

Down-regulation of heat shock protein 70 improves arsenic trioxide and 17-DMAG effects on constitutive signal transducer and activator of transcription 3 activity

Sampa Ghoshal · Indranil Rao · Justin C. Earp ·
William J. Jusko · Meir Wetzler

Received: 13 May 2009 / Accepted: 7 December 2009 / Published online: 25 December 2009
© Springer-Verlag 2009

Abstract

Purpose Signal transducer and activator of transcription 3 (STAT3) has been shown to be constitutively active in approximately 50% of patients with acute myeloid leukemia and is associated with worse outcome. Arsenic trioxide (ATO) synergizes with the heat shock protein (HSP) 90 inhibitor, 17-DMAG, to down-regulate STAT3 activity. However, both agents up-regulate HSP70, an anti-apoptotic protein. We therefore examined whether down-regulating HSP70 with short interference (si) RNA will affect ATO and 17-DMAG effects on constitutive STAT3 activity.

Experimental design A semi-mechanistic pharmacodynamic model was used to characterize concentration–effect relationships of ATO and 17-DMAG effects on constitutive STAT3 activity and HSP70 expression with or without siRNA against HSP70 in a cell line model.

Results Treatment with siRNA for HSP70 resulted in a stronger degree of synergism on down-regulation of

STAT3 activity by ATO and 17-DMAG. However, treatment with siRNA for HSP70 resulted in less synergism on up-regulation of HSP70 by the two drugs.

Conclusions Down-regulation of HSP70 improves ATO and 17-DMAG effects on constitutive STAT3 activity. These results further provide a basis for studying the combined role of ATO with a HSP90 inhibitor such as 17-DMAG in AML with constitutive STAT3 activity.

Keywords Pharmacodynamic modeling · Heat shock protein 70 · Heat shock protein 90 · Arsenic trioxide · Signal transducer and activator of transcription 3

Introduction

Signal transducer and activator of transcription 3 (STAT3) has been shown to be constitutively active in approximately 50% of acute myeloid leukemia (AML) cases and to correlate with adverse treatment outcome [1]. We have shown that arsenic trioxide (ATO) down-regulates constitutive STAT3 activity in AML cells within 6 h, without affecting cell survival until 48 h [2]. Since heat shock protein (HSP) 90 is implicated in maintaining the conformation, stability and function of key proteins involved in signal transduction pathways, we demonstrated that the different HSP90 inhibitors [geldanamycin, 17-allylamino-17-demethoxygeldanamycin (17-AAG), and 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (NSC 707545, 17-DMAG)] augment ATO's down-regulating effect on constitutive STAT3 (P-STAT3) [3]. Since 17-AAG has poor solubility, the water soluble derivative, 17-DMAG, which is more biologically available, was tested in the current study.

Sampa Ghoshal and Indranil Rao contributed equally to this work.

S. Ghoshal · M. Wetzler (✉)
Department of Medicine, Roswell Park Cancer Institute,
Leukemia Section, Elm and Carlton Streets,
Buffalo, NY 14263, USA
e-mail: meir.wetzler@roswellpark.org

I. Rao · J. C. Earp · W. J. Jusko
Department of Pharmaceutical Sciences,
State University of New York at Buffalo,
Buffalo, NY, USA

Present Address:

J. C. Earp
Pharmacometrics, Office of Clinical Pharmacology,
Food and Drug Administration, Silver Spring, MD, USA

Both ATO and the HSP90 inhibitors up-regulate HSP70, a protein known to inhibit apoptosis [4, 5]. We therefore asked whether down-regulating HSP70 with short interference (si) RNA would affect ATO and 17-DMAG effects on constitutive STAT3 activity.

When targeting HSP70, we had to consider all members of this chaperone protein [6]. The HSP70 family includes at least eight members with diverse biochemical functions including nascent protein folding, preventing denatured protein aggregation and modulating assembly and disassembly of protein complexes [6]. There are six cytosolic HSP70 proteins; of these, HSC70 or HSP70-8 (locus HSPA8, position 11q23.3-q25) is ubiquitously expressed in all cells. HSP70t (locus HSPA1L, position 6p21.3) and HSP70-2 (locus HSPA2, position 14q24.1) are testis-specific and almost non-detectable in other tissues. HSP70-6 (locus HSPA6, position 1cen-qter) is induced only in extreme stresses. HSP70-1A and HSP70-1B, collectively known as HSP72 (locus HSPA1A and HSPA1B, position 6p21.3), are also induced following extreme stresses. However, constitutive expression of HSP72 was observed in several malignancies [6, 7]. We thus targeted HSP70-1A and HSP70-1B (designated below as HSP70) in this study.

