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BRIEF REPORT
Response of Metastatic Recurrent Neuroblastoma to Nitisinone: A Modulator of Tyrosine Metabolism

Nathan L. Kobrinsky, MD[1,2]* and Diane E. Sjolander[1]

Nitisinone blocks the tyrosine pathway and may be effective in treating neuroblastoma. A 33-month-old male with heavily treated metastatic, recurrent, N-MYC amplified neuroblastoma received nitsinone (0.8 mg/kg/day escalated to 5.0 mg/kg/day). Dramatic tumor regression and resolution of pain without toxicity were observed. At 10 weeks, the tumor progressed. Nitisinone, low dose cyclophosphamide and doxorubicin subsequently produced a very good partial response. At 18 months the disease progressed. The child succumbed 21 months after starting nitisinone. Nitisinone produced an increase in tyrosine and catecholamine metabolite (HVA, VMA, and metanephrines) levels. Nitisinone may be a promising agent in metastatic neuroblastoma. Pediatr Blood Cancer 2006;46:517–520. © 2005 Wiley-Liss, Inc.

Key words: HVA; metanephrine; neuroblastoma; nitisinone; tyrosine; VMA

Neuroblastoma is the most common solid neoplasm of childhood outside of the central nervous system. Although the outcome is excellent for children with localized disease, for children with widespread disease, the outcome is poor. Regrettably, three quarters of affected children have widespread disease at diagnosis [1].

With multimodality therapy including intensive chemotherapy, surgery, radiation, and autologous stem cell transplant consolidation outcomes have improved. With this treatment strategy the 3 year event free survival for high risk neuroblastoma has been recently reported at $34\% \pm 4\%$ [2]. For those children that recur, the outcome is extremely poor. The majority succumb within months. A subset of chronic relapsers with a relatively prolonged survival, particularly with camptothecin-based therapy, is however recognized [3]. These subjects ultimately die of progressive disease. Clearly, there remains a need for improved treatment strategies for newly diagnosed and recurrent neuroblastoma.

Neuroblastoma, as a neural crest derived neoplasm, has a highly active tyrosine pathway with the resultant production of HVA, VMA, metanephrines, and other catecholamines [3–6]. Proximal blockade of this pathway may therefore raise intracellular tyrosine, alter the distribution of intracellular catecholamines, and facilitate the differentiation or cytotoxicity of neuroblastoma cells.

Nitisinone, originally developed as a triketone herbicide, induces proximal blockade of the tyrosine pathway by highly specific inhibition of the enzyme 4-hydroxyphenpyruvate dioxygenase through rapid and avid but reversible binding [7,8]. Nitisinone is approved by the Food and Drug Administration for the treatment of tyrosinemia type I [9]. A fatal hereditary liver disease, tyrosinemia type I results from a deficiency of fumarylacetoacetate hydrolase. The drug has also been reported to be effective in the treatment of the rare metabolic disease alkaptonuria, a disorder due to the deficiency of homogentisate 1,2 dioxygense [10]. Both of these disorders are in the tyrosine catabolic pathway [10].

In neuroblastoma, it is proposed that nitisinone may block the normal degradation pathway of tyrosine via homogentisic acid and increase tyrosine levels as well as dopamine, HVA, VMA, and other catecholamine metabolites (see Fig. 1). If these effects increase cytotoxicity and differentiation, nitisinone may be an effective agent for the treatment of neuroblastoma. This report describes the clinical and biochemical effects of nitisinone used alone and in combination with standard chemotherapy for the treatment of a child with widespread recurrent stage 4 MYCN amplified neuroblastoma.

