N-butyldeoxynojirimycin reduces growth and ganglioside content of experimental mouse brain tumours

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Summary Abnormalities in glycosphingolipid (GSL) biosynthesis have been implicated in the oncogenesis and malignancy of brain tumours. GSLs comprise the gangliosides and the neutral GSLs and are major components of the cell surface glycocalyx. N-butyldeoxynojirimycin (NB-DNJ) is an imino sugar that inhibits the glucosyltransferase catalysing the first step in GSL biosynthesis. The influence of NB-DNJ was studied on the growth and ganglioside composition of two 20-methylcholanthrene-induced experimental mouse brain tumours, EPEN and CT-2A, which were grown in vitro and in vivo. NB-DNJ (200 μM) inhibited the proliferation of the EPEN and CT-2A cells by 50%, but did not reduce cell viability. The drug, administered in the diet (2400 mg kg⁻¹) to adult syngeneic C57BL/6 mice, reduced the growth and ganglioside content of subcutaneous and intracerebral EPEN and CT-2A tumours by at least 50% compared to the untreated controls. NB-DNJ treatment also shifted the relative distribution of tumour gangliosides in accordance with the depletion of metabolic substrates. Side effects of NB-DNJ treatment were generally mild and included reductions in body and spleen weights and intestinal distension. We conclude that NB-DNJ may inhibit tumour growth through an effect on ganglioside biosynthesis and may be useful as a new chemotherapy for brain tumours. © 2001 Cancer Research Campaign

Keywords: glycosphingolipids; N-butyldeoxynojirimycin; glucosyltransferase; brain tumours; mouse; gangliosides; glioma

Despite advances in surgical technique and radiation therapy, the prognosis for malignant glioma patients remains dismal, with a 2-year survival rate of less than 20% (Prados and Levin, 2000). Among the brain tumours, malignant gliomas represent 13–22% and are the leading cause of death from cancer in persons 15 to 34 years of age (Harbaugh and Black, 1998). Brain tumours are the second leading cause of cancer death in children in the United States, and their incidence is on the rise (Schmidt, 1998). Presently, the medical treatment of cancer is based on surgery and radiation therapy which may or may not be followed by chemotherapy (Shapiro, 1999; Yung et al, 2000). However, invasiveness combined with multidrug resistance protects most malignant gliomas from direct chemical, radiological or surgical assault (Harbaugh and Black, 1998). Since malignant brain tumours remain as lethal today as they were in the 1970s, new therapeutic approaches are urgently needed (Davis et al, 1999; Fathallah-Shaykh, 1999).

The glycosphingolipids (GSLs) comprise the gangliosides and the neutral GSLs and are anchored in the outer surface of plasma membranes through a lipophilic ceramide tail (Hakomori, 1996; Bai and Seyfried, 1997). Gangliosides differ from the neutral GSLs in having at least one sialic acid molecule attached to the oligosaccharide head group. N-acetylneuraminic acid (NeuAc) and N-glycolyneuraminic acid are the major sialic acid species present in mouse tissues. Chemotherapy through slowing GSL synthesis has been proposed based on evidence that GSLs may modulate tumour growth, metastasis, immunity and multidrug resistance (Chang et al, 1997; Liu et al, 1999; Radin, 1999). Defects in GSL biosynthesis are found in most human cancers and may underlie the invasive and malignant properties of brain tumours (Merzak et al, 1995; Hakomori, 1996; Berra et al, 1997). GSLs can modulate the function of growth factor receptors and have recently been implicated as modulators of brain tumour angiogenesis and cell adhesion (Hakomori, 1996; Alessandri et al, 1997; Bai and Seyfried, 1997; Manfredi et al, 1997; Zeng et al, 2000). GSLs newly synthesized and shed by tumours can competitively bind to interleukin-2 (IL-2) receptors on T cells, hindering the proliferative response required for immunological rejection (Chang et al, 1997). Taken together, these findings suggest that the modification of GSL biosynthesis and expression could be effective in brain tumour therapy.

