Uptake of the Carborane Derivative of Cholesteryl Ester by Glioma Cancer Cells Is Mediated Through LDL Receptors

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Received November 14, 2003; accepted March 24, 2004

Purpose. This study was to elucidate the mechanism of cellular uptake of cholesteryl 1,12-dicarba-closo-dodecaboranel-carboxylate (BCH), a new anti-cancer carborane derivative of cholesteryl ester, by glioma cancer cells.

Methods. BCH (solubilized with liposomal formulation) was incubated with SF-763 and SF-767 glioma cell lines in the presence of different amounts of monoclonal anti-LDL receptor antibody for cellular uptake studies. Various amounts of lipoprotein deficient serum (LPDS) were also used during the uptake. The effect of calcium ion and low temperature on BCH uptake were investigated. In addition, the transfer of BCH from liposomes to low-density lipoprotein (LDL) particles was determined through gradient ultracentrifugation.

Results. BCH uptake by these glioma cells was significantly inhibited by the monoclonal antibody. The uptake by both cell lines was reversely correlated with the amount of LPDS. The presence of calcium ion promoted the BCH uptake, whereas the low temperature decreased the BCH uptake. After 16 h incubation, about 46% of BCH was transferred from liposomes to LDL particles.

Conclusions. These results strongly suggested that the cellular uptake of BCH (in liposomal formulation) by SF-763 and SF-767 glioma cell lines is mediated through LDL receptors.

KEY WORDS: BCH; BCH transfer; calcium ion; cellular uptake; glioma; LDL receptor; monoclonal anti-LDL receptor antibody; low temperature; LPDS.

INTRODUCTION

Cellular drug targeting requires site-specific deliveries to specific cell types. Two important steps involved in this process are the recognition and interaction of drugs or drug carriers with specific target cells and the delivery of therapeutics into the target cells with reduced uptake by the non-target cells (1). Such a process is either cell-surface receptor mediated by ligand-receptor interaction or cell-surface epitope mediated by antigen-antibody interaction. The cellular targeting is thus largely dependent on the specificity of the target cell surface proteins. It is known that many cancer cells overexpress certain types of cell surface receptors, including transferrin receptor (2,3), folate receptor (4–6), and LDL receptor (7–9), to meet the increased cell proliferation and growth requirement. One strategy of targeted drug delivery for cancer therapy is to take advantage of these overexpressed cell surface receptors for achieving more selective drug uptake. By incorporating the corresponding ligands for these surface receptors, anticancer drugs can be specifically delivered to the cancer cells.

Among various ligands for the over-expressed receptors on cancer cells, low-density lipoprotein (LDL) is unique (10). It is an endogenous lipoprotein complex that has an approximate size of 22–25 nm in diameter and serves as a natural carrier of cholesterol and cholesterol ester in blood circulation. Containing about 1500 molecules of cholesteryl esters, the core of one LDL particle is surrounded by about 800 molecules of phospholipids and 500 molecules of cholesterol in its outer polar shell. Apolipoprotein, which possesses unique binding site for LDL receptor, is incorporated into the particles by hydrophobic interaction. For normal cell growth, the cholesterol level is balanced by LDL receptor-mediated uptake and subcellular degradation of LDL. However, because of the increased growth and proliferation of cancer cells, high turnover of cellular cholesterol for membrane growth and metabolism is required. As a result, overexpressed LDL receptor level has been found in many cancer cells (7–9).

Owing to its natural carrier capability and targeting specificity, LDL has been extensively studied as an anticancer carrier for hydrophobic drugs or prodrugs (10–12). Recently, our laboratory has designed and synthesized an anti-cancer cholesterol-carborane conjugate, cholesteryl 1,12-dicarba-closo-dodecaboranel-carboxylate (BCH), for boron neutron capture cancer therapy (13). The conjugate mimics the structure of cholesteryl esters located in the hydrophobic core of LDL. Because of the structural similarity, this type of conjugates has the potential to interact with lipoproteins and to follow through the LDL pathway for targeted drug delivery. The *in vitro* cellular uptake of BCH by two cancer cell lines, glioma (glioblastoma multiforme) SF-763 and SF-767, has been previously studied (14). The results indicated that the cellular uptake of BCH by glioma cells was about 14 times higher than that by normal neuron cells and the uptake in glioma cells was up to 10 times higher than that required for successful cancer treatment. However, the mechanism of BCH uptake in cancer cells and the involvement of LDL receptor-mediated process are not clear.