The combined effects of ATO, 17-DMAG and HSP70 siRNA on constitutive STAT3 activity, HSP70 and HSP90 protein concentrations were assessed using the Ariens non-competitive interaction model [8, 9] with an interaction parameter (ψ). This relationship was selected owing to the differences in molecular structure and mechanisms of the test agents. Interaction parameters may be useful in various mechanism-based models to account for synergism or antagonism not predicted by the mechanistic expectations of the modeling scheme [10–12]. The estimated value of this parameter indicates the intensity of the drug–drug interaction when compared with the no-interaction value (i.e., the value that does not influence the underlying mechanistic model, based on single-drug effect alone). The interaction model is not limited to mass-law drug–receptor binding equations, but provides estimates of how much each drug contributes to the interaction after binding to their respective targets. These models were designed to study the effect(s) of down-regulating HSP70 on the known synergistic effect of ATO and 17-DMAG on constitutive STAT3 activity [3]. We therefore incorporated data from our previous study [3] into the current model.

Methods

Materials

All chemicals were purchased from Sigma Immunochemicals (St. Louis, MO) unless otherwise specified. 17-DMAG

was provided by Dr. Percy Ivy, NIH, National Cancer Institute, Bethesda, MD.

Cell line and culture conditions

The AML cell line, HEL, a cytokine-independent human erythroleukemia cell line that has constitutive STAT3 activity, served as a model system. The cells were exposed for 6 h to ATO and 17-DMAG with or without HSP70 siRNA or a mismatch (Dharmacon, Lafayette, CO).

siRNA electroporation

The following custom made siRNAs were used targeting HSPA1A and HSPA1B, 5'-CGACGGAGACAAGCCCAAG-3'. We used a version of HSPA1 siRNA with two mismatches (underlined): 5'-CGACCGGAGACAAGCGCAAG-3' as control. The siRNA was introduced into the cells via electroporation (BTX Electro Cell Manipulator 600, Genetronics, CA). This method was adapted from BTX Protocol No. 576. In every siRNA experiment, an electroporation control with media only was included. Exponentially growing HEL cells were washed in serum-free RPMI 1640 media and resuspended in the same media at a density of 1.2×10^7 cells/200 μ l. The voltage was set to 250 and the capacitance was at 250 μ F; 200 nM siRNA was used. The siRNA dosage was chosen, since in preliminary experiments 200 nM caused >75% down-regulation of HSP70 by western blotting while maintaining cell viability >70% (data not shown). A BTX disposable cuvette (model # 620) with a 2-mm gap was used. In preliminary experiments, HSP70 protein concentrations were measured at 24, 48 and 72 h; the most significant down-regulation was observed at 48 h (Fig. 1a). Therefore, cells were incubated with ATO and 17-DMAG for the last 6 h of the 48-h incubation. Cell viability was determined by the trypan blue dye (Life Technologies, Grand Island, NY) exclusion assay. Pilot studies were conducted to check the viability and growth rates of cells after electroporation; those did not differ from non-electroporated cells.

Reverse transcriptase polymerase chain reaction (RT-PCR)

The RNA was harvested from cell culture with RNeasy mini kit (Qiagen, Valencia, CA). Single stranded cDNA synthesis was made with Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) with oligo dT primers. The cDNA was used as a template in a PCR reaction to amplify different HSP 70s and the housekeeping gene actin. The reaction was performed as previously described [13]. The primers are described in Table 1. The samples

$$E = 100 \times \left[1 - \frac{\left[I_{\max,A} \times \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A} + I_{\max,B} \times \left(\frac{B}{\psi \times IC_{50,B}} \right)^{\gamma_B} + \left(I_{\max,A} + I_{\max,B} - I_{\max,A} \times I_{\max,B} \right) \times \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A} \times \left(\frac{B}{\psi \times IC_{50,B}} \right)^{\gamma_B} \right]}{1 + \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A} + \left(\frac{B}{\psi \times IC_{50,B}} \right)^{\gamma_B} + \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A} \times \left(\frac{B}{\psi \times IC_{50,B}} \right)^{\gamma_B}} \right] \quad (1)$$

Symbol A refers to the concentration of ATO and B refers to 17-DMAG and $I_{\max}$ is the fraction which represents the maximal capacity by which drug A or B can inhibit constitutive STAT3 activity when present alone. When $I_{\max} = 0$, it indicates no possible inhibition and when $I_{\max} = 1$, it indicates complete inhibition of response (on P-STAT3) at high concentrations. The IC_{50} is the concentration of drug A or B alone which elicits half the maximal response and γ is a power or curve shape coefficient.

