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Invited Review

Iron deprivation and cancer: a view beginning with studies of monoclonal antibodies against the transferrin receptor

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Summary. This review provides a perspective on the potential utility of iron deprivation treatments as components of cancer therapy. The perspective began to develop with investigations of the selective inhibitory effects on lymphocyte activation which were produced by monoclonal antibodies against the transferrin receptor. Those investigations led to the unexpected discovery that such antibodies would produce synergistic inhibition of lymphoid tumor growth in vitro when used in combination with the iron chelator deferoxamine. The perspective was further developed when additional studies in vivo indicated that combination iron deprivation treatment could prevent initial tumor outgrowth and cause regressions of established tumors in the 38C13 murine lymphoma model. The anti-tumor effects were accompanied by significant toxicities, however, and the analysis of the causes of those toxicities is now an important issue. The opportunities and problems which these results present are interpreted in the broader context of currently available information concerning the anti-tumor effects of deferoxamine and gallium nitrate in the pre-clinical and clinical settings, and questions for future research are presented.

Key words: Iron, Deferoxamine, Gallium, Monoclonal antibodies, Transferrin receptor, Cancer treatment

Introduction

There has been steadily growing interest in the potential usefulness of iron deprivation as a component of cancer therapy. There are currently two major forms of treatment that can be used to create iron deprivation in the clinical setting. The first is based on gallium nitrate and the second is based on the iron chelator deferoxamine. A third treatment, which is based on the use of monoclonal antibodies against the transferrin receptor, is now in early phase clinical trials. The purpose of this review is to communicate a perspective on the current status and future potential of these treatments, with special emphasis on the possibilities of using them in combination with each other and with other established forms of cancer treatment.

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Mabs against the transferrin receptor and deferoxamine produce synergistic inhibition of lymphoid tumor growth in vitro

Our laboratory began to study iron deprivation in relation to cancer treatment as a consequence of three unanticipated experimental results. The first unanticipated result was the development of a rat IgG monoclonal antibody against the murine transferrin receptor (IgG ATRA) (Kemp et al., 1987). We discovered this antibody while attempting to make hybridomas that would identify surface receptors involved in controlling the growth and differentiation of B lymphocytes.

The second unanticipated result was that our antibody displayed a surprisingly selective pattern of inhibition of lymphocyte activation protocols (Kemp et al., 1987). In particular, we observed that T cell activation (especially mixed lymphocyte reactions and the generation of cytotoxic T lymphocytes) was highly sensitive to ATRA mediated inhibition while B cell activation was unaffected. We therefore undertook a detailed comparison of our antibody and two other ATRAS and found that while there was some variation between the reagents, a similarly selective pattern of inhibition T and B lymphocyte activation protocols nevertheless appeared to be a general property of such reagents (Kemp et al., 1989).

The third unanticipated result involved hematopoietic tumors and followed from experiments that were undertaken with T cell clones. The T cell experiments had revealed that TH1 clones (those which make IL2 and Gamma Interferon) were more susceptible to ATRA mediated growth inhibition that were TH2 clones (those which make IL4 and IL5) (Thorson et al., 1991). The

studies pointed toward a qualitative difference in the way in which the two types of T cell clones respond to a similar insult in the process of transferrin receptor mediated iron uptake. The desire to understand the nature of varied responses to ATRA mediated inhibition of iron uptake drove us to repeat similar experiments with more easily managed uniform cell populations (hematopoietic tumors). In particular, we were driven to repeat an experiment that involved combined treatment with the IgG ATRA and the iron chelator deferoxamine (DFO). Much to our surprise, we observed that hematopoietic tumors exhibited synergistic growth inhibition when so treated (Kemp et al., 1990). The synergism was evident from the significant left shift in the DFO dose response curve seen with each tumor when the IgG ATRA was present.

The results were surprising because, in spite of the fact that we had shown that our IgG ATRA could inhibit normal lymphocyte activation, we had little reason to suspect that it would make a significant contribution to tumor growth inhibition. Indeed, our own data confirmed prior observations which indicated that, by themselves, IgG ATRAS were very poor growth inhibitors (Lesley and Schulte, 1985; Taetle et al., 1986). Interestingly, subsequent studies revealed that an individual IgG ATRA could inhibit iron uptake by hematopoietic tumors by as much as 80%, but that even this degree of injury was insufficient to cause a collapse of DNA synthesis (Kemp et al., 1992). As a result, we proposed a threshold model of iron deprivation injury. This model predicts that malignant cells will tolerate a significant impairment in iron acquisition but that a small incremental insult near a critical threshold will result in the activation of process that collapses DNA synthesis and causes cell death (Kemp et al., 1992). It will not seem surprising at this point in time to say that we now believe that the process in question is programmed cell death (apoptosis), but this will be dealt with subsequently.

