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Editorial

Neuroblastoma Therapy using Radiolabelled [^{131}I]meta-iodobenzylguanidine ([^{131}I]MIBG) in Combination with Other Agents

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INTRODUCTION

[^{131}I]META-IODOBENZYLGUANIDINE ([^{131}I]MIBG) is an effective single agent for the treatment of neuroblastoma. However, uptake of the drug in malignant sites is heterogeneous, suggesting that this therapy alone is unlikely to cure disease. Increasingly [^{131}I]MIBG is administered in combination with more conventional treatments in attempts to exploit the expression of several different targets in the rapidly evolving cells of tumours. Targetable pathways include: specific ligand binding, epitope expression, proliferation, angiogenesis, hypoxia and cell adhesion. Drugs targeting alterations in cell cycle progression could also be a part of this combination therapy.

The active uptake of MIBG into neuroblastoma cells is dependent on their expression of the noradrenaline transporter (NAT). Following the elucidation of the nucleotide sequence of the gene encoding the NAT [1], molecular assays have become available for semi-quantitation of its expression and recent efforts to increase MIBG accumulation in malignant cells have considered means of enhancing NAT gene transcription. The aim of such studies is to elevate the intracellular levels of the radiopharmaceutical so that cure, rather than temporary control of tumour growth, could be the outcome. The article in this issue by Meco and colleagues [2] (pp. 1227–1234) takes us a step closer to identifying cellular control mechanisms which may be implicated in the regulation of NAT gene transcription.

CLINICAL INVESTIGATIONS

In Europe, several different ways are being assessed of treating neuroblastoma by combining MIBG with other forms of therapy. In Amsterdam, [^{131}I]MIBG treatment has been given in conjunction with hyperbaric oxygen. This attempt to overcome the radioresistance of poorly oxygenated regions of tumours resulted in improved survival rates compared with [^{131}I]MIBG treatment alone [3]. In a separate study, the Amsterdam group administered MIBG as initial treatment of newly diagnosed patients before either chemotherapy or surgery [4]. This scheme seeks to reduce tumour volume to facilitate surgical excision without creating chemoresistant mutants. However, the [^{131}I]MIBG dose is fractionated and tumour growth may occur during the lengthy interval between administrations. Preoperative [^{131}I]MIBG treatment was found to be as effective as, but less toxic than, induction chemotherapy [5].

Clinical studies in the U.K. have used [^{131}I]MIBG followed by intensive multiagent chemotherapy and haemopoietic stem cell rescue with high-dose carboplatin and melphalan [6] or whole body irradiation [7]. A current UKCCSG clinical investigation involves several hospitals and aims to treat patients with newly presenting, advanced stage disease firstly with [^{131}I]MIBG and 5 days later with combination chemotherapy + radiotherapy. Toxicity will be assessed at an initial whole body dose of 1.5 Gy. Thereafter the MIBG component will be increased until autologous bone marrow or peripheral stem cell rescue is required. It is hoped to involve European centres to determine whether this combination scheme is superior to chemotherapy alone [8].

Klingebiel and colleagues [9] have conducted a pilot study of the treatment of stage 4 neuroblastoma patients with [^{131}I]MIBG, high-dose chemotherapy, peripheral blood stem cell rescue and immunotherapy using anti-GD2 murine or chimeric antibody. The impressive response rate resulting from this novel combination justifies a more extensive study involving a larger number of patients.

The Rome group have considerable experience of the use of [^{131}I]MIBG in combination with cisplatin. Cisplatin may have some activity as a radiosensitiser; more importantly, perhaps, is the observed potentiation of MIBG uptake by cisplatin given previously [10]. The schedule employed consists of two doses of cisplatin, both given 1 day before [^{131}I]MIBG. According to *in vitro* studies, the 24 h interval between the administration of the two treatments allows enhanced expression of the NAT gene and increased accumulation of MIBG. This combination of [^{131}I]MIBG and cisplatin administration to stage IV neuroblastoma patients has been successful in preliminary clinical studies but haematologic toxicity was also observed [11].

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OPTIMAL SEQUENCING

In order to determine the most effective sequence in which to administer the components of combination therapy which includes [^{131}I]MIBG and cytotoxic drugs, it is necessary to consider the radiological bystander effect associated with [^{131}I]MIBG treatment. Because the beta particles emitted by ^{131}I have path lengths of approximately 800 μm, cross-fire irradiation from cells targeted with [^{131}I]MIBG to neighbouring cells provides a significant proportion of the toxic effect of this radiopharmaceutical. Microdosimetric modelling suggests that the size of tumour deposit most likely to be sterilised by [^{131}I]MIBG is 2.5 to 5.0 mm [12]. Smaller aggregates of cells are underdosed because more of the beta-decay energy is absorbed out with the targeted mass. If conventional anticancer drug treatment is given before [^{131}I]MIBG, larger metastases will be reduced in size so that the benefit of cross-fire kill from subsequently administered [^{131}I]MIBG will be attenuated. Moreover, uptake of [^{131}I]MIBG (an energy-requiring process) will be inhibited by prior exposure to cytotoxins. For these reasons, it seems logical that [^{131}I]MIBG should precede chemotherapy. However, evidence is rapidly accumulating which indicates that treatment with DNA-damaging agents, such as ionising radiation, maturation-inducing compounds and cytotoxic drugs, can enhance active uptake of MIBG by neuroblastoma cells. This suggests that it may be prudent to administer a priming dose of conventional therapy before [^{131}I]MIBG [10].