Mammalian GSLs are synthesized by the stepwise addition of sugar residues to the oligosaccharide head group. This is accomplished through the action of a Golgi-bound multi-glycosyltransferase system, where the GSL product of one transferase serves as the substrate for another transferase (Sandhoff and van Echten, 1994; Seyfried et al, 1994). The small imino sugar, NB-DNJ, inhibits the ceramide-specific glucosyltransferase (GlcT-1) that catalyses the first step in GSL biosynthesis (Platt et al, 1994) (Figure 1). Since GlcCer is the common metabolic precursor for the synthesis of most gangliosides and neutral glycolipids, NB-DNJ treatment significantly reduces the content of all GSLs containing the GlcCer core structure (Platt et al, 1994). NB-DNJ is a derivative of the naturally occurring plant and microbial compound deoxynojirimycin (DNJ), and is water soluble in contrast to other, highly hydrophobic inhibitors of GSL.
biosynthesis (Platt and Butters, 1995). NB-DNJ is easily administered to adult mice in the diet without toxicity. Dietary administration of NB-DNJ prevents ganglioside GM2 accumulation in the brains of mice with Tay Sachs and Sandhoff diseases and depletes GSLs in metabolically active tissues of adult mice (Platt et al., 1994, 1997a, 1997b; Platt and Butters, 1995; Jeyakumar et al., 1999). NB-DNJ is therefore ideally suited as a pharmacological agent for targeting GSL biosynthesis in vivo.

NB-DNJ was used previously in clinical trials for Human Immunodeficiency Virus (HIV) infection and is currently used in clinical trials for the lipid storage disorder Gaucher’s disease (Fischl et al., 1991). Age- and sex-matched (6–8 week-old male) B6 mice were used as tumour recipients. All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committee.

### Experimental mouse brain tumours

Both EPEN and CT-2A experimental mouse brain tumours were used for these studies. The EPEN tumour was originally produced through implantation of 20-MC into the cerebral ventricle of a B6 mouse and had histological characteristics of an epedymoblastoma (Rubin, 1968). The CT-2A tumour was generated in our laboratory and arose in a B6 mouse 450 days after 20-MC implantation into the cerebral cortex as previously described (Seyfried et al., 1992). Histologically, the CT-2A brain tumour was broadly classified as a poorly differentiated highly malignant anaplastic astrocytoma. The EPEN tumour grows as a firm, cohesive, nonhaemorrhagic mass in vivo and as cell islands in vitro, while the CT-2A tumour grows as a soft, noncohesive, haemorrhagic mass in vivo and as a fusiform monolayer in vitro (Seyfried et al., 1992).

Also, the growth rate is significantly greater for the CT-2A tumour than for the EPEN tumour (Cotterchio and Seyfried, 1993).

### N-butyldeoxynojirimycin

NB-DNJ (OGT918; 219.3, MW) was obtained as a gift from Searle/Monsanto, St Louis, MO, and Oxford GlycoSciences, Abigdon, UK.

### Cell culture and NB-DNJ treatment

Approximately 2×10⁵ EPEN or CT-2A tumour cells were seeded in wells of a 24-well plate and cultured for 24 h in a humidified atmosphere of 95% air and 5% CO₂ at 37°C in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were cultured for an additional 8 days in the presence or absence of 200 μM NB-DNJ. A Coulter counter was used for cell counts of the control and treated cells, and the trypan blue exclusion procedure was used to assess the viability of tumour cells following NB-DNJ treatment (Kemp, 1968). The number of dead cells that incorporated trypan blue was counted using a haemacytometer.

### Subcutaneous and intracerebral tumour implantation

An EPEN or CT-2A flank-grown tumour was removed from a B6 mouse and was rinsed and diced in cold phosphate-buffered saline (PBS) at pH 7.4. The right flanks of recipient mice were shaved in order to facilitate tumour inoculation, detection, and growth assessment. Mice were anaesthetized with methofane (Metofane, Mallinckrodt Veterinary Inc, Mundelein, IL), and the tumour was implanted by the s.c. injection of 0.1 ml of minced tumour tissue suspended in 0.2 ml PBS by use of a 1 cc tuberculin syringe and 18-gauge needle. The inoculated mice were monitored daily for nodule formation. Tumours were implanted i.c. as previously described (Seyfried et al., 1987). Mice were anaesthetized with methofane, and their heads were shaved and then swabbed with 70% ethyl alcohol under sterile conditions. A midline incision was made on the posterior aspect of the head and the periosteum was swept laterally to expose the sagittal, coronal and lambdoidal sutures of the skull. A 1 mm burr hole was made, using an electric