IgG ATRAS and combination treatment with a high molecular weight form of deferoxamine inhibit lymphoid tumor growth in vivo

The next phase in our studies was that of testing whether combined iron deprivation treatment could be employed in vivo. We decided to employ the murine 38C13 tumor model. We did this for two principal reasons. The first reason was that 38C13 represented a well-studied model for in vivo therapeutic studies (Bergman and Haimovich, 1977; Maloney et al., 1985). The second reason was that 38C13 proved to be the sixth out of six tumors tested that exhibited synergistic growth inhibition when treated with an IgG ATRA and DFO in vitro.

The in vivo studies undertaken with the 38C13 model provided several interesting insights. One of the first such insights was that we were not able to achieve meaningful sustained plasma concentrations of DFO in mice when using DFO mesylate (the current standard therapeutic preparation). This was almost certainly due to the fact that DFO has a very short half-life in all species tested when administered as the mesylate salt and to the fact that mouse plasma degrades DFO four times faster than human plasma (Meyer-Brunot and Keberle, 1967). We were finally able to solve this problem by using the high molecular weight hydroxyethylstarch conjugate of DFO (HES-DFO) produced by Biomedical Frontiers, Inc. of Minneapolis, MN (Hallaway et al., 1989). This compound allowed us to achieve sustained DFO equivalent plasma concentrations in excess of 1mM.

When using HES-DFO and a single IgG ATRA, it was possible to show that the combination thereof produced synergistic, and nearly complete, inhibition of the initial outgrowth of the 38C13 tumor (Kemp et al., 1995). Neither HES-DFO nor the IgG ATRA was capable of producing a significant inhibitory effect when used alone, however. The lack of activity of the IgG ATRA alone was consistent with the expectations arising from the tissue culture experiments (Kemp et al., 1990, 1992) and, as we now realize, the lack of activity of HES-DFO alone might well have been expected. The latter realization came about as the result of work by Voest and colleagues. They showed (and we have since confirmed) that the typical tissue culture experiment underestimates the amount of DFO required to produce tumor growth inhibition if the amount of transferrin bound iron available to a tumor approaches that which is present in plasma (Voest et al., 1993). The actual concentration gradients of transferrin in normal interstitial tissues and in abnormal tumor beds are not well understood, however, and it is therefore not yet possible to devise an appropriate correction factor for the DFO or HES-DFO data obtained in vitro. In any event, in spite of the fact that the in vivo experiments clearly indicate that enough HES-DFO can reach extra-vascular tumor cells to allow a synergistic interaction with the IgG ATRA, the same experimental data nevertheless also indicate that the locally available HES-DFO has so far been inadequate for the detection of single agent anti-tumor activity against the 38C13 lymphoma.

In spite of the fact that combined treatment with HES-DFO and a single IgG ATRA had a dramatic inhibitory effect on initial tumor outgrowth, it was not curative and, moreover, it had no effect on tumors that had been allowed to become established for 6-7 days. Because of this, we investigated the possibility of using pairs of IgG ATRAS, alone or in combination with HES-DFO. We did this because of the prior work of White et al. (1990) who showed that some pairs of mouse anti-human IgG ATRAS were capable of producing synergistic anti-tumor effects. We were able to find a pair of rat anti-mouse IgG ATRAS that were capable of producing synergistic inhibition of 38C13 in vitro and tested them in vivo. The antibody pair was capable of producing regressions in a fraction of the mice bearing established tumors. Moreover, when the antibody pair

was combined with HES-DFO nearly all of the established tumors underwent regression (Kemp et al., 1995).

