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Scott Wadler · Edward L. Schwartz

Biologic agents as biochemical modulators: pharmacologic basis for the interaction of cytotoxic chemotherapeutic drugs and interferon

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Abstract Biochemical modulation of cytotoxic cancer chemotherapeutic agents is one means of enhancing the activity and selectivity of antitumor drugs. Traditionally this approach has utilized detailed information regarding a particular enzymatic reaction or biochemical pathway to develop potential modulating agents. In contrast, the reported clinical therapeutic activity of IFN in combination with cytotoxic agents has prompted a reexamination of the biochemical actions of the cytokine. Interferon elicits a number of cellular actions that might contribute to its pharmacologic activity, including both direct antitumor effects and host-mediated actions. The best understood are those related to the cytotoxicity of the fluoropyrimidine antimetabolites and include enzymatic reactions involved in fluoropyrimidine metabolic activation, catabolism, and interaction with its target enzyme. However, even in this instance, a mechanistic association of a specific pharmacologic action with therapeutic activity remains to be determined. These studies demonstrate that cytokines and other biologic agents may exert specific biochemical modulations that augment (or potentially attenuate) the activity of the cytotoxic chemotherapeutic agents.

Key words Biochemical modulators · Chemotherapeutic drugs · Interferon

Introduction

The success of a chemotherapeutic regimen for cancer is a function of the sensitivity of the tumor and the selectivity of the cytotoxic agents for the target cell. Since selectivity in general depends upon quantitative differences between malignant and normal cells, modulation of the cytotoxic activity by biochemical means is a potentially useful approach for the design of drug combinations. Experimental studies of biochemical modulators of cytotoxic anticancer drugs have most commonly utilized the antimetabolites, although modulation of the activity of alkylating agents, DNA-repair enzymes, and multidrug-resistance pathways have also been the focus of investigations. The translation of theoretical and experimental studies of biochemical modulators into clinical practice has been slow, however. The reduced folate leucovorin (LV) is probably the best example of a biochemical modulator that is widely used in clinical practice. LV has been used to increase selectivity and "rescue" normal cells from high doses of methotrexate and, more recently, to potentiate the binding of the active metabolite of 5-fluorouracil (5-FU) to its target site in the tumor cell. Modulation of 5-FU activity by LV is a commonly employed strategy in the design of therapy for advanced lesions of the colon and rectum and has been tested in the treatment of head and neck and breast cancers. Modulation of 5-FU activity with other agents such as *N*-(phosphonacetyl)-L-aspartate (PALA) and methotrexate has been studied clinically as well. The methotrexate and 5-FU combination is an example of the use of two agents that individually possess cytotoxic activity, whose use in a sequence-dependent manner would be predicted on the basis of biochemical considerations to have a greater than additive effect.

The rationale for employing biochemical modulation as a clinical strategy assumes a detailed knowledge of the specific biochemical pathways in the tumor cell and predictions based on in vitro observations about how modulation of these pathways will affect the actions of the cytotoxic drug at the cellular level. For example, the assumption that supplementation of cellular pools of reduced folate would augment the clinical efficacy of 5-FU was derived from an understanding of several complex biochemical processes that were studied *in vitro*: the multistep and divergent pathways by that 5-FU can be

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S. Wadler (✉) · E. L. Schwartz
Albert Einstein Cancer Center and Department of Oncology, Montefiore Medical Center, 111 East 210th Street, Bronx, NY 10467, USA

anabolized to its active metabolite fluorodeoxyuridylate (FdUMP), the cellular levels of the endogenous substrate deoxyuridine monophosphate (dUMP), the complex mechanism of binding of FdUMP to its target enzyme thymidylate synthase and the role of the stabilization of this complex by the reduced folate N^5,N^{10}-methylene tetrahydrofolate.

Clinically, the outcome of a biochemical modulating regimen will be influenced by additional complexities, including pharmacokinetic considerations of both the cytotoxic drug and the modulating agent at the systemic and cellular levels and, where appropriate, cell cycle kinetic considerations that may affect the metabolic pathways involved. Strategies to employ a variety of biochemical modulators in the clinic have previously been reviewed [35, 114]. Although the clinical outcome of a particular regimen utilizing a biochemical modulator may be difficult to predict from in vitro studies, computer simulations of enzyme-inhibitor combinations suggest that relatively small changes in the activity of key enzymes would be sufficient to change a drug interaction from antagonistic to synergistic [46]. Furthermore, the same combination can be synergistic in one cell type (e.g., the tumor cell) but not in a second cell type (e.g., the normal cell) [46], lending support to the concept of selective biochemical modulation.