In this research, we seek to elucidate the mechanism of BCH uptake by glioma cells. In order to determine whether the BCH uptake is LDL receptor-mediated, BCH uptake by the glioma cells in the presence of monoclonal anti-LDL receptor antibody was measured. The involvement of lipoprotein during the uptake process was studied by uptake measurement while supplementing the culture medium with a series of combinations of lipoprotein-deficient serum and normal serum. In addition, the effect of calcium ion, a required divalent metal ion for LDL receptor-mediated endocytosis, and the temperature on the BCH uptake were also investigated, respectively. The interaction of liposomal formulation of BCH with LDL was examined by incubation and subsequent separation through gradient ultracentrifugation followed by the determination of protein and boron content in each separated fraction.

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ABBREVIATIONS: BCH, cholesteryl 1,12-dicarba-closo-dodecaboranel-carboxylate; DPPC, DL-α-dipalmitoyl phosphatidylcholine; EMEM, Eagle's Minimum Essential Medium; LDL, low-density lipoprotein; LPDS, lipoprotein deficient serum.

MATERIALS AND METHODS

Materials

BCH was synthesized in our laboratory as described previously (13). DL-α-dipalmitoyl phosphatidylcholine (DPPC) and cholesterol were purchased from Sigma Chemicals (St. Louis, MO, USA). Human glioma cells, SF-763 and SF-767, were obtained from the tissue bank of the Brain Tumor Research Center (University of California-San Francisco, San Francisco, CA, USA). Eagle's Minimum Essential Media (EMEM), Dulbecco's phosphate-buffered saline (PBS), trypsin-EDTA, and gentamicin solution were obtained from Fisher Scientific Products, Inc. (Suwanee, GA, USA). Fetal bovine serum (FBS), human normal serum, and human lipoprotein-deficient serum (LPDS) were obtained from BioCell Laboratories, Inc. (Rancho Dominguez, CA, USA). Analytical grade chloroform, methanol, isopropanol, and other chemicals were obtained from J.T. Baker (Phillipsburg, NJ, USA).

Preparation of BCH Liposomal Formulation

Similar to the native cholesteryl esters, BCH is extremely hydrophobic and thus it was formulated in liposomes as used in the previous studies (14). The BCH liposomal formulation was prepared by the solvent evaporation method. In brief, 52 mg DPPC, 10 mg cholesterol, and 2.5 mg BCH were dissolved in approximately 6 ml chloroform in a round bottom flask. The chloroform was evaporated under vacuum in a rotary evaporator (Buchi Rotavap, RE121, Brinkmann Instruments, Westbury, NY, USA). The dried lipid film formed on the flask wall was hydrated with 10 ml PBS that had been preheated to 55°C. The flask was shaken in water bath at 55°C and 120 rpm for 5 h. Size reduction of the resulting multilamellar vesicles was carried out using Emulsiflex B3 device (Avestin, Ontario, Canada) for 10 cycles. The size distribution of the resulting small liposomes was measured using a Nicomp submicron particle sizer (Model 370, Nicomp, Santa Barbara, CA, USA).

Cell Culture Studies

Human glioma SF-763 and SF-767 cells were routinely grown in 75 cm^2 plastic cell culture flasks (Corning Inc., Corning, NY, USA) containing 10 ml EMEM supplemented with 10% FBS and 50 U/ml penicillin and 50 μg/ml streptomycin. The flasks were seeded with approximately 1×10^6 cells and placed in a humidified 5% CO_2 incubator at 37°C. The cultures were passaged twice a week to maintain the cells in exponential growth phase. During the uptake experiments, fresh culture medium supplemented with 10% human serum was used, and the cells were incubated with a specified quantity of BCH in liposome formulation. After the predetermined incubation period, the culture medium was removed; the cells were rinsed 3 times with PBS, then harvested with trypsin-EDTA and counted. The cells were centrifuged at 3500 rpm for 10 min. The cell pellets were stored at 4°C until analysis. For all the experiments, the number of replicates was three and ANOVA method was used for statistical evaluation.