Although these experiments have shown that combined iron deprivation treatment can have very significant anti-tumor effects in vivo, they have also shown that combined iron treatment produces toxicity in mice (Kemp et al., 1995). Thus, when treated with the combination of a single IgG ATRA and HES-DFO, about half of the mice developed unambiguous bacterial infections after 10 days. Moreover when mice were treated with a pair of IgG ATRAS and HES-DFO, some form of systemic stress became detectable by 6 days. The latter stress was manifested by hypoactivity and ruffled fur and was followed by death within 24-48 hours in the initial instances in which pre-emptive euthanasia was not performed. Microscopic foci of bacteria could be found in the livers of mice treated with the ATRA pair and DFO and we therefore believe that infection played some role in the toxicity arising from such treatment. While such toxicities may or may not be observed in human beings (and therefore do not preclude carefully constructed Phase I trials), they do represent obstacles to further experimentation with combined iron deprivation treatment. It is therefore necessary to undertake a detailed dissection of the pathophysiology involved in order to ascertain whether the toxicities can be prevented or reversed and we currently have such studies underway.

The broader context of iron deprivation: the status of deferoxamine

It is now appropriate to place our observations with DFO, HES-DFO, and IgG ATRAS into a broader context of information relating to iron deprivation and cancer therapy. We will begin with more background information about DFO. DFO, as the mesylate salt, is under active investigation as a component of multi-modality treatment regimens for disseminated neuroblastoma. Thus, after showing that DFO had single agent activity against neuroblastoma (Donfrancesco et al., 1990), Donfrancesco et al. (1993) went on to provide evidence that DFO could be employed in a multi-agent regimen with cyclophosphamide, etoposide, carboplatin, and thiotepa. A related trial is now being planned in the Untied States under the auspices of the Pediatric Oncology Group (C. Frantz, University of Maryland, personal communication). There is also a recent report of the use of DFO in conjunction with alpha interferon, adriamycin, tamoxifen, and ascorbic acid in the treatment of hepatoma (Kountouras et al., 1995).

These studies provide evidence that DFO can be employed against certain solid tumors. Prior to these reports, however, there was an earlier case report about the use of DFO in combination with ARA-C in the treatment of acute lymphoblastic leukemia (Estrov et al., 1987). That report, like our pre-clinical studies, suggests that DFO and/or HES-DFO may also be to play a role in the treatment of hematopoietic neoplasms. It is thus of interest to note that the Pediatric Oncology Group in the United States is now sponsoring an open trial for the evaluation of DFO in the treatment of recurrent T cell ALL (C. Frantz, University of Maryland, personal communication).

The mechanism of action of DFO, and thus the rationale for its use, is understood in part. DFO is a highly specific ferric iron chelator that enters cells by a passive mechanism and binds iron in labile pools (Keberle, 1964; Meyer-Brunot and Keberle, 1967; Lloyd et al., 1991). It seems reasonably clear that, at limiting doses, a principal effect of DFO treatment is to inhibit ribonucleotide reductase activity (Lederman et al., 1984) by virtue of reducing the amount of iron available to support the continuous regeneration of the free radical in the M2 subunit (Atkin et al., 1973; Thelander et al., 1983). It is also the case that relatively severe iron deprivation can interfere with mitochondrial electron transport function (Wharton et al., 1988). It should not be surprising to learn, however, that recent studies have suggested that the effects of DFO are potentially more complex than first anticipated. Thus, in addition to the fact that DFO is well known for its capacity to alter post-transcriptional regulation of the transferrin receptor and ferritin genes (Klausner et al., 1993), it has recently become apparent that DFO is also capable of modulating the transcription of several genes, including erythropoietin (Gleadle et al., 1995) and inducible nitric oxide synthase (Weiss et al., 1994). In any event, there is increasing reason to believe that acute iron deprivation is both cytostatic and cytotoxic for some tumors and that the latter effect is manifested by increased levels of apoptotic cell death (Fukuchi et al., 1994; Hileti et al., 1995; Ul-Haq et al., 1995). The fact that DFO induced iron deprivation is capable of inducing apoptosis in some tumors clearly strengthens the rationale for further investigation of DFO as an antineoplastic agent.

The broader context of iron deprivation: the status of gallium

A discussion of the broader context of iron deprivation treatment of cancer must include, and indeed at this point in time might well begin with, the metal gallium. Gallium, as usually given in the form of gallium nitrate, is known to be bound to transferrin and, once taken inside the cell, is thought to interfere with endosomal acidification (Chitambar and Seligman, 1986). Since the release of ferric iron from transferrin is dependent upon such acidification, it is therefore believed that this is the principal mechanism by which gallium interferes with iron uptake. Early screening studies showed that gallium had single agent activity against both solid tumors and hematopoietic neoplasms (Foster et al., 1986) and recent work has revealed that gallium is active against transitional cell carcinoma when given by intravenous infusion (Seligman and Crawford, 1991; Einhorn et al., 1994). Most recently,

gallium has been shown to have activity against refractory lymphoma when used in conjunction with hydroxyurea (Chitambar et al., 1996). Gallium is now being evaluated for activity in CNS neoplasms as a result of preclinical work which showed that it inhibited the growth of medulloblastoma in nude mice (Whelan et al., 1994a,b).