The interaction of ATO and 17-DMAG on the stimulation of HSP70 expression was characterized with the following stimulatory equation for non-competitive interaction.

one drug) by Chakraborty and Jusko [10, 11]. The interaction parameter, ψ , indicates the mutual influence of each drug on the IC_{50} of the other drug when present jointly. A value of $\psi < 1$ indicates a lesser value of IC_{50} , meaning less drug is required to achieve half-maximal effect (synergy) when compared with either present alone. A value of $\psi > 1$ indicates a higher value of IC_{50} , meaning more drug is required to achieve half-maximal effect (antagonism). A value of $\psi = 1$ indicates no effect on the IC_{50} value of either drug (no interaction).

When the concentration of either drug is zero, the equations take the form of the basic Hill function with the value of ψ assumed to be 1. In Eq. 1, when the concentration of drug B is zero

$$E = 100 \times \left[1 + \frac{\left[S_{\max,A} \times \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A} + S_{\max,B} \times \left(\frac{B}{\psi \times SC_{50,B}} \right)^{\gamma_B} + \left(S_{\max,A} + S_{\max,B} + S_{\max,A} \times S_{\max,B} \right) \times \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A} \times \left(\frac{B}{\psi \times SC_{50,B}} \right)^{\gamma_B} \right]}{1 + \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A} + \left(\frac{B}{\psi \times SC_{50,B}} \right)^{\gamma_B} + \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A} \times \left(\frac{B}{\psi \times SC_{50,B}} \right)^{\gamma_B}} \right] \quad (2)$$

Symbol A refers to the concentration of ATO, B refers to 17-DMAG, $S_{\max}$ is the maximum capacity of either drug on the stimulation of HSP70 when present alone and SC_{50} is the concentration which produces half the maximum effect when the drugs are present alone.

In the above equations, the values of $I_{\max}$ vary between 0 and 1, but the values of $S_{\max}$ are greater than zero with no upper limit. These equations were proposed by Ariens [8, 9] for drugs that interact non-competitively. An interaction parameter, ψ , was later included (multiplied by the IC_{50} of

$$E = 100 \times \left[1 - \frac{I_{\max,A} \times \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A}}{1 + \left(\frac{A}{\psi \times IC_{50,A}} \right)^{\gamma_A}} \right] \quad (3)$$

In Eq. 2 when the concentration of drug B is zero

$$E = 100 \times \left[1 + \frac{S_{\max,A} \times \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A}}{1 + \left(\frac{A}{\psi \times SC_{50,A}} \right)^{\gamma_A}} \right] \quad (4)$$

Non-linear regression was performed with ADAPT II software [14]. For both siRNA-treated and -control pairs, single-drug data were fitted to Eq. 3 for inhibition of P-STAT3 and Eq. 4 for the stimulation of HSP70 to resolve the pharmacologic parameters ($I_{\max}$, $S_{\max}$, IC_{50} , SC_{50} , and γ). From the P-STAT3 data, it is clear that complete inhibition of response was achieved and hence $I_{\max}$ was set to 1 for both siRNA-treated and -control datasets. The same $S_{\max}$ was used to fit both the siRNA-treated and -control data. Interaction data were then fitted with Eqs. 1 and 2. When fitting the interaction data, the pharmacologic parameters and γ obtained from Eqs. 3 and 4 were fixed and the interaction parameter ψ was the only parameter resolved.

Results

Expression of the HSP70 family members and down-regulation by ATO and 17-DMAG

The expression levels of the HSP70 family members in HEL cells are shown in Fig. 1a. The results demonstrate that HSP72 (collectively representing HSP 70-1A and HSP 70-1B) was the most abundant member. Further, HSC70 (HSPA8), which was also expressed in HEL cells, was affected by neither ATO nor 17-DMAG treatments (Fig. 1b). Therefore, only HSP72 (designated HSP70 below) was targeted by the siRNA.

Down-regulation of P-STAT3

The down-regulation of P-STAT3 activity by ATO for siRNA-treated and -control cells are shown in Fig. 2a, and the down-regulation of P-STAT3 activity by 17-DMAG for siRNA-treated and -control cells are shown in Fig. 2b. Fittings with Eq. 3 yielded the parameter estimates that are listed in Table 2. The $I_{\max}$ was fixed to 1, since it was evident from the data that complete down-regulation of P-STAT3 is possible. The $S_{\max}$ was kept the same for both the siRNA-treated and -control cells. The values of IC_{50} for both drugs are well in accordance with the findings of our previous work [3]. The IC_{50} values for both ATO and 17-DMAG decreased after treatment with siRNA for HSP70. The value of IC_{50} for ATO decreased from 1,301 to 1,064 nmol/l after treatment with siRNA for HSP70 indicating an increase in potency of ATO after the treatment. Similarly, the IC_{50} of 17-DMAG decreased from 450 to 157 nmol/l after treatment with siRNA for HSP70 indicating an increase in potency of 17-DMAG after the treatment. The interaction data were fitted with Eq. 1 to obtain the values of the interaction parameter, ψ , for both siRNA-treated and -control cells. The estimates of ψ are listed in Table 3. The value of ψ for the siRNA-control cells was 0.544 indicating mechanism-based synergy, which is in accordance with our previous work. Treatment with siRNA for HSP70 resulted in a ψ value of 0.041, which indicates a stronger degree of synergistic interaction