Determination of BCH in Cell Samples by High-Performance Liquid Chromatography

Cells containing BCH compound were dried by a freeze drying system (Labconco Corporation, Kansas City, MO, USA). BCH in the cells was extracted by a solvent mixture of methanol and isopropanol (50:50% v/v) and concentrated by Savant Speed-Vac concentrator system (Albertville, MN, USA). A Zorbax Stable Bond C-18 column (4.6 × 150 mm; particle size, 5 μm) coupled to a Waters 2690 HPLC system (Waters Incorporation, Milford, MA, USA) was used to analyze the BCH in samples using an existing method (15). The column was equilibrated with the mobile phase consisting of 50% methanol and 50% isopropanol at a flow rate of 0.5 ml/min. BCH was separated isocraticlly and measured at 220 nm.

Monoclonal Anti-LDL Receptor Antibody Production and Purification

Cell line of ATCC CRL-1691 (C7, Mouse hybridoma) is a highly specific monoclonal hybridoma and an experimental protocol detailed in the references was followed (16–18). The cell line was grown in Dulbecco's Modified Eagle's Medium supplemented with 4 mM L-glutamine, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. Two liters of culture supernatant was harvested by high-speed centrifugation at 4°C. The antibody-containing supernatant was mixed with a saturated ammonium sulfate solution at 50% v/v ratio. Precipitation of antibody was conducted at 4°C and the precipitate was harvested by high-speed centrifugation. The precipitate was resuspended in PBS and dialyzed against PBS at 4°C overnight. The antibody was further purified by affinity liquid chromatography using Affi-Gel Protein A MAPS II Kit (Bio-Rad Laboratories, Hercules, CA, USA). Briefly, 1 ml of Affi-Gel Protein A agarose was packed into a 1 × 10 cm Econo-Column chromatography column and equilibrated with 5 bed volume of binding buffer so that the effluent pH equals to the pH of the binding buffer (pH 9.0). Antibody sample was loaded to the column and then washed with 15 bed volume of binding buffer. The antibody was eluted with 5 bed volume of elution buffer. The column was regenerated with 5 bed volume of regeneration buffer. The purity of the antibody was confirmed by SDS-PAGE containing 12% acrylamide and being visualized with Coomassie blue staining. Additional ELISA experiment was also performed to verify the antibody purity. Protein concentration was measured by Bradford method.

Effect of Monoclonal Anti-LDL Receptor Antibody on the BCH Uptake by Glioma SF763 and SF767 Cells

Glioma SF763 and SF767 cells were grown in EMEM medium supplemented with 10% human normal serum and 2 mM of calcium ion. After the cells were grown to be about 80% confluent, 1 ml liposomal formulation of BCH (67.5 μg BCH/ml) was added to the cell culture and mixed completely. The lower BCH concentration was used in this experiment in order to observe higher differentiation of the antibody effect. Different amount of monoclonal anti-LDL receptor antibody with final concentration of 0.05, 0.1, 0.15, 0.2, and 0.25 mg/ml, respectively, was added to each cell culture. The culture was incubated at the same condition for 4 h (a relatively short incubation period to minimize receptor over-saturation) before the cells were trypsinized and harvested by centrifugation as described above. The amount of BCH in cells was determined by HPLC.

Effect of Lipoprotein on the BCH Uptake by the Glioma Cells

To determine the effect of lipoprotein on BCH uptake, 5%, 7%, 8%, 9%, and 10% of delipidized human serum was supplemented in the culture medium and, with a combination of the human normal serum (5%, 3%, 2%, 1%, and 0%, respectively), a total of 10% of serum was present in the medium. Cells were grown in 150 cm^2 flasks at the same condition as above and 1 ml liposomal formulation of BCH (154.5 μg BCH/ml) was added. After 16 h of incubation, the cells were treated as described above and the amount of BCH uptake by the cancer cells was measured by HPLC.