Questions for future investigation

At this point, it seems reasonable to predict that interest in iron deprivation treatment of cancer will continue to grow. It is therefore worth considering what questions will serve as the focal points for future investigations. One such question is this: In what ways can iron deprivation treatment be integrated with other established treatment modalities so as to produce maximal therapeutic benefit with minimal toxic side effects? While the existing studies provide some clues; i.e., DFO can be utilized with cyclophosphamide, etoposide, carboplatin, and thiotepa (Donfrancesco et al., 1993) or with alpha interferon, adriamycin, tamoxifen, and ascorbic acid (Kountouras et al., 1995) and gallium can be used with hydroxyurea (Chitambar et al., 1996), the actual basis for predicting what combinations might work against which tumors is clearly inadequate. This is because we do not yet have a complete understanding of how DFO, gallium, and ATRAS inhibit growth and induce apoptosis in cells (Fukuchi et al., 1994; Ul-Haq et al., 1995; Hileti et al., 1995). Should the inquiry begin with the assumption that dysfunctional DNA synthesis due to the inhibition of ribonucleotide reductase (Lederman et al., 1984) is the most important point of attack or should one give equal or greater attention to the possibility of pre-S phase inhibition of cyclin A protein synthesis (Lucas et al., 1995)?

A second question is as follows: In what ways can existing iron deprivation treatments be used together, either in combination or in sequence, to promote efficacy and minimize toxicity? The studies from our laboratory that have already been discussed provide some insight into both the potential benefits and the potential problems associated with the combined use of HES-DFO and ATRAS (Kemp et al., 1995). Since the existing clinical data indicate that neither HES-DFO nor an ATRA, when given singly, produce any significant toxic effects (Drs. Bo Hedlund and Ray Taetle, personal communications), and further since animal and human toxicities are often very different, consideration is now being given to a carefully constructed Phase I dose escalation trial of combination treatment with HES-DFO and IgG ATRAS (Dr. Ray Taetle, personal communication). Another potential approach is to give DFO, or possibly HES-DFO, prior to the administration of gallium. They have to be given in sequence because DFO chelates gallium and antagonizes its effect. Tissue culture experiments have already suggested that a sequential DFO-gallium approach produces an enhanced anti-tumor effect when either transitional cell carcinoma (Seligman et al., 1993) or breast carcinoma (Wang et al., 1996) is the experimental target and early clinical trials of sequential DFO-gallium treatment are now being undertaken for prostate cancer (R. Dreicer, personal communication). It may be unproductive to give gallium and ATRAS simultaneously since antibody mediated down regulation of the transferrin receptor (Kemp et al., 1990) is likely to interfere with transferrin-mediated gallium uptake, but sequential administration seems feasible, at least in principle.

A third question may be: What new types of iron deprivation strategies will become available for use in the near future and how might they be employed in cancer treatment? In this regard, research continues on oral iron chelating agents such as L1 (Kontoghiorghes, 1995) and additional recent work has been focused on variant forms of the iron chelating compound pyridoxal isonicotinoyl hydrazone (PIH). The PIH variants have shown significant anti-tumor effects in vitro and can also apparently bind and deliver gallium to cells (Richardson et al., 1995).

Finally, a fourth question is this: Do we really understand enough about normal cell biology to suppose that we are addressing all of the physiologically significant pathways of iron uptake? Thus, for example, is the iron binding capacity of the p97 molecule (Kennard et al., 1995) physiologically important in those tumors that express it? In addition, given the steady interest in the process, or processes, of transferrin independent iron uptake in vitro (Wright and Lake, 1990; Kaplan et al., 1991; Seligman et al., 1991; Qian and Morgan, 1991; Qian and Eaton, 1991; Inman and Wessling-Resnick, 1993; Olakanmi et al., 1994; Conrad et al., 1994; Thorstensen et al., 1995), to what extent might further analysis of the molecular basis of such phenomena provide new opportunities for investigating iron deprivation treatment of cancer in vivo? As in generally the case in science, there are more questions than answers and much work to be done!

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