Editorial

Is there a role for retinoids to treat minimal residual disease in neuroblastoma?

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Summary A variety of pre-clinical and clinical data point toward high drug levels of retinoids being required to achieve optimal efficacy against neuroblastoma. The results of the Kohler trial reported in this issue demonstrate that low-dose 13-cis-RA does not have clinical efficacy against neuroblastoma in a setting of minimal residual disease. A comparison of the Kohler trial with the US CCG trial provides clinical evidence that high-dose levels of retinoids are optimal for treating minimal residual disease in neuroblastoma. The comparison of high-dose and low-dose 13-cis-RA studies in neuroblastoma suggests the intriguing possibility that high dose, pulse schedules of other retinoids could be effective as therapeutic and chemopreventive agents in diseases where low-dose, chronic retinoid administration was not effective. Pre-clinical and perhaps clinical studies of the latter concept should be considered. © 2000 Cancer Research Campaign

Rationale for retinoids in neuroblastoma

Relapse from minimal residual disease occurs in over 50% of patients with high-risk neuroblastoma, despite intensive multi-modality therapy with haematopoietic stem cell support (Matthay et al, 1993). To improve outcome, therapies are required with novel mechanisms effective against residual tumour that was able to survive myeloablative doses of cytotoxic agents. The retinoid 13-cis-retinoic acid (13-cis-RA), also known as isotretinoin, is an isomer of all-trans-retinoic acid (ATRA) that occurs naturally, but in very low concentrations, and has been employed for therapy and chemoprevention of cancer (Smith et al, 1992). In vitro, both 13-cis-RA and ATRA caused differentiation, decreased proliferation and decreased *MYCN* expression in neuroblastoma cell lines, including some established from tumours refractory to cytotoxic chemotherapy (Sidell, 1982; Thiele et al, 1985; Sidell et al, 1986; Reynolds et al, 1991, 1994; Abemayor, 1992; Melino et al, 1997). Because of the strong activity of ATRA in acute promyelocytic leukaemia, an in vitro comparison was performed of the clinically achievable levels of 13-cis-RA (5 µM) and ATRA (0.5 µM). It was shown in 6 of 12 neuroblastoma cell lines that for ATRA and 13-cis-RA at clinically achievable levels, the two drugs were equal in activity, while for 6 of 12 lines 13-cis-RA had significantly better activity than did ATRA (Reynolds et al, 1994).

Anecdotal reports as well as a U.S. Children's Cancer Group (CCG) phase II trial of 13-cis-RA in children with neuroblastoma suggested only modest activity in recurrent disease when 13-cis-RA was given continuously at 100 mg/m²/day (Reynolds et al, 1991; Finklestein et al, 1992). In vitro testing of 13-cis-RA using multiple neuroblastoma cell lines demonstrated that levels of 5–10 µM caused growth arrest, which was sustained in some cell lines for weeks after removal of the 13-cis-RA from the culture medium. These data suggested that high-dose, pulse 13-cis-RA would be effective for clinical studies in neuroblastoma patients and might allow dose escalation above levels obtainable with continuous dosing. Based on the results of the in vitro modelling, a phase I dose escalation trial was designed in which patients were given higher doses of 13-cis-RA on an intermittent schedule, to allow recovery from toxicity (Villablanca et al, 1995). This phase I study was done in children with high-risk neuroblastoma following autologous bone marrow transplantation, and established that the high-dose intermittent schedule (using 13-cis-RA for 14 days consecutively out of every 28 days) had low toxicity and achieved levels known to be effective against neuroblastoma in vitro. The maximum tolerated dose was 160 mg/m² daily, which achieved peak levels of 7 µM. Three complete responses in bone marrow were observed in ten evaluable patients (Villablanca et al, 1995). These data showed that 13-cis-RA is well tolerated after intensive chemoradiotherapy, and suggested that it could have efficacy against minimal residual disease that causes relapse.

Phase III clinical trials of 13-cis-RA in neuroblastoma

Based on the laboratory and clinical studies of 13-cis-RA, the U.S. CCG designed a Phase III randomized trial for high-risk neuroblastoma. Children who were progression-free after completion of either intensive chemotherapy or myeloablative chemoradiotherapy and autologous bone marrow transplantation were assigned to either 6 months of high-dose intermittent 13-cis-RA or to no further treatment. The event-free survival (EFS) for the group randomized to 13-cis-RA ($n = 130$) was 46%, significantly higher than that of patients randomized to no further treatment ($n = 128$), at 29% ($P = 0.027$) (Matthay et al, 1999).

In this issue of *British Journal of Cancer*, Kohler and colleagues for the European Neuroblastoma Study Group (ENSG) report a double-blind randomized trial of low dose continuous 13-cis-RA given after completion of cytotoxic therapy for high-risk neuroblastoma. The results of the ENSG study differ from that of the CCG trial in that no difference in EFS was seen with the use of

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13-cis-RA given to patients in complete or very good partial remission after cytotoxic therapy. There are a number of possible reasons for this discrepancy. The most likely reason for the lack of efficacy in the ENSG trial is the low dose employed for 13-cis-RA. The study was begun in 1989, prior to publication of the data from the in vitro studies and the phase I trial that led to the CCG randomized study. The ENSG study was designed using a dose that was approximately 15% of that shown to be the maximum tolerated dose in the phase I study by Villablanca and colleagues (Villablanca et al, 1995) and of the subsequent randomized CCG phase III trial (Matthay et al, 1999). At that low dose, drug levels would be far below those shown to be effective for sustained growth arrest of neuroblastoma cell lines (Reynolds et al, 1991, 1994; Reynolds and Lie, 2000).