Effect of Calcium Ion on the BCH Uptake by the Glioma Cells

The binding of LDL to LDL receptor requires Ca^{2+} (16). The role of calcium ion in the binding is to provide positive charge (the binding site of LDL receptor is very negatively charged, calcium ion mediates the specific binding of LDL with LDL receptor) and required ionic strength. In order to further demonstrate whether LDL-receptor-mediated pathway was involved in the BCH uptake, Ca^{2+} was depleted from the medium during the BCH uptake experiment. The same medium but containing 2 mM Ca^{2+} was used as a control. After 16 h incubation of the cell culture with 1 ml BCH liposomal formulation (67.5 μg BCH/ml), cells were harvested as described above. Because the BCH became much lower in rest of the studies (calcium ion, temperature, and density separation), the inductively coupled plasma (ICP) method (14) was used to achieve the appropriate sensitivity for BCH measurement. Briefly, 200 μl of concentrated (70%) nitric acid was added to each harvested cells and vortexed thoroughly. After 2 h, the cells were completely disrupted and appeared to be clear solution. Deionized water (800 μl) was added and vortexed prior to analysis for boron content by ICP.

Effect of Low Temperature on the BCH Uptake by the Glioma Cells

At 4°C, the binding of LDL and LDL receptor reaches equilibrium after 3 to 4 h incubation, and no significant internalization occurs (19). Thus if LDL receptor-mediated pathway is involved in BCH uptake, the content of BCH taken by the cells at 4°C should become much lower than that taken by the cells at 37°C. Glioma cell lines SF-763 and SF-767 were cultivated and the BCH uptake experiment was conducted at 4°C and 37°C, respectively. An incubation time of 4 h was used, during which the binding of LDL and LDL receptor reached equilibrium (19). The cells were then harvested as described above and cellular content of BCH was determined by ICP.

Separation of LDL from Liposome After Incubation

BCH liposomal formulation (0.2 ml, 124.04 μg boron /ml) was incubated with human LDL (0.2 ml) at 37°C and 5% CO_2 for 16 h. In a disposable glass tube, 10 μl of Nile Red (100 mg/ml in acetone) was added, and the solvent was removed by evaporation. The incubation mixture of liposome and LDL was transferred to the disposable tube and incubated with Nile Red at room temperature for 30 min. The mixture was pipetted into a 8.9 ml polyallomer centrifuge tube (16 × 60 mm, Beckman Instruments, Palo Alto, CA, USA). A discontinuous gradient was formed by carefully layering 4 ml of salt solution (d = 1.063) above the mixture, followed by layering with 4 ml of another salt solution (d = 1.006). All the salt solutions were prepared by adjusting the density of phosphate-buffered saline with solid potassium bromide. As the control experiments, 0.2 ml of liposomes (containing no BCH) and 0.2 ml of LDL was individually pipetted into separate tubes and layered with the same salt solutions as above after incubation with Nile Red. All the tubes were balanced carefully with salt solution (d = 1.006) and ultracentrifuged at 100,000 × *g* (90 Ti rotor, Preparative Ultracentrifuge Optima, Beckman Instruments) and 20°C for 24 h. After centrifugation, the liposome or LDL layer was visualized at UV light (254 nm) in dark room. The separated layers of liposome and LDL from the mixture were carefully transferred into different tubes. The protein content in each fraction was determined by Bradford method. The boron content in each fraction was determined by ICP.

RESULTS

Purification of Monoclonal Anti-LDL Receptor Antibody

Because CRL-1691 cell line is a highly specific monoclonal hybridoma, the specificity of the monoclonal antibody is warranted (16–18). The purification of the produced monoclonal antibody followed two laboratory procedures: ammonium sulfate precipitation and affinity chromatography. By using 45–50% saturated ammonium sulfate, most of the serum proteins contained in the culture medium was eliminated. The subsequent affinity chromatography further purified the monoclonal antibody. After these two procedures, the antibody purity was examined by SDS-PAGE. The result is shown in Fig. 1. Monoclonal antibody molecules have two heavy chains and two light chains, which are linked by disulfide bridges. In SDS-PAGE, the disulfide bridges were broken and thus two bands, which have approximate molecular weight of 50,000 Da and 25,000 Da, respectively, appeared. Figure 1 shows that the prepared monoclonal anti-LDL receptor antibody was pure and ready to be used for the sub-