EXPERIMENTAL COMBINATION THERAPY

A number of *in vitro* studies have provided insights into mechanisms of interaction between MIBG and cytotoxic or differentiative treatments which may influence the scheduling of the components of combination therapy. Agents which increase the uptake of MIBG by neuroblastoma cells in culture include ionising radiation [13], phorbol esters, retinoids [14] and cisplatin [10;2]. By virtue of their interaction with DNA, all of these agents can upregulate the expression of genes encoding damage-recognition proteins such as p53, whose loss of function is a common event in the development of many cancers but which has a very low frequency of mutation in neuroblastoma [15]. p53 is a transcription factor which, through sequence-specific DNA binding to various p53-responsive elements [16], modulates the expression of a range of target genes following DNA damage. It is possible that the cytotoxin-mediated increase in cellular accumulation of MIBG results from transcriptional transactivation of the NAT gene via a putative p53-like consensus sequence in the promoter. It is now necessary to determine the sequence of the NAT gene promoter in order to specify transcription regulatory elements. This will enable the identification of other agents which could act in synergy with MIBG to improve tumour-selective therapy.

In this issue of the *European Journal of Cancer*, Meco and coworkers (pp. 1227–1234) [2] report enhanced uptake of MIBG following exposure of neuroblastoma cells to graded doses of cisplatin and doxorubicin. In the case of doxorubicin, this appeared to involve upregulation of transcription of the NAT gene. The increased MIBG uptake by cisplatin may be due to transcriptional controls as well as influences upon existing NAT molecules and the balance between these two modes of action may be concentration-dependent. No evidence of differentiative effects was observed. An interesting finding was the significant correlation between drug-induced G_2/M blockade and active uptake of MIBG. This seemed to be cycle stage-specific, since cells arrested in S phase exhibited no stimulation of MIBG accumulation, suggesting negative regulation of NAT expression or activity during mitosis. Support for this mode of stimulatory activity could be obtained by comparison of the capacity for active uptake of MIBG by cells which are capable of G_2/M blockade with those which exhibit disrupted mitotic arrest. If confirmed, this would lend support to strategies designed to sensitise tumour cells to MIBG targeted radiotherapy by manipulation of the G_2/M checkpoint.

Meco and colleagues [2] also demonstrated enhanced uptake of MIBG in human tumour xenografts 2 and 3 days after administration of cisplatin or doxorubicin. Unfortunately, drug pretreatment also resulted in greater concentration of radiopharmaceutical in a variety of normal organs, suggesting that some non-target tissues may be adversely affected by combination therapy involving the administration of cytotoxins followed by MIBG. If the increased accumulation in non-malignant sites is due to upregulation of NAT expression, then negative regulatory elements in the NAT promoter should be identified in order to design pharmacological approaches to the minimisation of normal tissue toxicity.

Recently Cunningham and colleagues [17] examined the toxicity *in vitro* of four different preparations of benzylguanidine: [^{123}I]MIBG, [^{125}I]MIBG, [^{131}I]MIBG and [^{211}At]MABG. The alpha particle-emitting agent [^{211}At]MABG was the most potent and it is hoped that the cyclon-produced radiohalogen [^{211}At]-astatine will become more generally available. The Auger electron-emitting conjugates [^{123}I]MIBG and [^{125}I]MIBG were toxic to single cells but less effective in the treatment of multicellular spheroids than the beta emitter [^{131}I]MIBG. These findings suggest that short-range emitters would be well suited to the treatment of circulating tumour cells or small clumps whereas long-range, beta emitters would be superior in the treatment of subclinical metastases or macroscopic tumours. These experimental results provide support for a clinical strategy using cocktails of radioconjugates in targeted radiotherapy.

At Duke University, North Carolina, U.S.A., a new analogue (3-[^{211}At]astato-4-fluorobenzylguanidine) has been synthesised which exhibited superior NAT-mediated uptake and retention in neuroblastoma cells compared with [^{211}At]MABG but was more susceptible to dehalogenation *in vivo* [18]. We await with interest the production of further novel targeting radiopharmaceuticals from this group.

Gene therapy techniques remain at an early stage but there is now exciting evidence showing that cells transfected with the NAT gene developed impressive capacity for MIBG uptake and succumbed to treatment with [^{131}I]MIBG in a dose-dependent manner [19]. Suitable vectors and specific, promoter control of expression of NAT transgenes must be developed to assess the feasibility of combining gene transfection with radionuclide therapy using MIBG as the molecular vehicle.

More is known about the specific biochemical and molecular features of neuroblastoma than any other childhood cancer. It is expected that we will soon witness specification of treatment based on the biological characteristics of tumours in individual patients. Studies of the type described by Meco and colleagues [2] will allow us to devise the most rational use of MIBG in order to fulfil its therapeutic potential.

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