptake buffer was 25 mM N-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid/Tris (pH 7.5), 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM D-glucose. When the uptake of 3-O-methyl-D-glucose was measured, D-glucose was not added to the uptake buffer. The exact procedure for uptake measurement in these cells has been described previously (5, 6).

Cyclosporin A is a cyclic undecapeptide that has potent immunosuppressive activity (1). It is currently in wide use in organ transplant patients to prevent graft rejection (2). Cyclosporin A treatment, however, is associated with many toxic effects (2). In comparison with much investigated toxic effects such as nephrotoxicity, hepatotoxicity, and hypertension, much less is known about the influence of cyclosporin A treatment on pregnancy. There are two reports currently available that review the course and outcome of pregnancy in patients receiving cyclosporin A (3, 4). A striking observation in both reports is an increased occurrence of intrauterine growth retardation during treatment with the drug. This raises the possibility of a direct effect of cyclosporin A on fetal growth. Because reduced placental transfer of nutrients is one of the well-known causative factors of reduced fetal growth, we investigated, using a human placental choriocarcinoma cell line as a model, the effects of cyclosporin A on several placental transport systems mediating the transfer of glucose and amino acids from mother to fetus. The results of the investigation show that the transport system responsible for the transfer of taurine is selectively impaired by the drug, whereas the other transport systems are either stimulated or not affected.

MATERIALS AND METHODS

Materials. All radiolabeled compounds were purchased from DuPont-New England Nuclear (Boston, MA). The specific radioactivities of these compounds were as follows: [2-³H(n)]taurine, 25.6 Ci/mmol; L-[4,5-³H(n)]leucine, 53.0 Ci/mmol; L-

RESULTS AND DISCUSSION

There are multiple transport systems in the human placenta that mediate the transfer of amino acids from mother to fetus (7). At least three systems serve neutral α -amino acids (systems A, ASC, and L) (8, 9) and one each for β -amino acids (system β) (10), acidic amino acids (system X_{AG}⁻) (11), and basic amino acids (system y⁺) (12). Placental transfer of glucose is mediated by a Na⁺-independent, facilitative glucose carrier (13). We investigated the effects of 5 μ M cyclosporin A on these transport systems in the JAR human placental choriocarcinoma cell line by using the following substrates: taurine (system β), alanine (system ASC), leucine (system L), proline (system A), lysine (system y⁺), aspartic acid (system X_{AG}⁻), and 3-O-methyl-D-glucose (glucose carrier). The results are summarized in Table 1. The amino acid transport systems A, ASC, and y⁺ were not influenced by cyclosporin A, and neither was the glucose transport system. The systems L and X_{AG}⁻ were also not inhibited, but rather significantly stimulated by cyclosporin A. The only amino acid transport system that was impaired by the drug was the β system. At 5 μ M, cyclosporin A caused approximately 50% inhibition of this system.

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Table 1. Effects of cyclosporin A on amino acid and glucose transport systems in JAR human placental choriocarcinoma cell line*

Substrate	Transport system	Transport (pmol/mg protein/3 min)		% Control	p value
		Control	Cyclosporin A		
Amino acids					
Taurine	β	0.46 $\pm$ 0.02	0.25 $\pm$ 0.01	54	<0.001
Alanine	ASC	129.00 $\pm$ 5.00	127.00 $\pm$ 4.00	99	0.8
Leucine	L	3.70 $\pm$ 0.11	4.60 $\pm$ 0.15	125	<0.001
Proline	A	3.01 $\pm$ 0.09	3.22 $\pm$ 0.04	107	0.14
Lysine	y^+	59.94 $\pm$ 2.25	55.20 $\pm$ 1.81	92	0.15
Aspartic acid	X_{AG}^-	15.40 $\pm$ 0.60	18.23 $\pm$ 0.47	118	0.005
Sugar					
3-O-Methyl-D-glucose	Glucose carrier	0.12 $\pm$ 0.01	0.13 $\pm$ 0.01	108	0.19

* The values are means $\pm$ SEM. Monolayer cultures were treated for 12 h at 37°C with either cremophor (control) or cyclosporin A (stock solution made in cremophor). After the treatment, transport of amino acids and glucose was measured for 3 min at 22°C using radiolabeled substrates. Final concentration: cyclosporin A, 5 μ M; cremophor, 0.024%; taurine, 20 nM; alanine, 1 μ M; leucine, 50 nM; proline, 0.5 μ M; lysine, 5 μ M; aspartic acid, 0.15 μ M; and 3-O-methyl-D-glucose, 0.1 μ M.

Physiologic concentrations of taurine in maternal blood are in the micromolar range. Because the above-described experiments were done with 20 nM taurine, it was of interest to determine if transport would be affected by cyclosporin A at higher concentrations of taurine. We used a taurine concentration of 10 μ M, which is close to the physiologic range and is enough to saturate the taurine transport system in the JAR cell (5). Under these conditions, cyclosporin A (5 μ M) was found to inhibit taurine uptake by 55% (uptake values were 30.1 $\pm$ 0.6 and 13.5 $\pm$ 0.6 pmol/mg protein/3 min in the absence and presence of cyclosporin A, respectively).

Therapeutic concentrations of cyclosporin A in blood are in the range of 0.1–0.6 μ M (14), but occurrence of higher concentrations is not uncommon (3, 15). Figure 1 describes the dose-response relationship for the inhibitory effect of cyclosporin A on taurine transport in human placental choriocarcinoma cells. The inhibition was significant (15 $\pm$ 4%, p < 0.005) even at a cyclosporin A concentration as low as 0.5 μ M, and the inhibition increased with increasing concentrations of the drug. At a con-

centration of 5 μ M, the inhibition of taurine transport was found to be approximately 45%.