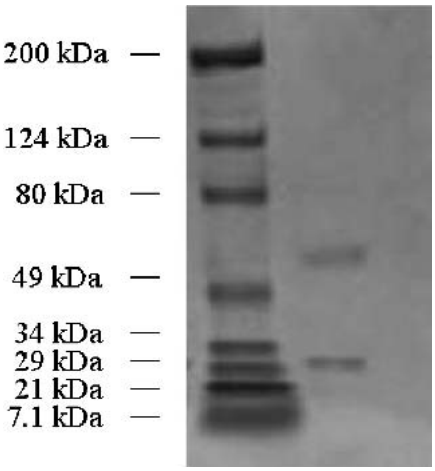

Fig. 1. SDS-PAGE (12% acrylamide) of the purified monoclonal anti-LDL receptor antibody.

sequent studies. Additional ELISA experiment also verified the result.

Effect of Monoclonal Anti-LDL Receptor Antibody on BCH Uptake

If the LDL receptor-mediated pathway is involved in the BCH uptake, the uptake will be affected by the presence of the antibody specific to LDL receptor. The BCH uptake experiment was thus conducted in the presence of different concentrations of the anti-LDL receptor antibody. The result is shown in Fig. 2. In the presence of anti-LDL receptor antibody, the uptake of BCH by the glioma cells was significantly reduced. The inhibition of BCH uptake by glioma cells was positively correlated with the amount of antibody in the culture medium. The reduction of BCH uptake by antibody reached the maximum effect in the presence of 0.1 mg/ml antibody. The result suggested that LDL receptor played an important role in the BCH uptake by glioma cell lines.

Effect of Lipoprotein Concentration on BCH Uptake

The possible involvement of lipoprotein in the BCH uptake process was examined by the depletion of lipoprotein from the serum, which was supplemented to the cell culture medium. During the BCH uptake experiment, a combination of normal human serum and lipoprotein deficient serum was supplemented to the culture medium, with a total of 10% serum amount in the medium. The BCH uptake by glioma cells was determined and the result is shown in Fig. 3. The more the lipoproteins depleted from the culture medium, the less BCH was taken up by the cells. It appeared that the presence of lipoprotein was a significant factor for the BCH uptake.

Effect of Calcium Ion on BCH Uptake

To further illustrate the involvement of LDL receptor-mediated pathway in the BCH uptake, the effect of Ca^{2+} on BCH uptake was determined by completely depleting Ca^{2+} from the culture medium. The uptake of BCH in the presence and absence of Ca^{2+} in culture medium is shown in Fig. 4. The BCH uptake in the presence of calcium ion was about 2.7 times as much as that in the absence of calcium ion for SF-763 cells. For SF-767 cells, the BCH uptake in the presence of calcium ion is about 1.5 times as much as that in the absence of calcium ion. Ca^{2+}, as a mediator for the LDL receptor-mediated pathway, appeared critical for BCH uptake by glioma cells.

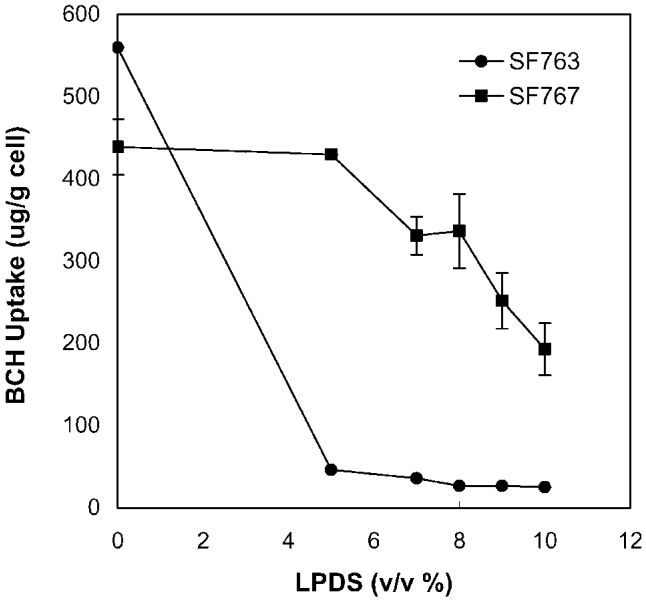

Fig. 3. The effect of lipoprotein deficient serum on BCH uptake by glioma cell lines SF-763 and SF-767.