CASE REPORT

The subject, a 33-month-old white male, presented with right hip and back pain, pancytopenia, and proptosis. A biopsy of a large right adrenal mass was performed and the diagnosis of stage 4 MYCN amplified, unfavorable histology neuroblastoma was established. Other sites of involvement included the liver, spleen, multiple bones, bone marrow, and retro-orbital tissues. Urinary VMA, HVA, and metanephrines were markedly elevated. He was treated with intensive chemotherapy including cisplatin and doxorubicin

[1]Pediatric Oncology, Roger Maris Cancer Center, Fargo, North Dakota; [2]Department of Pediatrics, Faculty of Medicine, University of North Dakota, Grand Forks, North Dakota

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*Correspondence to: Nathan L. Kobrinsky, Pediatric Oncology, Roger Maris Cancer Center, 820 4th Street North, Fargo, North Dakota 58122. E-mail: nathankobrinsky@meritcare.com

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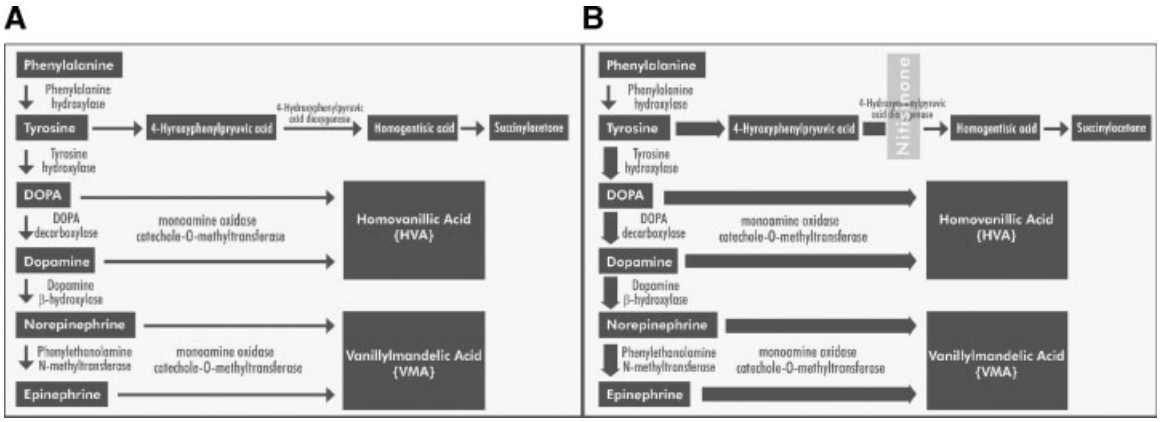

Fig. 1. **A**: Catecholamine metabolism in neuroblastoma. **B**: Catecholamine metabolism with nitisinone. Note proximal build-up of tyrosine and increased production of HVA, VMA, and other catecholamine metabolites.

followed by ifosfamide, mesna, and carboplatin over a 12 week period. Clearing of all disease sites except for a small residual mass in the right adrenal was accomplished. Surgical resection of the remaining right adrenal mass was then performed.

Four weeks later, he underwent an autologous stem cell transplant consolidation. His post-transplant course was complicated by renal failure, respiratory failure, grade four enteritis, and prolonged pancytopenia. He required ventilatory support and dialysis for over a month and prolonged blood product support. He gradually recovered. Cis-retinoic acid was then administered for 12 weeks.

He developed bone pain, proptosis, and a recurrent right adrenal mass 20 months after his initial diagnosis. At first recurrence he was treated with topotecan. He achieved another partial response lasting for 30 weeks. At second progression, he was treated with hydroxyurea, actinomycin D, and doxorubicin. He achieved a partial response lasting for 14 weeks. At his third episode of disease progression, he was treated with hydroxyurea, vincristine, methotrexate, and cyclophosphamide. He achieved a partial response lasting for 4 weeks. At fourth progression, he was treated with arsenic trioxide. Increasing bone pain, a progressive increase in the size of subcutaneous nodules, rising catecholamine metabolites, and progressive pancytopenia were observed. Arsenic trioxide was discontinued after 5 weeks of therapy. Nitisinone was initiated 1 week thereafter. At this time, the patient had pancytopenia from bone marrow replacement by tumor, proptosis, and subcutaneous nodules over the scalp, splenomegaly, hepatomegaly, and a mass in the region of the right adrenal. The patient had severe bone pain and was unable to walk. Continuous narcotic analgesics were required.