In contrast to the classic modulating agents that are incorporated into drug combinations for their putative effect on a specific biochemical locus, biologic agents have pleiotropic cellular actions and have been studied clinically with only a minimal understanding of their biochemical actions. There is evidence, however, that biologic agents may in fact function in a manner analogous to that of biochemical modulating agents, and their multiple cellular effects could contribute to their overall efficacy in combination chemotherapy. Using IFN as an example, we review the biochemical and pharmacologic basis for chemo/biotherapeutic combinations for the treatment of malignancies.

Interferon in combination chemotherapy

Interferon (IFN) has been shown to have additive and synergistic effects on a broad range of anticancer agents, including alkylating agents, antimetabolites, anthracycline antibiotics, and drugs that interact with microtubules (reviewed in [106]). These investigations include studies in cell culture, animal tumors, and human xenografts. Historically, biologic agents such as IFN were presumed to be immunopotentiating agents, whose role was to complement the action of cytotoxic agents. One hypothesis, for example, supposed that a biologic agent, with its greater degree of immunologic specificity but weaker cytotoxic action, could be employed to remove residual tumor cells not eradicated by the more potent cytotoxic agents [36]. Although the immunologic actions of IFN have been documented, the limited studies reported have not resolved the significance of these actions to the potentiating effect of IFN in combination chemotherapy (see [106]). IFN has well-documented antiviral and negative growth-regulatory properties against a variety of experimental tumor models. The mechanisms for the antiproliferative actions of IFN remain to be determined, and it is not clear whether these or other actions of IFN will be the primary basis for the IFNs' interaction with cytotoxic drugs.

Mechanisms of action of IFNs

The classification and biochemical actions of the IFN families have previously been described (reviewed in [60]), and whereas the classes of IFN differ in their precise mode of action, certain characteristics are common to all of them. IFN regulates the expression of specific IFN-inducible or -suppressible genes with a corresponding increase or decrease in expression of their mRNAs and an up- or down-regulation of the corresponding cellular proteins. Although the spectrum of genes regulated by each family of IFNs is different and partially dependent on the cell type studied, there is a wide overlap in the target genes regulated among the different families of IFNs. The first step in the pharmacologic actions of IFN is the binding of the IFN molecule to a specific receptor on the cell membrane [93]. A change in the rate of transcription of IFN-inducible genes occurs within minutes to hours of IFN binding. The increase in gene transcription correlates with the level of occupancy of IFN receptors on the cell membrane.

A number of studies have begun to characterize the signal transduction mechanism of IFN in various cell systems. Although this mechanism differs among the different families of IFNs, certain similarities persist between the type I (α and β) IFNs and type II IFN (γ), and common pathways have recently been identified for IFNs and other families of cytokines and growth factors [58, 83, 84, 96]. Although some studies have implicated cyclic adenosine monophosphate (AMP) as a second messenger for the antiproliferative but not the antiviral activity of IFN γ in human macrophages [89], others have found that manipulation of second messenger systems does not affect IFN-induced gene transcription [62]. In these studies, protein kinases have been implicated as common elements of signal transduction [50, 62, 81]; recent studies have provided direct evidence that three members of the JAK family of non-receptor kinases (e.g., JAK1, JAK2, and Tyk2) are involved in the IFN-response pathway [73, 103, 113]. These pathways include preformed cytoplasmic proteins that are phosphorylated and thereby activated by IFN binding, then assemble in the cytoplasm and are translocated to the cell nucleus, where they bind to promoter regions to control the transcription of IFN-responsive genes [50, 88].