Fig. 2 Single-drug exposures of ATO and 17-DMAG for the down-regulation of P-STAT3 (a, b) and up-regulation of HSP70 (c, d). Red circles, observed expression of P-STAT3/HSP70 after drug exposures of siRNA-control cells; red line, predicted expression of P-STAT3/HSP70 after drug exposures of siRNA-control cells. Blue squares, observed expression of P-STAT3/HSP70 after drug exposures of siRNA-treated cells; blue line, predicted expression of P-STAT3/HSP70 after drug exposures of siRNA-treated cells

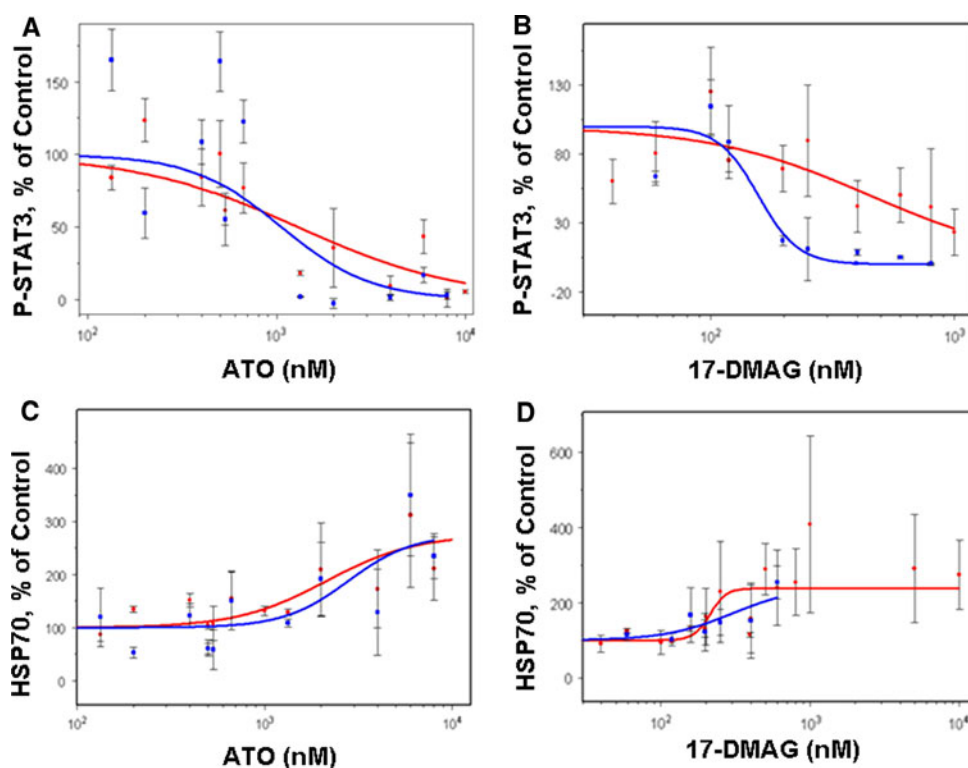


Table 2 Parameter estimates for single-drug effects on P-STAT3 down-regulation and HSP70 up-regulation for siRNA-treated and -control cells

				No siRNA		siRNA	
				Estimate	CV%	Estimate	CV%
P-STAT3	ATO	IC ₅₀ (nmol/l)		1,301	29.6	1,064	21.4
		γ		NA	NA	1.83	15.2
	17-DMAG	IC ₅₀ (nmol/l)		450	14.7	157	9.7
		γ		1.32	25.6	5.30	30.1
HSP70	ATO	$S_{\max}$		1.76	34.7	1.76	Fixed
		SC ₅₀ (nmol/l)		2,142	62.4	1,794	27.7
		γ		1.82	58.6	2.53	47.8
	17-DMAG	$S_{\max}$		1.42	11.2	1.42	Fixed
		SC ₅₀ (nmol/l)		215	8.31	300	23.9
		γ		7.86	59.8	1.94	41.8

of the two drugs in the presence of the siRNA against HSP70. Thus, it could be concluded that the effect of ATO and 17-DMAG on their respective IC₅₀ values was more pronounced when the cells were treated with siRNA when compared to control cells.