Nitisinone Protocol

Consent to use nitisinone on a compassionate basis was obtained from the Institutional Review Board. Informed consent was subsequently obtained from the family.

A physical examination, hematologic and biochemical profile, fasting phenylalanine and tyrosine levels and urinary VMA, HVA, and metanephrines were obtained at baseline and at 2 week intervals. Imaging studies were obtained at baseline, at recurrence (10 weeks) and at 3-month intervals thereafter.

Nitisinone was administered orally at an initial dose of 0.8 mg/kg/day PO in two divided doses. This dose was based on the dose recommended for children with tyrosinemia type I of 1 mg/kg/day also administered in two divided doses [9,10]. The dose was escalated every 4 weeks. Dose escalation was held at 5.0 mg/kg/day.

Tumor progression was documented by bone scan at 10 weeks and by the development of subcutaneous nodules at 12 weeks. At this time, a moderate dose chemotherapy regimen was added to nitisinone: nitisinone 5.0 mg/kg PO daily; vincristine 1.5 mg/m^2; cyclophosphamide 1,000 mg/m^2; doxorubicin 25 mg/m^2. Despite previous vincristine, cyclophosphamide and doxorubicin resistance, a very good partial response was observed following two courses of therapy (see Fig. 2). Subsequently, a low dose maintenance chemotherapy regimen, developed at the St. Jude's Children's Research Hospital, was administered in combination with nitisinone: nitisinone 5.0 mg/kg PO daily; cyclophosphamide 150 mg/m^2 PO days 1–7; doxorubicin 35 mg/m^2 IV day 8. This chemotherapy regimen was repeated at 3 week intervals. The patient's response was maintained for 7 months.

RESULTS

Nitisinone Toxicity

Nitisinone has been reported to produce corneal irritation, thought to be due to high levels of tyrosine. For patients with tyrosinemia type 1, this side effect has been successfully treated by severe dietary restrictions of tyrosine and phenylalanine [10].

Since an elevation of tyrosine levels was a desired effect of nitisinone therapy in neuroblastoma treatment, tyrosine and

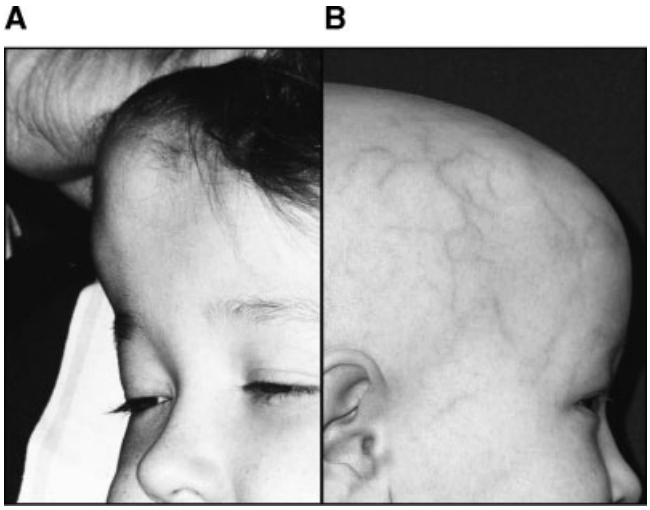

Fig. 2. **A**: Proptosis of the right eye and subcutaneous nodules on the scalp at recurrence following single agent nitisinone. **B**: Decrease in proptosis and subcutaneous nodules following combination of nitisinone, cyclophosphamide, and doxorubicin despite previous cyclophosphamide and doxorubicin resistance.

phenylalanine were not restricted. In fact, a diet rich in tyrosine (including cheese, vanilla, bananas, chocolate, smoked fish, and meat) was encouraged to further raise tyrosine levels. Grade two conjunctivitis not requiring intervention and not progressive was observed. No other side effects were identified.