At least four IFN-responsive transcription regulatory-protein complexes have been identified by DNA-binding gel-shift assays. ISGF3 (IFN-stimulated gene factor) is a multiprotein complex activated by IFN α. It consists of three preformed cytoplasmic proteins that are phosphory-

Table 1 Selected biochemical actions of IFN and potential mechanisms by which they might contribute to the modulating activity of IFN toward cytotoxic drugs

Action	Potential mechanism of interaction	Reference
Inhibition of thymidine utilization	Interaction with pyrimidine antimetabolites	[15, 31, 63]
Inhibition of dihydropyrimidine dehydrogenase activity	Reduced catabolism of fluoropyrimidines	[40, 115]
Induction of thymidine phosphorylase activity	Potentiation of metabolic activation of fluoropyrimidines	[91, 92]
Induction of double-stranded RNA-activated protein kinase activity	Possible tumor-suppressor gene	[54, 66]
Modulation of thymidylate synthase activity	Interaction with pyrimidine antimetabolites	[10, 51]
Modulation of DNA damage and repair	Augmentation of drug-induced cell death	[43]
Inhibition of angiogenesis	Restriction of tumor growth	[25, 26]

lated in response to IFN. The activated ISGF3 translocates to the nucleus, where it binds a fourth protein to form a tight DNA-binding complex [29, 50, 88, 94]. ISGF2 is also known as IRF-1 (IFN-regulatory factor), is induced by IFN or virus treatment, and activates both IFN and IFN-responsive genes, although it is not the sole activator of these genes [68]. IRF-2 is also induced by IFN, but only after the induction of IRF-1; furthermore, IRF-2 appears to repress the expression of IFN-induced genes [27]. Overexpression of IRF-2 in NIH 3T3 cells caused the cells to become transformed and increased their tumorigenicity in nude mice; the transformed phenotype was reversed by coexpression of the IRF-1 gene [39]. ICSBP (IFN consensus sequence-binding protein) has structural similarities to the IRF genes, may be induced only in response to IFN γ, and also appears to repress the expression of IFN-induced genes [74].

The promoter elements of IFN-responsive genes have been characterized and found to bind the IFN-activated protein complexes [38]. The IFN α-generated complex binds to a *cis*-acting consensus sequence called the IFN-stimulated response element (ISRE) [28, 61]. Other elements have been identified, including those showing partial overlap with the ISRE, those possessing negative and positive regulatory domains, and those that bind factors activated by IFNs α and γ [67, 97].

IFN-induced genes

Various mRNAs are induced by IFNs that are translated into IFN-inducible proteins. Over 30 IFN-inducible proteins have been described, including 2′-5′-oligo adenylate synthetase, major histocompatibility complex (MHC) class-I and -II proteins, and a recently described protein (p27) found to be overexpressed in breast carcinoma [80]. Others are likely to be identified as different cell types and tumors are studied. The majority of the known IFN-induced genes have not been characterized, and their functions are unknown. Furthermore, the full implications of IFN induction of any of these proteins is unknown, and their role in potentiating the activity of chemotherapeutic agents is unclear. In studies in patients with chronic myelogenous leukemia, induction of four IFN-stimulated genes was observed in vivo in patients with both IFN-sensitive and -resistant disease [101]. Clearly, only a subset of the IFN-induced changes in gene expression is likely to be relevant to a particular cellular action of IFN.

Specific biochemical actions of IFN with potential relevance to its modulation of the activity of cytotoxic drugs

A number of biochemical activities whose modulation by IFN may contribute to the antitumor activity and/or chemotherapy-potentiating activity have been identified (Table 1).

Double-stranded RNA-activated protein kinase

Double-stranded RNA-activated protein kinase (dsRNA-PK, also known as P1/eIF2 kinase and human p68 kinase) is induced by IFN. The enzyme is activated by double-stranded RNA and stem-loop structures in single-stranded RNA. The enzyme has two kinase activities upon activation by RNA: autophosphorylation and subsequent phosphorylation of the α subunit of eukaryotic translation initiation-factor eIF2, that cause inhibition of protein synthesis. The latter property is thought to play a major role in the antiviral actions of IFN. Recent transfection studies using wild-type and mutant dsRNA-PK suggest that it may also function as a tumor suppressor gene [54, 66]; the relevance of this observation to the antiproliferative and potentiating activities of IFN are not known.

Thymidine utilization

Thymidine utilization is inhibited by IFN via actions on both thymidine transport and decreases in thymidine kinase activity in treated cells [15, 31, 63]. These actions appear not to be sufficient to account for either the antiproliferative actions of IFN or its ability to enhance the cytotoxicity of 5-FU; however, they could conceivably contribute to the biochemical modulatory activity of IFN on antimetabolites. Studies done in the absence of thymidine in the medium or with thymidine kinase-deficient variants suggest, however, that changes in thymidine utilization are not involved in the potentiating effect of IFN on 5-FU cytotoxicity [42].