Isobolograms were constructed for both siRNA-treated and -control cells for the combinations of ATO and 17-DMAG (Fig. 3a, b). The lines represent all the possible combinations of ATO and 17-DMAG that result in 50% of maximal inhibition of P-STAT3. The solid lines represent the model fitted to the data, and the dashed lines represent the no-interaction model ($\psi = 1$). The isobolograms were generated by the method described in our previous work [3]. Fig. 3 indicates that for both the siRNA-treated and -control cells, the interaction line lies beneath the no-interaction line indicating mechanism-based synergy. However, for siRNA-treated cells, the interaction lies further away from the no-interaction line indicating a stronger synergy as also indicated by the interaction parameter value of 0.041 compared to 0.544 for the control cells.

Three-dimensional figures were generated (Fig. 4a, b). Tightening of the surface toward the origin is indicative of more synergistic interaction (since the effect on the IC₅₀ values are more pronounced). In the siRNA-treated cells, Fig. 4b, the surface is more tightened toward the origin when compared with the control cells, Fig. 4a.

Table 3 Estimates of interaction parameter, ψ , for siRNA-treated and -control cells

	Before siRNA treatment Ψ Estimate (CV%)	After siRNA treatment Ψ Estimate (CV%)
ATO and 17-DMAG on P-STAT3	0.544 (44)	0.041 (43)
ATO and 17-DMAG on HSP70	0.243 (295)	0.413 (NA)

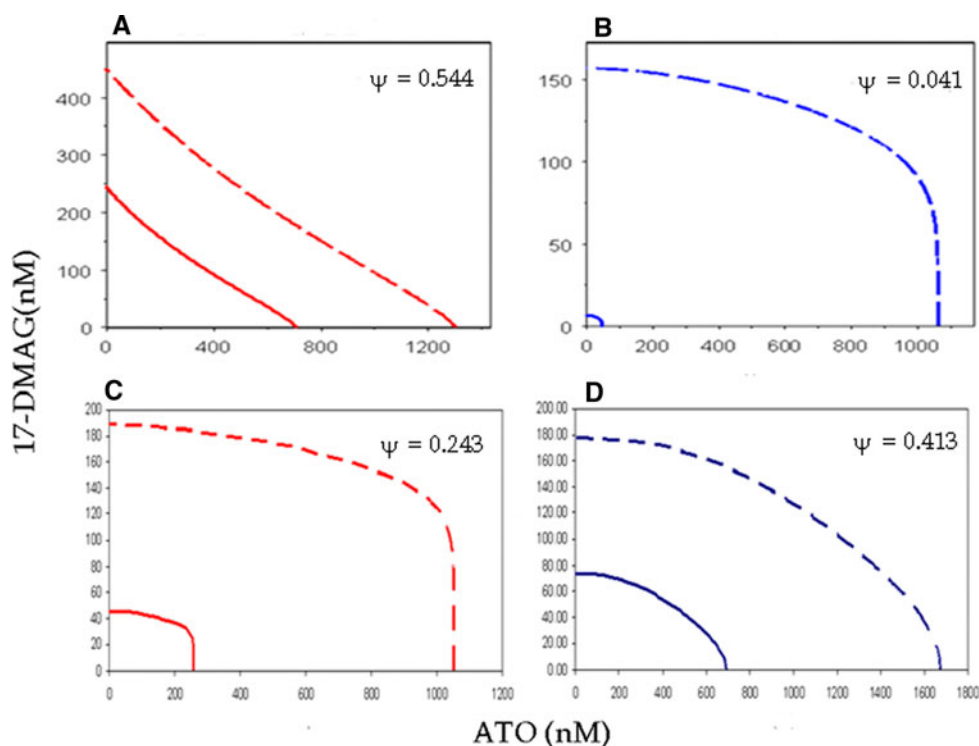
NA not available

Up-regulation of HSP70

Up-regulation of HSP70 activity by ATO for siRNA-treated and -control cells is shown in Fig. 2c, and the up-regulation of HSP70 activity by 17-DMAG for siRNA-treated and -control cells is shown in Fig. 2d. As seen in the case of P-STAT3 down-regulation, fitting of single-drug data with Eq. 4 characterized the data. The fitted parameter estimates are listed in Table 2. The $S_{\max}$ was kept the same for both the siRNA-treated and -control cells. The values of SC₅₀ for both drugs were very close with those obtained in our previous work [3]. The SC₅₀ values for both ATO and 17-DMAG increased after treatment with HSP70 siRNA indicating a decrease in the potency of the two drugs after treatment. The value of SC₅₀ for ATO increased from 2,142 to 2,794 nmol/l after treatment with HSP70 siRNA indicating a considerable decrease in the potency of the drug. Similarly, after treatment with HSP70 siRNA, the SC₅₀ of 17-DMAG increased from 215 to 300 nmol/l, indicating a decrease in the potency of ATO and 17-DMAG. The value of the interaction parameter, ψ , was obtained by fitting the interaction data of both siRNA-treated and -control cells. The estimates of the interaction parameter, ψ , are listed in Table 3. The value of ψ for the siRNA-control cells was 0.243 indicating strong synergy. After treatment with HSP70 siRNA, the value of ψ was 0.413, which indicates a decrease in the degree of the synergistic interaction of the two drugs. Thus, after treating the cells with HSP70 siRNA, the IC₅₀ values for ATO and 17-DMAG increased and potency decreased.