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**MATERIALS AND METHODS**

**Mice**

The C57BL/6 (B6) strain was obtained from the Jackson Laboratory (Bar Harbor, ME). All B6 mice used in this study were propagated in the animal room of the Biology Department, Boston College, using animal husbandry conditions described previously (Flavin et al, 1991). Age- and sex-matched (6–8 week-old male)
drill, over the right parietal region behind the coronal suture and lateral to the sagittal suture. A 1 mm³ tumour fragment was then implanted into the right cerebral hemisphere of each anaesthetized recipient B6 mouse using a trocar. The skin edges were immediately approximated using sterile surgical forceps and fused using collodion (JT Baker, Inc, Phillipsburg, NJ). The animals recovered spontaneously and were monitored and returned to their cages when fully active.

**In vivo NB-DNJ treatment and tumour growth assessment**

B6 mice bearing s.c. or i.c. tumors were randomized into either control or treated groups and were housed 2 per cage. NB-DNJ was administered in the diet as previously described (Platt et al, 1997a, 1997b). Mice in the control group received ground mouse chow (Agway Prolab Rat/Mouse/Hamster 3000, PMI Feeds, Inc, St Louis, MO). Mice in the treated group received ground mouse chow containing NB-DNJ (2400 mg NB-DNJ kg body weight⁻¹ day⁻¹). Previous studies showed that this dietary dosage of NB-DNJ reduces GSL content in liver and lymphoid organs by 50–70% and is well tolerated in B6 mice over a 2-week treatment period (Platt et al, 1997b). The diet and compound (both as dry solids) were mixed thoroughly before use, stored in an airtight container at room temperature, and were used within 7 days of mixing. Both control and NB-DNJ-containing diets were provided ad libitum and were administered in glass vials that were fixed to the cage bottom. Water was also provided ad libitum.

Tumours grown s.c. were measured with calipers by the method of Looney as previously described (Cotterchio and Seyfried, 1993). Tumour volume was calculated by the formula for the approximation of the volume of a sphere as Volume = 1/2 (Length × Width × Height). Tumour volume was measured every other day throughout the treatment period. Since EPEN tumour growth rate is slower than that of the CT-2A tumour, it took longer for the EPEN tumour to reach a measurable size (30–40 mm³). Consequently, NB-DNJ treatment of the EPEN or CT-2A tumours grown s.c. was initiated on day 14 or on day 6 post-inoculation, respectively. Treatment of the EPEN or CT-2A tumours was terminated on day 28 or on day 14 post-inoculation, respectively, when the control s.c. tumours reached an approximate mean volume of 1000 mm³.

NB-DNJ treatment was initiated 6 days after i.c. implantation for both the EPEN and the CT-2A tumours. This ensured vigorous tumour growth at the initiation of drug treatment. Unlike s.c. tumour growth, cranium size constrains i.c. tumour growth. Consequently, NB-DNJ treatment was terminated on day 15 post-implantation for both tumours. In addition to tumour volume measurements, mouse body weights were measured to the nearest 0.1 g and behavioural activity (ambulation and grooming) was monitored. At the termination of drug treatment, all mice were sacrificed by cervical dislocation and tumour, brain, liver and spleen tissue was collected, frozen and then lyophilized. In an independent study, healthy mice were treated with NB-DNJ (2400 mg kg⁻¹ d⁻¹) for 9 days to measure serum and tissue NB-DNJ content.