Thymidine phosphorylase

Thymidine phosphorylase (TP) activity is induced up to 10-fold by IFN in colon and breast carcinoma cells in vitro and in peripheral blood mononuclear cells in patients being treated with IFN [91, 92]. Enzyme-activity levels of TP vary widely in different tissues and have been found to be elevated in human tumor samples as compared with surrounding normal parenchymal tissue [117]. The enzyme catalyzes the reversible conversion of thymine to thymidine. 5-FU is also a substrate for the enzyme, being converted to 5-fluorodeoxyuridine (5-FdUrd), the first step in one pathway for the metabolic activation of 5-FU. The antitumor activity of 5′-deoxy-5-fluorouridine [17] is also potentiated by IFN under conditions in that TP activity is induced, as this nucleoside must first be converted to the free base before it can exert its cytotoxic activity.

Dihydropyrimidine dehydrogenase

Dihydropyrimidine dehydrogenase is responsible for the catabolism of 5-FU in vivo [40], and has been found to be decreased in cells from patients treated with IFN [115]. Patients treated with IFN have elevated plasma levels of 5-FU, and the magnitude of changes in 5-FU catabolism observed during IFN treatment in the cells was similar to the magnitude of decrease in the clearance of 5-FU seen in other studies [33]. The relative contributions of host-mediated effects of IFN on metabolism versus direct antitumor actions remain to be determined.

Thymidylate synthase

Thymidylate synthase (TS) is required for the de novo synthesis of thymidylate, and cellular levels of the protein are subject to transcriptional and posttranscriptional regulation, including autoregulation of TS mRNA translation by TS protein [11]. Treatment of cells with 5-FU, that binds to TS and inhibits its activity, leads to the elevation of cellular TS in some cell lines, presumably due to the reversal of the inhibitory effect of TS protein on its own translation [10]. The mRNA-specific negative feedback control mechanism has been postulated to be a change in the conformation of TS, that results in enhancement of TS binding to its own mRNA [51]. When used in combination with 5-FU, IFN γ prevented the elevation in TS levels seen with 5-FU alone [10]; in other studies, this effect was not seen with IFN α [43]. The mechanism for the IFN γ action is unknown but could be related to the aforementioned effect of IFN on translation.

DNA damage and repair

DNA damage and repair play an integral role in the pharmacologic actions of the majority of antitumor agents used clinically. DNA damage is usually measured as single- and double-stranded DNA breaks, that may be a consequence of the misincorporation of a nucleoside analog into the DNA, misrepair of the incorporated fraudulent base, or other drug effects on the repair machinery. A study suggests that IFN increased the amount of single- and double-strand breaks detected in 5-FU-treated cells [43]. It was not determined in this study whether the effect of IFN could be generalized to other agents that damage DNA or whether it was specific to 5-FU (i.e., due to effects on 5-FU incorporation or excision). In other studies, IFN was found to inhibit the activity of DNA polymerase-α, although the relevance of this observation to DNA repair remains to be determined [102].

Cytochrome P450 and drug-metabolizing enzymes

Cytochrome P450 and drug-metabolizing enzymes are responsible for the activation and inactivation of many cancer chemotherapeutic agents. IFN has been shown to inhibit the activity and reduce the levels of microsomal cytochrome P-450, cytochrome P-450 reductase, cytochrome b_5, and cytoplasmic glutathione S-transferase [70]. Treatment with the IFN inducer poly I:poly C resulted in a decrease in mRNA for P-450 1A1, 1A2, 2C11, and 2E1 [85], and in an experimental mouse tumor model, murine IFN both elevated and depressed various microsomal enzymes [2].

Inhibition of angiogenesis

Inhibition of angiogenesis can severely restrict tumor growth in experimental systems [26]. Partly on the basis of evidence that the IFNs could inhibit capillary endothelial cell migration and lymphocyte-induced angiogenesis [6, 95], the cytokine became the first angiogenesis inhibitor to reach clinical trials. It has recently been reported that IFN α is active in the treatment of life-threatening pediatric hemangiomas [25]. It remains to be seen if this activity of the IFNs contributes to the activity they exert against neoplastic tumors when given in combination with cytotoxic agents. Of interest are recent reports that the IFN-induced gene TP appears to be identical to the putative angiogenic factor platelet-derived endothelial-cell growth factor (PD-ECGF) [30, 69].