Isobolograms were constructed for siRNA-treated cells for the combinations of ATO and 17-DMAG (Fig. 3c, d). Again, the lines represent all the possible combinations of ATO and 17-DMAG that result in 50% of maximal stimulation of HSP70. The solid lines represent the model fitted to the data, and the dashed lines represent no-interaction ($\psi = 1$). The figures indicate that for both the siRNA-treated and -control cells, the interaction line lies beneath

Fig. 3 Isobolograms for ATO and 17-DMAG on down-regulation of P-STAT3 expression (**a, b**), and HSP70 up-regulation (**c, d**). *Dashed lines*, mechanism-based additivity, that is, no-interaction line; *solid line*, predicted interaction curve with the value of the interaction fitted. **a, c** siRNA-control; **b, d** siRNA-treated



the no-interaction line indicating mechanism-based synergy. However, for siRNA-treated cells, the interaction lies nearer to the no-interaction line indicating less strong synergy as also indicated by the interaction parameter value of 0.413 compared to 0.243 for the siRNA-control cells.

Three-dimensional figures were generated (Fig. 4c, d). In the siRNA-control cells, Fig. 4c, the surface is more tightened toward the origin when compared to the treated cells, Fig. 4d, indicating that the synergistic effect has been reduced after treatment with siRNA for HSP70.

Drug–drug effect on cell survival

There was no effect of either combination on cell death at 6 or 24 h. ATO at 50% of the IC_{50} induced significant cell death at 48 h ($\sim 50\%$), while 17-DMAG resulted in only modest cell death at 50% of the IC_{50} ($\sim 10\%$). The addition of siRNA to ATO did not affect cell death but adding siRNA to 17-DMAG resulted in 50% cell death (compared to 10% with 17-DMAG alone). The control-siRNA had no effect on cell survival. The addition of siRNA to 50% of the IC_{50} of ATO and 17-DMAG at 48 h did not affect the 50% cell death observed with the combination.

Discussion

In a previous study, we have shown that ATO and HSP90 inhibitors synergize to inhibit P-STAT3 and enhance their

anti-leukemia activity [3]. This synergy occurred despite a synergistic up-regulation of HSP70, a protein known to inhibit apoptosis. Pharmacodynamic models were therefore applied in the present study to study the effect of ATO and 17-DMAG on the down-regulation of P-STAT3 while inhibiting HSP70 with siRNA. These models not only supported our previous findings but also proved that the degree of synergistic interaction between the two agents for the down-regulation of P-STAT3 increased in siRNA-treated AML cells. Moreover, the concomitant synergy which was observed in the up-regulation of HSP70 decreased in the presence of siRNA. The same semi-mechanistic pharmacodynamic model was used as in our previous work [3]. The degree of synergy was determined with the estimation of the interaction parameter, ψ .

The IC_{50} values for down-regulation of P-STAT3 for both agents (ATO and 17-DMAG) decreased in the siRNA treated cells, and the SC_{50} values for the up-regulation of HSP70 for both agents increased in the siRNA-treated AML cells. The decrease in IC_{50} values due to the treatment does not indicate that the degree of synergy would also increase with the combination of drugs. An increase in the IC_{50} value is only indicative of an enhancement of the potency of drugs. Similarly, an increase in the SC_{50} values due to a treatment is only indicative of a decrease in potency of the drugs. Two drugs might show an increase in the degree of synergy despite a decrease of potency. Greco et al. [15] showed that despite a decrease in the potency of Trimetrexate and AG2034 in the presence of 78 μM folic