BCH Uptake by Glioma Cells at 4°C

At 4°C, LDL receptor-mediated internalization of LDL becomes not significant (19). Therefore, the uptake of BCH

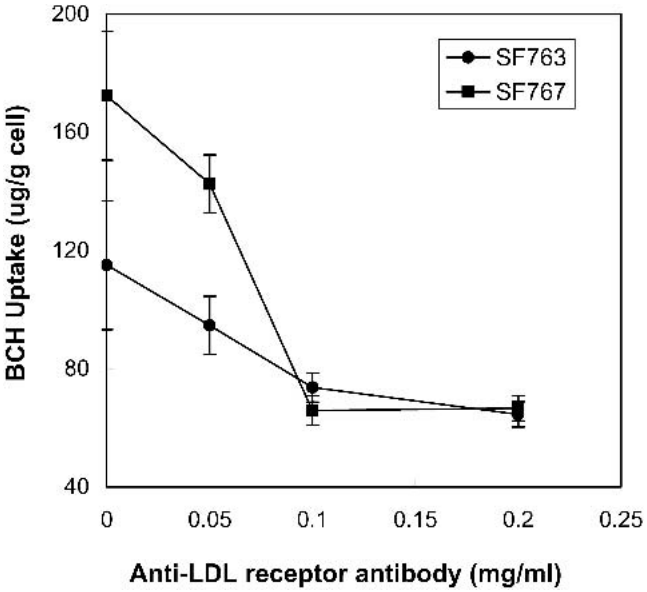

Fig. 2. The effect of monoclonal anti-LDL receptor antibody on BCH uptake by glioma cell lines SF-763 and SF-767.

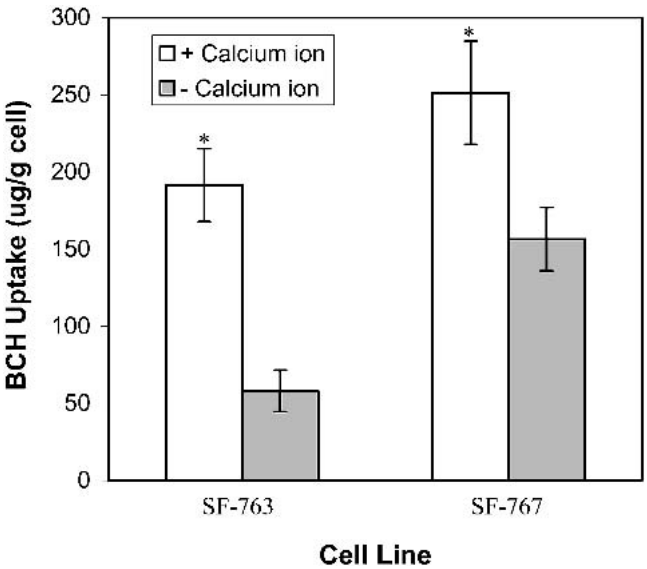

Fig. 4. The effect of Ca^{2+} on BCH uptake by glioma cell lines SF-763 and SF-767 (white bar, with 2 mM Ca^{2+}; gray bar, without Ca^{2+}).

by the glioma cells at this temperature should also be insignificant if LDL receptor-mediated pathway plays a major role in BCH uptake. The BCH uptake experiment was conducted at 4°C and 37°C, respectively, with the same conditions as described above. The result is demonstrated in Fig. 5. It can be seen that very limited amount of BCH was taken up by the glioma cells at 4°C as compared to that in 37°C. This result further confirmed that a receptor-mediated mechanism was involved in BCH uptake by the glioma cells.