Antitumor effects of IFN in combination with chemotherapeutic agents

An extensive number of studies have demonstrated synergistic cytotoxicity for combinations of chemotherapeutic drugs and IFN, although there have been substantially fewer studies that have identified specific cellular targets or mechanisms that might explain the synergistic interactions observed in vitro or in vivo. We summarize below

those studies in that information on the nature of potential biochemical interactions has been reported.

IFN and 5-FU

The most extensive studies on the interaction of IFN with a cytotoxic agent have involved 5-FU [109]. At the cellular level, 5-FU is anabolized in a sequential fashion to three active forms: 5-fluorouridine triphosphate (FUTP), 5-fluorodeoxyuridine triphosphate (FdUTP) and 5-fluorodeoxyuridylate (FdUMP). The latter binds covalently to the enzyme TS in the presence of reduced folate to form a ternary complex that blocks the binding of the endogenous substrate deoxyuridine monophosphate (dUMP), resulting in the inhibition of deoxythymidine monophosphate (dTMP) synthesis. As discussed above, IFN induced the activity of the 5-FU-anabolic enzyme TP, and this increase was accompanied by an elevation in the cellular levels of FdUMP in colon carcinoma cells [91]. The total cellular incorporation of 5-FU and other 5-FU anabolites was not affected, suggesting that there was some specificity in the induction of this enzyme. Similarly, studies using ^{19}F magnetic resonance spectroscopy for noninvasive monitoring of 5-FU and its metabolites suggest that IFN increased the formation of active metabolites of 5-FU in liver metastases in patients being treated with 5-FU and IFN [23].

In addition to augmented FdUMP levels, the addition of IFN to 5-FU may also result in direct effects on TS activity. In intact HL-60 cells, treatment with IFN resulted in impaired TS activity on days 1–4 of cell culture and increased the sensitivity of the cells to inhibition of TS by 5-FU [19, 20]. In H630 and GC_3/cl human colon cancer cells, in contrast, treatment with IFN had no direct effect on TS levels or catalytic activity [10, 43]. As described above, however, in the H630 cells, IFN-γ almost completely abrogated the rise in TS levels resulting from 5-FU treatment [10].

Although TS may be a target either directly or indirectly for IFN action, evidence suggests that IFN-induced enhancement of 5-FU inhibition of TS alone is inadequate to explain the cytotoxic interaction. For example, treatment with IFN failed to augment the cytotoxicity of the quinazoline-based TS inhibitor CB3717 in vitro [41]. Other loci of 5-FU/IFN interaction have been identified. For example, IFN treatment augmented the 5-FU-induced depletion of pools of deoxythymidine triphosphate (dTTP) in two human colon-cancer cell lines (personal communication), enhanced the formation of 5-FU-induced DNA single-strand breaks [43], and reduced the clearance of 5-FU in patients being treated with the combination [33]. Also of interest was the observation that 5-FU increased the sensitivity of tumor cells to natural killer cells that had been activated by IFN [82]. Hence, there is evidence for both direct interactions of 5-FU and IFN on the tumor cell and indirect pharmacokinetic and immunomodulatory host effects [59, 72].

IFN and doxorubicin

In vivo studies in immunoincompetent mice demonstrated greater-than-additive activity for the combination of IFN and doxorubicin against explanted human breast cancers [1]. One potential source of this interaction is at the level of the hepatic microsomal enzymes, that metabolize doxorubicin to both inactive and clinically active compounds. Although murine IFN both elevated and depressed various microsomal enzymes in an experimental mouse tumor model, there was no effect on the antitumor action of doxorubicin [1]. In the same murine system, human IFN (that had no effect on the murine P-450 system) in combination with doxorubicin was more effective than doxorubicin alone, providing evidence that the synergy exhibited by the combination was likely due to a direct antitumor action. A recent clinical trial has demonstrated the absence of pharmacokinetic effects of IFN on doxorubicin. Among patients treated with the combination of cyclophosphamide, 5-FU, doxorubicin, and IFN α (1–2 MU/m^2), no effect was seen on the peak concentration, half-life, area under the curve, or clearance of doxorubicin [100].

A second putative cellular target for IFN action in doxorubicin-sensitive cells is the multidrug-resistance-related membrane protein P-glycoprotein (PGP). In LoVo/dx, human colon cancer cells made resistant to doxorubicin, levels of PGP are increased and accumulation of doxorubicin is decreased. Pretreatment of these cells with IFN α resulted in an increase in the cytotoxic effects of doxorubicin and an increase in the cellular accumulation of doxorubicin in the absence in a change in the expression of PGP as measured by flow cytometry using the monoclonal antibody MRK16 [87], supporting a role for IFN α as a modulator of the multidrug-resistance phenotype.