**Analysis of gangliosides**

Gangliosides were isolated and purified from lyophilized tumour, brain and liver tissue by our previously described methods (Seyfried et al, 1978; Seyfried et al, 1987; El-Abbadi and Seyfried, 1994). Briefly, total lipids were extracted in chloroform:methanol (1:1, v/v). The Folch procedure was used to partition gangliosides into the upper aqueous phase (Folch et al 1957). The upper phase was converted to a chloroform:methanol:water ratio of 30:60:8 (v/v/v) for ion exchange chromatography (Seyfried et al, 1978). Samples were then applied to a DEAE-Sephadex A-25 (Pharmacia Biotech) column (acetate form) and non-acidic glycolipids were eluted by washing with chloroform:methanol: water (30:60:8, v/v/v). The gangliosides were then eluted from the column with chloroform:methanol:0.8 M sodium acetate (30:60:8, v/v/v). Residual phosphatides and ganglioside inner esters were removed by a mild base treatment in 0.1 N sodium hydroxide at 37°C for 1.5 h. C18 Bond Elut columns (Varian) were used to remove salts and base from ganglioside fractions. Quantification of N-acetylgalactosaminic acid (NeuAc) and N-glycolyneuraminic acid (NeuGa) was determined using a gas-liquid chromatography procedure (Yu and Ledeen, 1970). The resorcinol reaction was also used to analyse ganglioside sialic acid concentration in mouse brains as previously described (Seyfried et al, 1978). Sialic acid concentration was expressed as μg 100 mg dry weight⁻¹. The distribution of tumour gangliosides was examined using high performance thin-layer chromatography (HPTLC) as we previously described (Cotterchio and Seyfried, 1993). Ganglioside sialic acid was visualized on the HPTLC by the resorcinol-hydrochloric acid spray (Svennerholm, 1957).

**Detection of NB-DNJ in serum and tissue of healthy mice**

Serum and tissue NB-DNJ levels were quantified as previously described (Andersson et al, 2000). Healthy 6–8 week-old-male B6 mice were treated for 9 days with NB-DNJ at 2400 mg kg⁻¹ d⁻¹. Mice were sacrificed by carbon dioxide inhalation. Serum was collected and stored at −20°C. Brain and liver tissue was collected and stored at −80°C. Serum and supernatant of liver homogenate (130 mg ml⁻¹ in 10% methanol) were centrifuged 3 times through a Millipore Ultrafree filter, after an internal standard (N-3-methylbutyl-DNJ) had been added to the samples. The pooled filtrates were purified on a hydrochloric acid-preconditioned SCX column, eluted with 1% ammonia in methanol, dried down, and resuspended in water. The samples were further purified on a C18 column (methyl preconditioning, water wash, and methanol elution), and then quantified by HPLC (Dionex CS10 hpcce chromatography, isocratic elution with 50 mM sodium sulphate and 5% acetonitrile, pulsed amperometric detector).

**Histological examination**

Control and NB-DNJ-treated brains bearing the EPEN or the CT-2A tumours were dissected and fixed in formalin for 24 hours. Specimens were embedded in paraffin and sectioned at 5 μm thickness. For routine histological examination of tumour morphology, the EPEN and the CT-2A tumour sections were stained with haematoxylin-eosin (H&E) and examined by light microscopy (Zeiss, West Germany).

**RESULTS**

**Influence of NB-DNJ on in vitro EPEN and CT-2A cell growth**

NB-DNJ (200 μM) significantly reduced the number of EPEN
cells by 50% and the number of CT-2A cells by 48% compared to untreated cells after 8 days of treatment (Figure 2). NB-DNJ was not cytotoxic since the percentage of trypan blue positive cells (2–3%) was similar in both the control and treated flasks for both tumour lines. These data indicate that NB-DNJ inhibited EPEN and CT-2A cell proliferation without enhancing cytotoxicity.

Influence of NB-DNJ on the EPEN and the CT-2A tumour growth in vivo

Based on the anti-proliferative effect of NB-DNJ on EPEN and CT-2A tumour cells in vitro, we examined the effect of NB-DNJ on the growth of both brain tumours in vivo. NB-DNJ (2400 mg kg\(^{-1}\) day\(^{-1}\)) significantly reduced both s.c. and i.c. growth of the EPEN and the CT-2A tumours (Figures 3 and 4). The inhibitory effect of NB-DNJ treatment on s.c. growth was apparent for both tumours by the fourth or sixth day of treatment. By the end of treatment, the EPEN and the CT-2A tumour volumes were reduced by 55% and 72%, respectively, compared to tumours in the untreated control mice (Figure 3). The greater inhibitory effect of NB-DNJ on the CT-2A tumours than on the EPEN tumours may result from the faster growth rate of the CT-2A tumours compared to the EPEN tumours.