Clinical Effects

Immediately after starting nitisinone, bone pain and the need for narcotic analgesics resolved. The subcutaneous nodules on the scalp and proptosis of the right eye significantly decreased but did not completely resolve.

At 10 weeks, proptosis of the right eye, scalp lesions, abdominal pain, and bone pain recurred. With the addition of low dose cyclophosphamide and doxorubicin, a dramatic clinical response was observed. Pain immediately resolved. Proptosis and subcutaneous nodules gradually regressed (Fig. 2). Extensive liver disease (Fig. 3) and bone disease (Fig. 4) similarly regressed.

His disease rapidly progressed 18 months and he succumbed 21 months after beginning nitisinone therapy. Throughout the majority of this time, he was pain free, attended school, and had an excellent quality of life.

Biochemical Effects

The effects of nitisinone (dose 0.8 mg/kg/day) on fasting plasma tyrosine and phenylalanine levels and on 24-hr urine catecholamine metabolites are summarized in Table I. At 14 days after starting therapy, tyrosine levels were increased by more than eightfold. Urinary catecholamines were more than doubled.

At all doses of nitisinone tested, the fasting tyrosine level was >350 μmol/L (normal <115 μmol/L). A dose response was noted with tyrosine levels consistently >600 μmol/L at doses of nitisinone above 2.0 mg/kg/day ($R^2 = 0.7$, $P = 0.05$). No effect on phenylalanine levels was observed.

DISCUSSION

Nitisinone, when used as a single agent, demonstrated anti-tumor activity sustained for 10 weeks in a child with recurrent MYCN amplified metastatic neuroblastoma. At recurrence, the drug induced a very good partial response when combined with low dose cyclophosphamide and doxorubicin. This response was sustained for 18 months.

Administration of nitisinone was associated with a marked increase in plasma tyrosine and in urine catecholamines. These observations support the hypothesis that high intracellular tyrosine levels and/or altered intracellular catecholamine metabolites may induce differentiation and/or

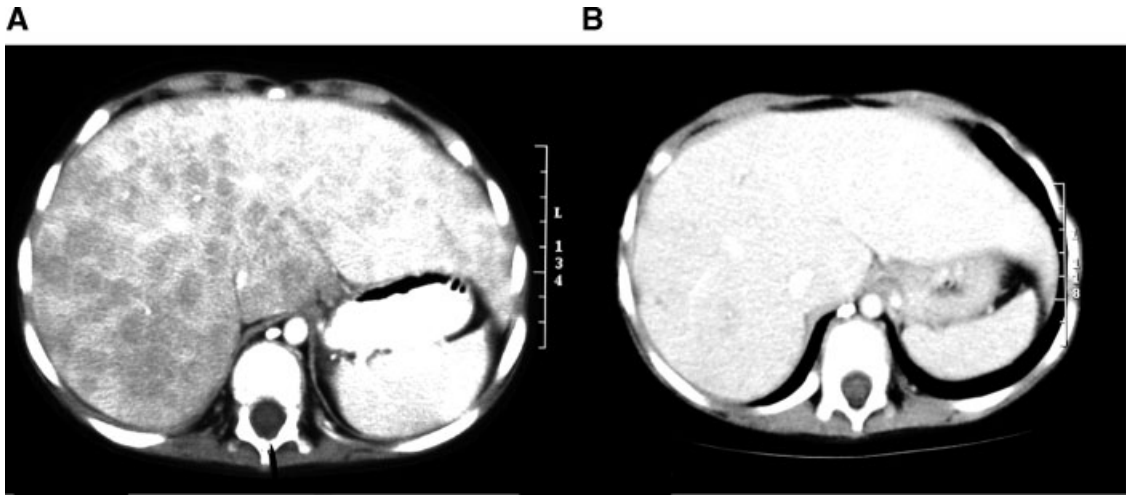

Fig. 3. **A**: CT scans obtained at recurrence. **B**: CT scans obtained following three courses of nitisinone, cyclophosphamide, and doxorubicin demonstrate resolution of extensive hepatic metastases. Both panels are the same magnification.