IFN and alkylating agents

Early experiments in an immunoincompetent mouse model demonstrated a greater-than-additive anticancer effect for the combination of IFN and cyclophosphamide [1] or ifosfamide [22]. A study in CDF_1 mice implanted with the murine leukemia P388 demonstrated exquisite schedule dependency for the interaction of IFN and cyclophosphamide against these tumors [5]. As the cytochrome P-450 system plays an essential role in the anabolism of cyclophosphamide and as IFN down-regulates this system (see above), the locus of interaction of these agents is unclear. In a mouse model, treatment with either IFN or the IFN inducer poly I:poly C resulted in decreased clearance of cyclophosphamide and a prolonged half-life for the drug [45]. In the same system the rate of formation of activated cyclophosphamide was delayed and peak levels of activated cyclophosphamide were lower than those in controls, reflecting a 29%–37% decrease in oxidation of the drug by hepatic microsomal enzymes. A single report examining the effects of IFN on melphalan pharmacokinetics in ten patients with multiple myeloma demonstrated a decrease in

plasma levels of drug and area under the curve when IFN and melphalan were employed in combination [18].

Potential interactions of IFN and alkylating agents gained considerable importance following reports that IFN enhanced the clinical effects of alkylating-agent-based chemotherapy regimens in patients with non-Hodgkin's lymphoma. In a large trial conducted by the Eastern Cooperative Oncology Group (ECOG), the addition of IFN to CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) resulted in an increase in the time to treatment failure and the duration of complete response despite the administration of IFN as sequential rather than concomitant therapy [98]. Preliminary reports from two European trials confirm that IFN may enhance the disease-free survival and overall survival of chemotherapy in patients with high tumor-burden follicular lymphoma and stage III and IV low-grade malignant non-Hodgkin's lymphoma [37, 99]. The ECOG trial and the European studies differed in the doses of chemotherapy employed, in the schedule on that IFN was given (sequential in the ECOG trial and concomitant in the European trial), and in the inclusion of patients with follicular large-cell lymphoma in one trial. Thus, a rigorous comparison of these trials is difficult. It is encouraging that in these somewhat disparate populations, IFN enhanced the benefits of chemotherapy. The precise mechanism of interaction likely differs in these different trials, raising additional questions about how best to employ biologic agents in combination with chemotherapeutic drugs.

Clinical implications

Early clinical trials of IFN and cytotoxic drugs were initiated in the 1980s largely on the basis of encouraging in vivo data for combinations of IFN plus doxorubicin or cisplatin against explanted human tumor xenografts in immunodeficient mice [1]. Enthusiasm for IFN/cytotoxic combinations diminished following the completion of phase I trials, which demonstrated excessive toxicities [32, 86, 112]. More recent trials combining IFN with 5-FU, for example, have demonstrated clinical activity for the combination with acceptable toxicities. A summary of recent and ongoing clinical trials employing IFN in combination with chemotherapeutic agents in selected disease sites is presented in Table 2.

Table 2 Clinical trials of chemotherapeutic agents + IFN by disease site. (*FU* 5-Fluorouracil, *LV* leucovorin, *DTIC* dacarbazine, *ORR* objective response rate, *PK* pharmacokinetic, *RT* radiation therapy, *CDDP* cisplatin, *Dox* doxorubicin, *PALA N*-(phosphonacetyl)-L-aspartate, *CY* cyclophosphamide, *HU* hydroxyurea, *CML* chronic myelogenous leukemia)