Total tumour dry weight was used to determine i.c. growth at the end of the treatment period. Dry weight provides a more accurate assessment of tumour growth than wet weight because it eliminates variability due to differences in tissue water content associated with oedema. The dry weights of the NB-DNJ-treated EPEN and CT-2A tumours were reduced by 71% and 55%, respectively, compared to tumours in the untreated control mice (Figure 4). The greater inhibitory effect of NB-DNJ on the EPEN tumours than on the CT-2A tumours may result from the smaller size of the EPEN than the CT-2A tumour at the initiation of drug treatment (day 6). Taken together, these findings indicate that NB-DNJ effectively inhibits the growth of the EPEN and the CT-2A tumours in both subcutaneous and intracerebral environments.

Influence of NB-DNJ on tumour, liver and brain ganglioside content

Since NB-DNJ is a known inhibitor of GSL biosynthesis, we...
compared tumour, liver and brain ganglioside content in untreated and NB-DNJ-treated mice. NB-DNJ significantly reduced the total ganglioside content of both the EPEN and CT-2A tumours grown either s.c. or i.c. (Table 1). Ganglioside concentration in the NB-DNJ-treated EPEN and CT-2A tumours grown s.c. was reduced by 56% and 39%, respectively. Ganglioside concentration of the treated tumours grown i.c. was reduced by 49% and 50%, respectively. The reduction in ganglioside content was observed for gangliosides containing both NeuAc and NeuGc sialic acids. In general, NB-DNJ treatment caused a greater reduction in NeuGc-containing gangliosides relative to NeuAc-containing gangliosides (Table 1). NB-DNJ also significantly reduced total liver ganglioside content by 74% (Table 1). Although total brain ganglioside concentration (µg sialic acid 100 mg dry weight± SEM) was about 11% lower in the NB-DNJ-treated mice (454 ± 19, n = 3) than in the untreated control mice (508 ± 10, n = 3), the difference was not statistically significant (determined by the two-tailed t-test). GSLs within the brain have a relatively slow turnover, rendering brain ganglioside biosynthesis less susceptible to inhibition by NB-DNJ. These findings suggest that NB-DNJ inhibition of ganglioside synthesis is greater in the more metabolically active tissues than in the less metabolically active tissues.

Influence of NB-DNJ on tumour, liver and brain ganglioside distribution

The HPTLC distribution of gangliosides in the untreated EPEN and CT-2A tumours grown s.c. or i.c. was similar to the distribution that we reported previously for these tumours (Seyfried et al, 1987; Cotterchio and Seyfried, 1993; El-Abbadi and Seyfried, 1994). GM3 is the only ganglioside synthesized by cultured EPEN cells, while gangliosides in the ‘a’ metabolic pathway (GM3, GM2, GM1, GD1a) are the major gangliosides synthesized by cultured CT-2A cells. Most gangliosides in the tumours are derived from tumour-infiltrating host cells or from the serum (Seyfried et al, 1996; Ecedy et al, 1998; Seyfried et al, 1998). The significant reduction in total ganglioside content in the NB-DNJ-treated tumours (Table 1) was associated with a noticeable shift in ganglioside distribution from the structurally simple (GM3) to the structurally complex gangliosides (GD1a, GT1b) (Figure 5). NB-DNJ treatment had no significant effect on the distribution of brain or liver gangliosides (data not shown).

**NB-DNJ content in serum and tissue of healthy mice**

To determine the tissue bioavailability, NB-DNJ levels were measured in the brain, liver and serum of healthy B6 mice after 9 days of treatment at 2400 mg kg⁻¹ d⁻¹. The concentration of NB-DNJ in the serum was 58 µM ± 4.4 (n = 3), an amount consistent with steady-state serum levels previously reported (Platt et al, 1997b). NB-DNJ concentration in the liver was 4.6 ± 0.7 µg g⁻¹ wet weight (n = 3), which was more than 10-fold higher than that in the brain (0.3 ± 0.1 µg g⁻¹ wet weight, n = 3). These findings suggest that NB-DNJ uptake is greater in the more metabolically active liver tissue than in the less metabolically active brain tissue or that the blood-brain barrier restricts brain uptake.