Other differences, which may have influenced the outcome, include the somewhat later start of the 13-cis-RA in the European trial, at a median of 341 days from diagnosis, compared to an average of 290 days in the Children's Cancer Group study. Beginning 13-cis-RA relatively soon after cytotoxic therapy, before tumour cells can begin to grow, may be critical for this agent to be most effective. The greater efficacy of 13-cis-RA against minimal disease (compared to a larger tumour burden) is supported by the fact that the CCG study showed that the most significant effect of the 13-cis-retinoic acid was in children in apparent complete remission, with no significant difference seen when the analysis was restricted to those in partial remission (Matthay et al, 1999). Although the ENSG study selectively treated only children whose disease was in complete or very good partial remission after chemotherapy, it is possible that the longer interval from ablative chemotherapy to the use of the differentiating agent allowed regrowth of tumour. The new US cooperative paediatric clinical trials group, the Children's Oncology Group, will test 13-cis-RA after myeloablative therapy even earlier, beginning 8 weeks after haematopoietic stem cell transplantation.

Compliance with the use of 13-cis-RA is a potential problem in small children, since currently the only available formulation is in capsules. For small children, the dosing may be sub-optimal, with the inability to take the capsules requiring removal from the capsule and administration with food, as done in the CCG study, or else not administering to very young children, as in the ENSG study. Since no pharmacokinetic measurements were obtained in the Kohler study, compliance cannot be verified. In the Kohler study, both induction regimens and myeloablative therapy (employed in 126 of the 175 randomized patients) were treatment centre-dependent, leading to a considerable variation in the different therapies given to patients prior to beginning 13-cis-RA. Furthermore, patient numbers were smaller in the ENSG study relative to the US CCG study. All of these issues could have diminished the power of the ENSG study to identify a positive benefit for 13-cis-RA.

The pre-clinical and clinical data on use of 13-cis-RA against neuroblastoma indicate that dosing, scheduling, and beginning treatment with 13-cis-RA at a time when tumour burden is low are all important elements in the efficacy of 13-cis-RA. The failure of 13-cis-RA to improve survival in the Kohler study provides further evidence supporting the use of optimal doses and schedules of retinoids when employed as anti-neoplastic agents.

NEW RETINOIDS AND POSSIBLE FUTURE RETINOID COMBINATIONS

Although 13-cis-RA improved the survival of patients with high-risk neuroblastoma, resistance to 13-cis-RA and ATRA occurs in neuroblastoma. If agents can be identified that are effective against retinoic acid-resistant neuroblastoma at drug levels obtainable in patients, further improvements in survival may be achieved (Reynolds and Lie, 2000). N-(4-hydroxyphenyl)-retinamide (4-HPR), or fenretinide, is a synthetic retinoid that is cytotoxic for tumour cells. In contrast to 13-cis-RA and ATRA, 4-HPR does not induce maturational changes, but causes apoptosis (Delia et al, 1993; Ziv et al, 1994; Supino et al, 1996) and 4-HPR has shown activity against cell lines known to be resistant to ATRA (Delia et al, 1993; Sheikh et al, 1995; Kazmi et al, 1996; Supino et al, 1996). 4-HPR has been reported to inhibit the growth of neuroblastoma cell lines in vitro at 4-HPR concentrations of $1–10 \mu M$ in a dose dependent manner (Di Vinci et al, 1994; Mariotti et al, 1994; Ponzoni et al, 1995), and 4-HPR was highly active against RA-resistant neuroblastoma cell lines at 5 to $10 \mu M$ drug levels (Reynolds et al, 1997).

Although until recently 4-HPR was only used at low doses, toxicity of 4-HPR in clinical trials has been minimal (Cobleigh et al, 1993; Formelli et al, 1993; Costa et al, 1995) and no haematologic toxicity has been reported. The major clinical toxicity of 4-HPR is decreased night vision, due to decreased plasma retinol levels (Decensi et al, 1997). Initial results of our US CCG phase I trial in children have shown no systemic toxicity of oral 4-HPR to date, even at higher doses that have achieved 4-HPR plasma levels of 3 to $7.5 \mu M$ (Basniewski et al, 1999). A phase II trial of oral 4-HPR as a single agent in recurrent neuroblastoma is planned within the Childrens Oncology Group, once the ongoing CCG phase I trial is complete.

Recent studies have shown that $5–10 \mu M$ 4-HPR stimulates large increases of ceramide in neuroblastoma cell lines, which is likely one of the mechanisms by which anti-tumour cytotoxicity is achieved with 4-HPR (Maurer et al, 1999). Neuroblastoma cell lines established at relapse after myeloablative therapy often have acquired a sustained resistance during the course of therapy to drugs which act via traditional cytotoxic mechanisms (Keshelava et al, 1998). However, such cell lines can be sensitive to high levels of 4-HPR, perhaps due to 4-HPR achieving tumour cell cytotoxicity via novel mechanisms of action (Maurer et al, 1999). The activity of 4-HPR against drug-resistant neuroblastoma cell lines, including cell lines resistant to ATRA and 13-cis-RA (Reynolds et al, 1997), suggests that high-dose 4-HPR may be effective against tumour cells that persist after current therapeutic approaches. Future trials may also employ 4-HPR in combination with agents that modulate ceramide metabolism so as to increase the anti-tumour activity of 4-HPR (Maurer et al, 2000).

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