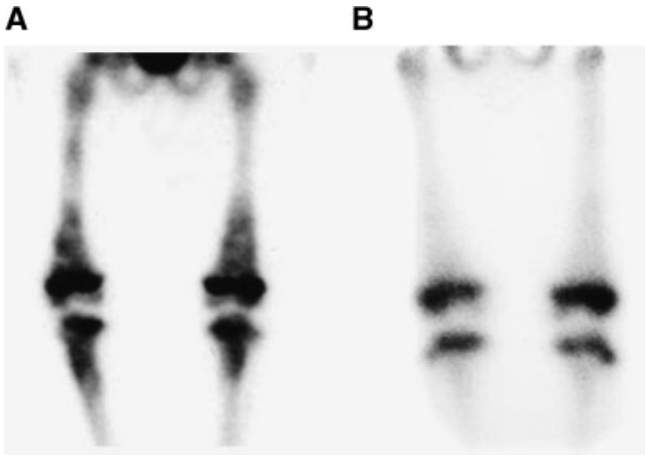

Fig. 4. **A**: Bone scans obtained at recurrence. **B**: Bone scans obtained following three courses of nitisinone, cyclophosphamide, and doxorubicin demonstrate resolution of extensive skeletal metastases.

may be cytotoxic to neuroblastoma cells. The observed clinical response may also be explained by down regulation of tyrosine kinase by high intracellular tyrosine levels. Clearly, other mechanisms may explain these observations.

Elevations of tyrosine may be observed at doses as low as 0.1 mg/kg, e.g., in subjects with alkaptonuria [10]. Typically, the dose recommended for subjects with tyrosinemia type 1 is 1 mg/kg PO daily. This dose is generally well tolerated, except for occasional reports of conjunctivitis. Conjunctivitis was not observed in the patient treated in this report. The maximum dose of nitisinone used (5 mg/kg/day) was limited by cost of the medication rather than toxicity of the drug.

The findings presented are quite preliminary and may not be representative of recurrent MYNC amplified neuroblastoma in general. Clearly, further studies using in vitro modeling systems to further characterize this preliminary clinical observation would be of great benefit. Such studies would (1) confirm these observations in large numbers of neuroblastoma cell lines, (2) help define optimal dosing, and (3) help identify the best chemotherapy agents with which nitisinone should be combined. Such in vitro studies would give direction to future clinical trials of nitisinone in recurrent neuroblastoma. Nitisinone may have a role to play in the treatment of other neural crest derived tumors, which have highly active tyrosine metabolic pathways. Such tumors may include melanoma, pheochromocytoma, small cell lung cancer and neuroectodermal tumors including Ewings sarcoma, medulloblastoma and primitive neuroectodermal tumor (PNET). In vitro studies would be helpful in defining which of these neoplasms respond to this unique agent.

TABLE I. Effects of Nitisinone on Tyrosine Metabolism in Metastatic Neuroblastoma

	Pre-treatment	Post-treatment
Serum		
Tyrosine	62 μmol/L	527 μmol/L
Phenylalanine	56 μmol/L	55 μmol/L
Urine		
Norepinephrine	50 μg/24 hr	88 μg/24 hr
Dopamine	1,608 μg/24 hr	4,205 μg/24 hr
Metanephrine	113 μg/24 hr	183 μg/24 hr
Normetanephrine	5,700 μg/24 hr	10,250 μg/24 hr
Metanephrine (total)	5,813 μg/24 hr	10,250 μg/24 hr
VMA	247 μg/mg creatinine	288 μg/mg creatinine
HVA	87 μg/mg creatinine	85 μg/mg creatinine

The metabolic effects of nitisinone without concurrent chemotherapy are reported. The nitisinone dose 0.8 mg/kg/day was given orally for 14 days.

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