More recent evidence indicates that FK506, another immune suppressive agent, is superior to cyclosporin A for use in organ transplant recipients (16). FK506 is a macrolide antibiotic and is chemically very different from cyclosporin A. The therapeutic concentrations of this drug are 10–100 times less than those of cyclosporin A. There is also a significant reduction in the occurrence of undesirable side effects with FK506 compared with cyclosporin A. However, no information is available in the literature with regard to the influence of FK506 on pregnancy and fetal development. With this in mind, we studied the effect of FK506 on taurine transport in human placental choriocarcinoma cells. As can be seen in Figure 1, there was no inhibition of taurine transport in these cells by FK506 even at a concentration as high as 5 μ M, which is 2 to 3 orders of magnitude higher than therapeutic concentrations. It thus appears that there will be no impairment of placental transfer of taurine in pregnant mothers receiving FK506.

Cyclosporin A and FK506 produce immunosuppression via distinct receptors, which, though different proteins, possess peptidylprolyl-*cis-trans*-isomerizing activity (17). Our observations that taurine transport in the placental cell line is inhibited by cyclosporin A but not by FK506 indicate that the specific receptors may not be responsible for the inhibition. There is another cellular protein, calmodulin, that binds cyclosporin A, but with a much lesser affinity than cyclophilin (18). Cyclosporin A binds to calmodulin in a calcium-dependent manner and inhibits calmodulin-dependent cellular processes. Therefore, we investigated the likelihood of the calmodulin antagonistic property of cyclosporin A being responsible for the inhibition of taurine transport observed in the placental cell line. This was done by studying the influence of three calmodulin antagonists, W-7, calmidazolium, and CGS 9343B, on taurine transport in these cells. To serve as controls, the effects of these compounds on two other transport systems were also determined, one stimulated by cyclosporin A (system L) and the other unaffected by cyclosporin A (system ASC). The results in Table 2 show that these calmodulin antagonists produced effects that were similar to those of cyclosporin A. These compounds inhibited the β system, stimulated the L system, and did not influence the ASC system. These data suggest, but do not prove, that the inhibitory effect of cyclosporin A on taurine transport may be due to the calmodulin antagonistic property of the drug.

Taurine is the most abundant free amino acid in a number of tissues. The nutritional needs of humans for taurine are partly met by dietary sources and partly by endogenous biosynthesis. The biosynthetic ability is very low at fetal and neonatal stages and progressively increases with age to reach adult levels. On the contrary, tissue concentrations of taurine exhibit a reciprocal pattern, being highest in fetal life and lowest in adult life (19).

Fig. 1. Effects of increasing concentrations of cyclosporin A or FK506 on taurine transport in the JAR human placental choriocarcinoma cell line. Cells were treated with increasing concentrations of either cyclosporin A (○) or FK506 (●) for 12 h, after which the 3-min transport of taurine (20 nM) was measured. Stock solutions of cyclosporin A and FK506 were made in cremophor and DMSO, respectively. The corresponding control cells were therefore treated in a similar way with either cremophor or DMSO alone. The values are mean $\pm$ SEM. *, p < 0.005; **, p < 0.001.

Table 2. Influence of calmodulin antagonists on amino acid transport systems in JAR human placental choriocarcinoma cell line*

Amino acid	Transport system	Amino acid transport (% control)			
		Control	W-7	Calmidazolium	CGS 9343 B
Taurine	β	100 $\pm$ 4	58 $\pm$ 4	53 $\pm$ 2	47 $\pm$ 3
Leucine	L	100 $\pm$ 1	148 $\pm$ 1	131 $\pm$ 1	161 $\pm$ 3
Alanine	ASC	100 $\pm$ 2	104 $\pm$ 3	102 $\pm$ 1	109 $\pm$ 3

* The values are means $\pm$ SEM. Cells were treated with or without W-7 (25 μ M), calmidazolium (3 μ M), or CGS 9343 B (10 μ M) for 12 h, after which the 3-min transport of taurine (20 nM), leucine (50 nM), or alanine (1 μ M) was measured.

Thus, taurine seems to have an important role in fetal and neonatal development in humans, and yet the human fetus and newborn have the least biosynthetic ability. Milk contains high concentrations of taurine, and intestinal absorption is the primary route by which the newborn gets this amino acid. Placental transfer from the mother is the only mechanism for meeting the nutritional needs for this amino acid in fetal life. Human placenta possesses a very active mechanism to transport taurine. One recent study analyzed the relationship between placental taurine and low birth weight infants (20). The concentrations of taurine in the placentas of the hypotrophic infants are significantly lower than those in the placentas of normal birth weight infants. In another study (21), provoking taurine deficiency and impairment of placental taurine transfer in pregnant rats caused a significant fall in the placental content of taurine. In addition, compared to fetuses taken from control mothers with no taurine deficiency, a significant reduction was noted in the weight of the placentas, the whole body, the brain, and the liver of the fetuses taken from the mothers with taurine deficiency. These studies provide evidence of the crucial role of taurine in fetal development. Interference with the placental transfer of taurine is thus expected to interfere with the normal development of the fetus. The selective impairment of taurine transport in human placental choriocarcinoma cells by cyclosporin A indicates that placental transfer of taurine may be compromised by cyclosporin A treatment in pregnant mothers. This may be at least one of the most likely causative factors involved in the cyclosporin A-induced intra-uterine growth retardation.

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