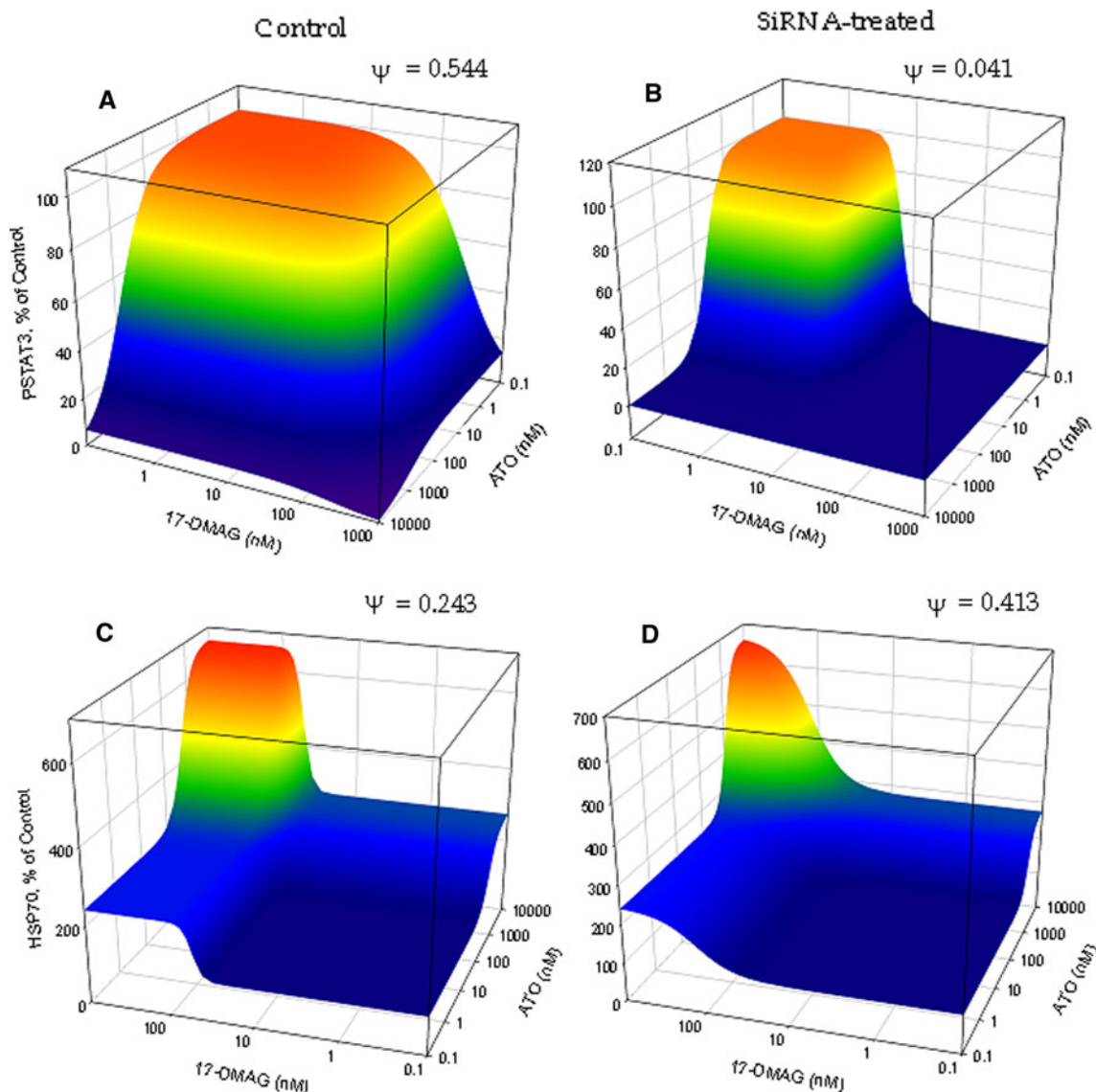


Fig. 4 Three-dimensional plots of drug effect on P-STAT3 down-regulation and HSP70 up-regulation for ATO and 17-DMAG combination. Surfaces are the model predictions based on the fitted

parameters. **a, b** Surfaces for the down-regulation of P-STAT3; **c, d** surfaces for the up-regulation of HSP70

acid, there was an increase in the degree of synergy for the two drugs.

In our previous work, it was observed that ATO and Geldanamycin had the most synergistic effect on the down-regulation of P-STAT3. ATO and Geldanamycin, on the other hand, had an antagonistic effect on the up-regulation of HSP70. Our findings here were similar. The degree of synergistic action was increased after treating the AML cells with siRNA for HSP70. The degree of synergistic action for the up-regulation of HSP70 was decreased. This means, that in clinical settings, the concomitant administration of a HSP70 inhibitor, such as KNK437 [16], a HSP70 antisense [17] or delivering siRNA via peptide

transduction domains [18] along with ATO and 17-DMAG may have a potential therapeutic benefit.

In this analysis, Isobolograms were used to depict the degree of interaction. Isobolograms are an excellent tool to depict the degree of interaction in comparison with no-interaction. Moreover, isobolograms also help one to determine the nature of interaction of the two agents. An isobologram-line similar to a straight line indicates that each combination of the two agents have the same relative total concentration [3] of the two drugs. Deviation from the straight line indicates that the total concentration to achieve 50% of maximal effect varies for different combinations. This phenomenon is more pronounced in case of the

siRNA-treated cells where the interaction is more synergistic and there is an observed change in the nature of the interaction of the two drugs.