Transfer of BCH in Liposomal Formulation to LDL

After liposomal formulation of BCH was incubated with LDL, the liposomal fraction and the LDL fraction were separated by ultracentrifugation, as shown in Fig. 6. LDL particles had higher density than liposome particles and, thus, the bottom layer contained LDL and the upper layer contained liposome. This was also confirmed by the position of LDL layer or liposome layer in centrifuge tube during the control experiments. No protein content was detected in the liposome layer. However, in the LDL layer, BCH was detected with a concentration of 54.7 μg boron (or 281.7 μg BCH)/ml. The BCH content in the liposome layer was 65.1 μg boron (or 335.3 μg BCH)/ml as compared to 124.04 μg boron (or 638.8 μg BCH)/ml before the incubation with LDL particles. Therefore, after 16 h incubation, about 46% of BCH compound was transferred to LDL particles.

DISCUSSION

Cellular drug uptake is affected by many factors such as cell types, growth stage of the cells and culture medium conditions. In our previous studies, the BCH uptakes in brain cancerous glioma cells (SF-763 and SF-767 cells) and brain normal cells (HCN-1a cells) were compared. The cell culture was scheduled in an appropriate way such that all three cell lines were in a similar growth stage before the BCH liposomal formulation was added to the culture medium. After 16 h incubation with the BCH liposomal formulation, the cells

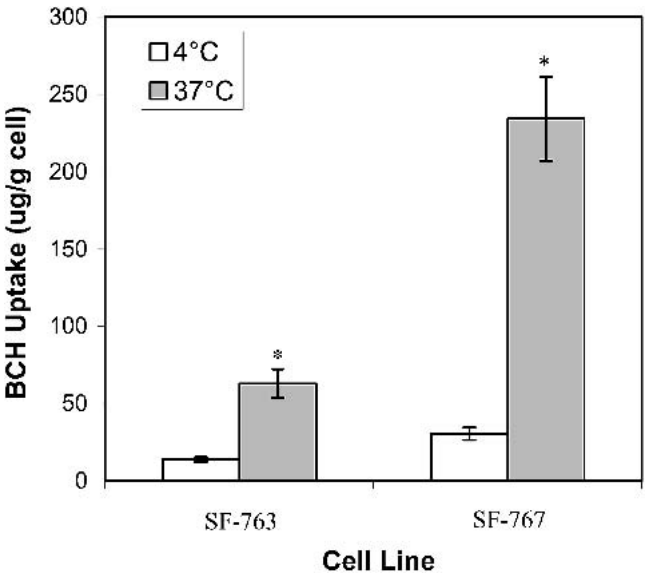

Fig. 5. The effect of temperature on BCH uptake by glioma cell lines SF-763 and SF-767 (white bar, 4°C; gray bar, 37°C).

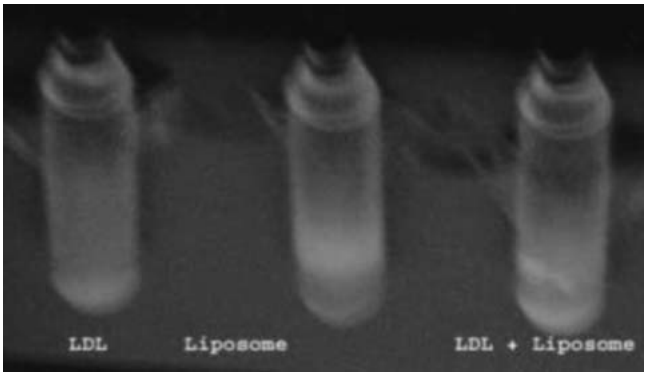

Fig. 6. Separation of LDL and liposome particles by gradient ultracentrifugation.

were harvested by trypsinization and centrifugation. The amount of BCH uptake by cell lines HCN-1a, SF-767, and SF-763 was determined by HPLC, and the results are shown in Table I. The amount of BCH uptake by the glioma cell lines was 14 times as high as that by the normal human neuron cell line. The amount of BCH accumulated in the glioma cells was also much higher than that required for successful boron neutron capture therapy (20). BCH is a carborane derivative of cholesteryl esters. Because the endogenous cholesteryl esters is naturally located in the oily core of lipoproteins and taken up by cells through the LDL-mediated process, it is postulated that the BCH uptake involves the lipoprotein pathway and the LDL receptors. To address this question, the present research focuses on the elucidation of the involvement of LDL receptors during BCH uptake process.