Disease site	Stage	Phase	IFN with	Other agent	Comments	References
Colorectal	Advanced	II	FU	–	Several studies; overall response rate, 26%–63%	[14, 16, 27, 44, 49, 75, 107, 108, 110]
	Advanced	III	FU	–	3 studies; FU+IFN = FU+LV FU+IFN = FU	[24, 52, 116]
	Adjuvant	III	FU	LV	In progress	–
	PK	I	FU	–	PK advantage for FU+IFN vs FU alone in some studies	[12, 33, 64, 76, 90, 100]
	Advanced	I–II	FU	LV	Equivocal as compared with FU+LV (ORR, 6%–53%)	[3, 8, 34, 53, 55, 56, 71, 77, 79]
Esophagus	Advanced	II	FU	–	2 studies; ORR, 25%–27%	[48, 111]
	Advanced	II	FU	RT	In progress	–
	Advanced	II	FU	CDDP	In progress	–
Melanoma	Advanced	III	DTIC	–	DTIC+IFN; survival better vs DTIC alone	[21]
Head and neck	Advanced	II	FU	–	No benefit	[105]
	Advanced	II	CDDP	–	Possible benefit	[104]
Lymphoma (follicular)	Advanced	III	CY/Dox	–	IFN improved survival, increased time to failure	[98, 99]
Myeloma	Advanced	III	CY	–	IFN maintenance improved survival?	[78]
Bladder	Advanced	II	FU	CDDP	in progress	[65]
Glioma	Local	II	FU	–	Possible activity	[7]
Pancreas	Advanced	II	FU	–	No activity	[13]
	Advanced	II	FU	LV	Possible activity	[4]
Gastric	Advanced	II	FU	PALA	In progress	–
	Advanced	II	FU	LV	Possible activity	[47]
Ovary	Local	II	CDDP	–	Treatment i.p.; in progress	–
CML	Advanced	II	HU	–	Possible activity	[101]

The combination of 5-FU plus IFN has appeared promising in the treatment of advanced colorectal cancer in eight phase II trials employing intermediate-dose 5-FU and IFN. Among 202 patients treated on these trials, there have been 76 responders (38%) [14, 16, 27, 44, 49, 75, 107, 110]. The results of phase III trials testing the combination of 5-FU plus IFN versus 5-FU plus LV or single-agent 5-FU have been less promising. In a multicenter trial conducted primarily in Europe, the combination of 5-FU plus IFN was no better than 5-FU employed alone in patients with advanced colorectal carcinoma [52]. In a second multicenter study conducted primarily in the United States, the combination of 5-FU plus IFN was not substantially better than treatment with 5-FU plus leucovorin (LV), although the toxicity patterns were different [116]. Several pharmacokinetics studies have been completed that demonstrated a pharmacokinetic advantage for the addition of IFN to 5-FU, that resulted in decreased clearance of 5-FU and higher plasma levels [12, 33, 90]. The role of adjuvant therapy with 5-FU/LV plus IFN versus 5-FU/LV alone is currently being tested by the National Surgical Adjuvant Breast and Bowel Project.

Another promising area for combinations of IFNs with cytotoxic agents has involved the treatment of follicular lymphomas. Following several encouraging preliminary trials, two large phase III trials conducted by the ECOG [98] and a multiinstitutional French consortium [99] have demonstrated a benefit in time to failure and/or in overall survival for combination therapy with IFN. A third area of interest is the combination of dacarbazine (DTIC) plus IFN in advanced melanoma. Although both drugs have only modest activity against metastatic melanoma, in a study from South Africa, the combination of agents resulted in an improvement in the objective response, complete response and overall survival with acceptable toxicities [21]. Finally, several preliminary trials employing IFN in combination with one or more cytotoxic agents have reported promising responses in esophageal cancer with 5-FU [48, 111] and in squamous-cell carcinoma of the head and neck with cisplatin (D. Vlock, personal communication).

Conclusions

Research on the interaction of IFN with specific cytotoxic agents has evolved from a series of observations demonstrating the synergistic interaction of these drugs to a description of mechanisms by that these interactions might occur at the cellular level. In most instances these data are preliminary, but they provide further hypotheses for testing. In the case of IFN and 5-FU, detailed biochemical information is available that may explain the synergy observed for these agents at the cellular level. With more detailed knowledge of the signal-transduction pathways of IFN and other cellular effects of IFN, studies of IFN/anticancer drug interactions at the molecular level may be more fruitful. For example, this review does not address the evidence that IFN can affect the expression of oncogenes, the phosphorylation of cell-cycle regulatory proteins, the transition of cells from the G_0 to the G_1 phase, or the induction of cell differentiation pathways, all of which represent potential therapeutic targets. Furthermore, as clinical trials employing IFN and cytotoxic agents progress and additional data become available regarding the clinical benefits of combination therapy, more detailed explanations of the mechanisms by which IFN and cytotoxic agents interact will be useful, possibly essential, for the design of the next generation of combination regimens.

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