Down-regulation of HSP70 improved 17-DMAG's effect on cell death suggesting that the anti-apoptotic effect of HSP70 up-regulation following exposure to 17-DMAG is more pronounced compared with ATO. However, this study was conducted in vitro and the actual survival effect(s) should be tested in vivo.

Enhancement of anti-leukemia activity of a HSP90 inhibitor with abrogation of HSP70 induction was previously demonstrated by Guo et al. [19], but our results showing that down-regulation of HSP70 improves ATO and 17-DMAG effects on P-STAT3 have not been published before. These results further support the concept of studying the combined role of ATO with a HSP90 inhibitor such as 17-DMAG in AML with constitutive STAT3 activity.

Acknowledgments This study was supported partially by grants from the National Cancer Institute Grants CA16056 and CA99238, National Institute of Health Grant GM57980 and by The Heidi Leukemia Research Fund (Buffalo, NY).

References

- Benekli M, Xia Z, Donohue KA et al (2002) Constitutive activity of signal transducer and activator of transcription 3 protein in acute myeloid leukemia blasts is associated with short disease-free survival. *Blood* 99:252–257
- Wetzler M, Brady MT, Tracy E et al (2006) Arsenic trioxide affects signal transducer and activator of transcription proteins through alteration of protein tyrosine kinase phosphorylation. *Clin Cancer Res* 12:6817–6825
- Wetzler M, Earp JC, Brady MT, Keng MK, Jusko WJ (2007) Synergism between arsenic trioxide and heat shock protein 90 inhibitors on signal transducer and activator of transcription protein 3 activity—pharmacodynamic drug–drug interaction modeling. *Clin Cancer Res* 13:2261–2270
- Takayama S, Reed JC, Homma S (2003) Heat-shock proteins as regulators of apoptosis. *Oncogene* 22:9041–9047
- Beere HM (2004) “The stress of dying”: the role of heat shock proteins in the regulation of apoptosis. *J Cell Sci* 117:2641–2651
- Rohde M, Daugaard M, Jensen MH, Helin K, Nylandsted J, Jaattela M (2005) Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. *Genes Dev* 19:570–582
- Powers MV, Clarke PA, Workman P (2008) Dual targeting of HSC70 and HSP72 inhibits HSP90 function and induces tumor-specific apoptosis. *Cancer Cell* 14:250–262
- Ariens EJ, Van Rossum JM, Simonis AM (1957) Affinity, intrinsic activity and drug interactions. *Pharmacol Rev* 9:218–236
- Ariens EJ, Simonis AM (1964) A molecular basis for drug action. *J Pharm Pharmacol* 16:137–157
- Greco WR, Bravo G, Parsons JC (1995) The search for synergy: a critical review from a response surface perspective. *Pharmacol Rev* 47:331–385
- Chakraborty A, Jusko WJ (2002) Pharmacodynamic interaction of recombinant human interleukin-10 and prednisolone using in vitro whole blood lymphocyte proliferation. *J Pharm Sci* 91:1334–1342
- Chow FS, Jusko WJ (2004) Immunosuppressive interactions among calcium channel antagonists and selected corticosteroids and macrolides using human whole blood lymphocytes. *Drug Metab Pharmacokinet* 19:413–421
- Drake AS, Brady MT, Wang XH, et al. (2008) Targeting 11q23 positive acute leukemia cells with high molecular weight-melanoma associated antigen-specific monoclonal antibodies. *Cancer Immunol Immunother* 58:415–427
- D'Argenio DZ, Schumitzky A (1997) ADAPT II users guide: pharmacokinetic/pharmacodynamic systems analysis software. Biomedical Simulations Resource, Los Angeles
- Faessel HM, Slocum HK, Jackson RC et al (1998) Super in vitro synergy between inhibitors of dihydrofolate reductase and inhibitors of other folate-requiring enzymes: the critical role of polyglutamylolation. *Cancer Res* 58:3036–3050
- Yokota S, Kitahara M, Nagata K (2000) Benzylidene lactam compound, KNK437, a novel inhibitor of acquisition of thermotolerance and heat shock protein induction in human colon carcinoma cells. *Cancer Res* 60:2942–2948
- Rayburn ER, Zhang R (2008) Antisense, RNAi, and gene silencing strategies for therapy: mission possible or impossible? *Drug Discov Today* 13:513–521
- Eguchi A, Dowdy SF (2009) siRNA delivery using peptide transduction domains. *Trends Pharmacol Sci* 30:341–345
- Guo F, Rocha K, Bali P et al (2005) Abrogation of heat shock protein 70 induction as a strategy to increase antileukemia activity of heat shock protein 90 inhibitor 17-allylamino-demethoxy geldanamycin. *Cancer Res* 65:10536–10544