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Novel role of triazenes in haematological malignancies: Pilot study of Temozolomide, Lomeguatrib and IL-2 in the chemo-immunotherapy of acute leukaemia

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ABSTRACT

Previous studies indicated that dacarbazine and Temozolomide could be highly effective against refractory acute leukaemia. Their activity relies mainly on the generation of methyl adducts at the O^6-position of guanine in DNA. High levels of O^6-methylguanine-DNA methyltransferase (MGMT) or a defective mismatch repair (MMR) system, are associated with cellular resistance to triazenes. The MGMT inhibitor, O^6-(4-bromothenyl)guanine (Lomeguatrib), can restore *in vitro* sensitivity to Temozolomide in MMR-proficient blasts. In the early 1970s we discovered that, *in vivo*, triazene compounds induce the appearance of novel transplantation antigens in murine leukaemia ("Chemical Xenogenization", CX). Non-self peptides presented by class I MHC molecules are generated by triazene-induced somatic mutations, affecting retroviral sequences that are detectable in the mouse genome. Moreover, preliminary experiments suggested that human cancer cells can also undergo CX. Therefore, we designed a chemo-immunotherapy strategy in leukaemic patients as follows: (a) *cytoreduction and a hypothetical CX phase*, i.e. treatment with Lomeguatrib (to suppress MGMT activity) and Temozolomide (to kill sensitive blasts and to presumably induce CX in resistant leukaemic cells); (b) *immune response recovery phase* using interleukin-2 (to possibly restore an immune response and take advantage of the hypothetical, triazene-induced CX). Here we present the results of pilot study which is in progress in patients with refractory/relapsed acute leukaemia. In all tested cases, Lomeguatrib suppressed MGMT activity *in vivo*. Six out of eight patients showed partial or complete disappearance of blast cells in peripheral blood or in bone marrow. We observed severe and long-lasting myelosuppression, accompanied by limited non-haematological toxicity. Up to now, two patients are alive (after

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9 and 10 months, respectively), four died of opportunistic infections and two of progressive disease. This investigation confirms the potential role of triazenes in leukaemia and highlights the contribution of Lomeguatrib in overcoming drug resistance. Further studies are required to establish whether Temozolomide can induce CX in human leukaemia, and thus offer a new approach to control minimal residual disease.

1. Introduction

Temozolomide is a methylating triazene compound that spontaneously decomposes into 5-(3-methyl-1-triazeno)imidazole-4-carboxamide, the active metabolite of dacarbazine [1]. Unlike dacarbazine, Temozolomide penetrates the blood–brain barrier [2]. Therefore, it is active against primary and metastatic tumors in the brain. The drug has recently been approved for the treatment of recurrent high-grade glioma and is in phase II/III clinical trials for the treatment of melanoma and other solid neoplasias.

Clinical experience on the use of methylating triazene compounds in acute leukaemia [3,4] and myelodysplasia [5] is currently limited, but these studies have demonstrated that both dacarbazine and Temozolomide possess antineoplastic activity in leukaemic patients. Therefore triazenes, which have a long history of clinical application in melanoma [6], lymphoma [7,8], sarcoma [9] and primary and metastatic brain tumors [10,11], provide a novel and, up to now largely unexplored, class of antineoplastic agents available for the treatment of acute leukaemia.

The mechanism underlying the activity of methylating triazene compounds is mainly based on methylation of the O^6-position of guanine. Upon DNA replication, O^6-methylguanine triggers a cascade of intracellular signals leading to cell cycle arrest and apoptosis [12–17]. The N3 and N7 atoms of purine bases of DNA are also methylated [2,18–20], and N3-methyladenine is considered a potentially toxic lesion if not repaired by the base excision repair system [18–20].

In the early seventies, a special feature of the pharmacodynamic activity of triazene compounds was discovered by our group in murine leukaemia models [21–26]. Mice, predominantly of H-2^d haplotype strain, were inoculated with fully histocompatible leukaemia cells and treated with dacarbazine for 4–6 transplant generations. Malignant cells collected from dacarbazine-treated donors were strongly immunogenic and antigenic, and were rejected by histocompatible hosts [21]. This effect was found not to be the result of drug-induced selection of highly immunogenic clones pre-existing in mixed leukaemia cell population [26]. In fact, we were able to demonstrate that dacarbazine-treated cells present novel transplantation antigens that are not detectable in the cell line of origin [26,27]. The mechanism underlying this phenomenon, which has been called “chemical xenogenization” (CX) [28] was found to be based on triazene-induced somatic mutations, leading to the appearance of non-self peptides presented by the class I major histocompatibility complex (MHC) [29,30]. Initially, the mutational theory proposed to explain CX was supported indirectly by experiments indicating that quinacrine, an antimutagenic compound, was able to entirely suppress CX [31]. Later, direct molecular evidence was obtained, showing that, in murine leukaemia cell lines, CX was the result of point mutations affecting retroviral sequences that are detectable in the mouse genome [29]. Of particular interest was the observation that CX was distinguishable from chemosensitivity. In fact, mouse leukaemias, rendered totally resistant to triazenes *in vitro*, were still fully susceptible to CX [32]. In addition, the L1210 Ha cell line, utilized in a number of *in vivo* experiments, was found to be almost entirely resistant to the cytotoxic effects of dacarbazine, but extremely sensitive to CX [33].

Further studies were performed by D’Atri et al. [34] to establish whether malignant cells of human origin would be susceptible to CX. Cancer cells of a human lung adenocarcinoma cell line were exposed to 4-(3-methyl-1-triazeno)benzoic acid *in vitro* and used to elicit an *in vitro* cytotoxic T lymphocyte (CTL) response. Thereafter, CTL were cloned and tested against untreated or triazene-treated target cells. The results showed that selected clones were able to specifically kill triazene-treated but not untreated control cells, thus suggesting that a CX phenomenon could be also detected in human malignant cells.

The discovery of CX enabled the design of a novel chemo-immunotherapy regimen against murine leukaemia [35]. Leukaemia-bearing mice were treated with dacarbazine (cytoreduction and CX phase, accompanied by a profound immuno-depression, [28]) followed by conditioning with cyclophosphamide and transfer of syngeneic lymphocytes (immune response recovery phase). Thereafter, the mice were treated with an antineoplastic agent (i.e. BCNU) able to provide anti-leukaemia synergistic effects when combined with host’s graft responses [36]. This preclinical protocol was found to be active and showed its potential for future clinical application in human leukaemia [35]. Further studies demonstrated that interleukin-2 (IL-2) could be successfully used in place of lymphocyte transfer to attain the recovery of the immune response in mice bearing Lewis lung carcinoma subjected to dacarbazine-mediated CX *in vivo* [37]. The possibility that IL-2 could restore immunological reactivity in immuno-competent cells exposed to triazenes was also demonstrated in *in vitro* studies [38].

2. MGMT and its inhibition

Given that the mechanism of cytotoxicity of triazenes involves methylation of the O^6-position of guanine in DNA, it is clear that the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) [reviewed in Refs. [39–42]] and the mismatch repair (MMR) system [reviewed in Refs. [43–45]], play pivotal roles in the cytotoxic and CX-inducing effects of methylating triazenes. Thus, high levels of MGMT can be responsible for target cell resistance to these compounds