Table 1 Influence of NB-DNJ on ganglioside content in experimental mouse brain tumours and liver

| Tissue Growth Environment | Treatment  | N*  | Total NeuAc µg 100 mg⁻¹ dry weight | NeuGc µg 100 mg⁻¹ dry weight | % NeuGc |
|---------------------------|-----------|-----|-----------------------------------|-----------------------------|--------|
| EPEN                      |           |     |                                   |                             |        |
| SC                        | Control   | 4   | 44.4 ± 5.2                        | 38.1 ± 5.1                  | 14     |
|                           | NB-DNJ    | 6   | 19.5 ± 2.0**                      | 16.0 ± 1.6**                | 18     |
|                           | IC        | 3   | 68.5 ± 0.6                        | 60.5 ± 1.2                  | 12     |
|                           | NB-DNJ    | 3   | 35.0 ± 3.5**                      | 32.6 ± 2.9**                | 7      |
| CT-2A                     |           |     |                                   |                             |        |
| SC                        | Control   | 6   | 62.2 ± 5.9                        | 39.2 ± 3.8                  | 37     |
|                           | NB-DNJ    | 6   | 37.8 ± 4.0*                       | 28.7 ± 2.7                  | 24     |
|                           | IC        | 6   | 61.6 ± 3.3                        | 43.1 ± 3.7                  | 30     |
|                           | NB-DNJ    | 6   | 30.9 ± 2.9**                      | 25.2 ± 2.7*                 | 18     |
| Liver                     |           |     |                                   |                             |        |
|                           | Control   | 3   | 30.5 ± 2.9                        | 1.95 ± 0.2                  | 94     |
|                           | NB-DNJ    | 4   | 11.9 ± 1.2**                      | 2.7 ± 0.2                   | 77     |

*N, number of independent tumour or liver samples studied. Values are expressed as means ± the SEM. NeuAc, N-acetylneuraminic acid; NeuGc, N-glycolyneuraminic acid. ©, intracerebral; SC, subcutaneous. Asterisks indicate that the control and treated tissue differ at the P < 0.01 (*) and P < 0.001 (**) levels as determined by the two-tailed t-test.

Figure 5 High-performance thin-layer chromatogram showing the effects of NB-DNJ treatment on EPEN (A) and CT-2A (B) tumour ganglioside distribution. Approximately 2 µg of ganglioside sialic acid were spotted for each sample. The plate was developed by one ascending elution with chloroform:methanol:water (50:45:10, v/v/v) that contained 0.02% CaCl₂•2H₂O. The bands were visualized by resorcinol spray (50). In (A) and (B), lanes 1, ganglioside standards; lanes 2, adult mouse brain gangliosides; lanes 3, control s.c. tumours; lanes 4, NB-DNJ-treated s.c. tumours; lanes 5, control i.c. tumours; lanes 6, NB-DNJ-treated i.c. tumours.

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administration over the short treatment period.

**DISCUSSION**

Our data show that the small imino sugar, NB-DNJ, significantly inhibited the growth rate and the ganglioside content of 2 different experimental mouse brain tumours, the EPEN and the CT-2A. The reduction of ganglioside content in the i.c.-grown tumours and the detection of NB-DNJ in the brain tissue of normal mice suggests that NB-DNJ passes the blood–brain barrier, consistent with previous studies (Platt et al, 1997a; Jeyakumar et al, 1999; Andersson et al, 2000). In contrast to most known chemotherapeutic molecules, NB-DNJ was non-cytotoxic and was generally well tolerated by the B6 mouse host. Since gangliosides modulate the activities of multiple cell surface receptors and adhesion molecules that participate in cell proliferation, migration, and angiogenesis (Hakomori, 1996; Alessandrini et al, 1997), it is possible that the NB-DNJ-linked inhibition of ganglioside biosynthesis is responsible for the reduced tumour growth. We do not exclude the possibility that NB-DNJ may also reduce tumour growth through effects on N-linked glycosylation or on caloric intake (Weindruch and Walford, 1988; Karlsson et al, 1993; Platt and Butters, 1995). It is unlikely that NB-DNJ reduces brain tumour growth through an effect on apoptosis because no noticeable differences were seen between the control and the NB-DNJ-treated tumours for the number of dead cells in vitro or for cell pyknosis or tissue necrosis in vivo. Additional studies will be needed to determine the mechanisms by which NB-DNJ inhibits brain tumour growth and whether reduced tumour growth and ganglioside content are correlated with NB-DNJ dosage.