If the cellular uptake of BCH is LDL receptor-mediated, the difference of BCH uptake between the glioma cells and normal neuron cells becomes self-explanatory, because glioma cell lines SF-763 and SF-767 overexpress LDL receptors on the cell surface as compared with the normal cells (9). Since the internalization of natural LDL by cells is LDL receptor-mediated, the binding of the LDL receptors by anti-LDL receptor antibodies could significantly reduce LDL uptake by the cells (16). Our results demonstrated the reduction of BCH content in glioma cells when monoclonal anti-LDL receptor antibody was present in the culture medium. This strongly suggested the possibility of the involvement of LDL and LDL receptor in BCH uptake by the glioma cells.

Furthermore, if LDL and LDL receptor are involved in the BCH uptake, the depletion of LDL from the culture medium can lead to a significant reduction of cellular uptake of BCH. Our result showed that the cellular uptake of BCH by the glioma cells was positively correlated with the amount of LDL present in the culture medium. The result further inferred that LDL functioned as a carrier of BCH during the BCH uptake by the glioma cells. In both experiments (i.e., when monoclonal anti-LDL receptor antibody was added to the culture medium, and when normal human serum was delipidized), there was a basal level of BCH uptake by the glioma cells even when the concentration of monoclonal anti-LDL receptor antibody in the culture medium reached very high (Fig. 2) or when the normal serum was completely delipidized. It is very likely that the cellular uptake of BCH in liposomal formulation was also partially based on the physical diffusion or the fusion of liposome particles with the cell membrane but the extent of uptake was much lower.

Table I. Cellular Uptake of BCH by Brain Cancerous Cells and Normal Cells

Cell line	Cellular BCH uptake* (μg boron/g cell)
Normal neuron cell, HCN-1a	19.9 ± 2.9
Glioma cancer cell, SF-763	240.1 ± 22.3
Glioma cancer cell, SF-767	283.4 ± 48.1

* Expressed in μg boron per gram of cell. The value can be converted to μg BCH per gram of cell with a multiplication of 5.15.

The specific binding of LDL with LDL receptor, which is the first step of receptor-mediated pathway, requires divalent calcium ion (19). By depleting the calcium ion in the culture medium, cellular BCH uptake was reduced. Furthermore, the receptor-mediated endocytosis is temperature dependent, and at 4°C, LDL is only bound to the cell surface and the internalization is not significant (19). The determination of BCH uptake at different temperature helped to confirm whether receptor-mediated mechanism was involved. When the cellular uptake experiment was conducted at 4°C, much smaller amount of BCH was detected in the cells as compared to that at 37°C. Therefore, it again indicated indirectly the involvement of a LDL receptor-mediated process during the BCH uptake. It is noted that membrane fluidity might also be affected, possibly contributing in part to the difference.

Based on these results, BCH in liposomal formulation appeared taken up by the glioma cells via LDL receptor-mediated endocytosis. Because liposome and LDL are both lipid-based particles, they can interact with each other by fusion, lipid transfer or lipid exchange. Our experimental results further demonstrated that large portion of BCH was transferred to LDL particles after 16 h of incubation of the BCH liposomal formulation with LDL. However, whether there was lipid transfer or lipid exchange between liposomes and LDL particles remain unknown.

In conclusion, the inhibition of BCH uptake by the presence of anti-LDL receptor antibody and the positive correlation of BCH uptake with lipoprotein amount in the culture medium, together with the effect of calcium ion and temperature on the BCH uptake, have strongly suggested the involvement of LDL receptor-mediated pathway in BCH uptake by the glioma cells. The BCH in liposomal formulation was also found to transfer in significant amount to LDL particles when the BCH liposomal formulation was incubated with LDL particles.

ACKNOWLEDGMENTS

This publication was made possible by Grant Number GM 53969 (SØ) from NIH and by University of Georgia Engineering Grant.

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