We found that NB-DNJ reduced the content of NeuGc-containing gangliosides to a greater extent than that of NeuAc-containing gangliosides in the i.c.-grown brain tumours. We previously showed that most NeuGc-containing gangliosides present in mouse brain tumours and in human glioma xenografts are derived from tumour-associated host cells (macrophages) and from the mouse serum (Ecsedy et al, 1998, 1999; Seyfried et al, 1998). Since most NeuGc-containing gangliosides in mouse serum are synthesized in the liver (Cotterchio and Seyfried, 1994), we suggest that the marked reduction of NeuG-containing gangliosides in the i.c.-grown brain tumours results in large part from the NB-DNJ-induced inhibition of ganglioside biosynthesis in the liver. It is not yet known if NB-DNJ might also reduce tumour NeuGc content through an effect on tumour-associated macrophages.

We recently showed that gene-linked changes in ganglioside distribution could influence angiogenesis in the EPEN tumour (Manfredi et al, 1999). Specifically, the neosynthesis of complex gangliosides (GM2, GM1, and GD1a) with a concurrent decrease in GM3 enhanced angiogenesis. These shifts in ganglioside distribution, however, were not associated with changes in total cellular ganglioside content. Although we found similar ganglioside shifts in the NB-DNJ-treated tumours, these shifts were associated with blocked ganglioside synthesis and significant reductions in total ganglioside content. Further studies are needed to determine if the effects of NB-DNJ on tumour ganglioside distribution also influence angiogenesis.

The NB-DNJ-induced shift in tumour ganglioside distribution from the more simple structures (GM3) to the more complex gangliosides (GM1, GD1a) in the CT-2A tumour results from a depletion of simple upstream metabolic precursors (glucosyl-
ceramide and lactosylceramide) together with the conversion of already synthesized precursors to ganglioside end products (GD1a). A similar phenomenon was described previously in mouse embryos treated with N-butyldeoxynojirimycin (NB-DGJ), a structural analogue of NB-DNJ, and is consistent with the substrate availability hypothesis of ganglioside biosynthesis (Seyfried et al, 1994; Brigande et al, 1998). The shift in ganglioside distribution in the EPEN tumour likely results from the depletion of GM3 synthesis in the proliferating tumour cells. Such a depletion will differentially magnify those gangliosides (GM1, GD1a) in the less proliferative host cells e.g., macrophages. Hence, NB-DNJ may influence tumour ganglioside composition through effects on both the tumour cells and the host cells.

Mammalian systems lack enzymes that metabolize the imino sugars and, as a consequence, NB-DNJ is secreted intact via the kidney (Platt and Butters, 1995, 1998). For this reason, secondary metabolites do not complicate the biological effects of NB-DNJ. The 5 μM steady-state serum level of NB-DNJ that we detected in the B6 mice after 9 days of dietary administration (2400 mg kg–1d–1) was similar to that found previously for inhibition of GSL biosynthesis in normal and pathological mouse tissues (Platt et al, 1997a, 1997b; Jeyakumar et al, 1999). In these previous studies, NB-DNJ treatment caused extensive depletion of GSLs in the liver and the lymphoid organs of healthy B6 mice and reduced the major side effect reported in patients treated with NB-DNJ, namely decrease in blood glucose levels, by 75% (Sottocornola E (1997) Glycosphingolipid expression in solid tumours and transformed cell lines. Indian J Biochem Biophys 34: 170–177). Additional data relative to the major side effects of NB-DNJ, efficacy of NB-DNJ in treating Gaucher’s disease and pharmacokinetics of NB-DNJ are available in a recent overview article which also describes the molecular basis for the clinical efficacy of NB-DNJ (Sottocornola E, 1997).

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