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Modulation of α-interferon's antiviral and clinical effects by aspirin, acetaminophen, and prednisone in healthy volunteers ☆

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Abstract

The magnitude and duration of the antiviral and clinical effect of α-interferon was measured in healthy volunteers. A single 3 million unit intramuscular dose of interferon was given either alone (controls) or after 72 h of concomitant medications. These medications included either aspirin (650 mg every 4 h), acetaminophen (650 mg every 4 h), or prednisone (40 mg per day). Peripheral blood mononuclear cells were assayed for resistance to vesicular stomatitis virus infection and induction of 2′-5′-oligoadenylate synthetase activity as evidence of interferon's antiviral effect. Co-administration of acetaminophen increased both antiviral parameters by more than 70% ($P < 0.05$) and reduced symptoms after interferon dosing, compared to controls. Aspirin and prednisone did not demonstrate any significant differences from controls in antiviral effect. As a group, acetaminophen, aspirin, and prednisone reduced the clinical symptoms by 47% compared to controls ($P = 0.03$) after interferon dosing, although individual drug comparisons failed to

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reach statistical significance. Independent of treatment group, the changes in antiviral markers after interferon dosing correlated closely with each other ($r = 0.72$, $P < 0.001$), but neither correlated with symptoms or fever ($r < 0.30$, $P > 0.05$). Acetaminophen enhances the antiviral effects of a single intramuscular dose of α-interferon, considering the parameters measured in these healthy volunteers.

Keywords: α-Interferon; Acetaminophen; Aspirin; Prednisone; 2′,5′-Oligoadenylate synthetase; Vesicular stomatitis virus; Drug interaction

1. Introduction

α-Interferon (IFN) has proved to be efficacious for the treatment of several viral infections, including hepatitis C, hepatitis B, and papilloma virus-related condyloma acuminata (Eron et al., 1986; Friedman-Kien et al., 1988; Hoofnagle et al., 1988; Davis et al., 1989; Di Bisceglie et al., 1989). Patients commonly experience acute dose-limiting side effects, however, after IFN administration in the range of doses currently used for FDA-licensed indications (Quesada et al., 1986). Non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and prednisone have all been reported to reduce IFN-associated symptoms (Mora et al., 1984; Fossa et al., 1990; Visco et al., 1991). This effect may occur through their inhibition of interferon-induced prostaglandin E (PGE) synthesis in the brain (Flower and Vane, 1972; Yaron et al., 1977; Fuse et al., 1982; Dinarello et al., 1984). PGE's effect on the hypothalamus has been reported to be the cause of IFN-associated fever (Yaron et al., 1977; Dinarello et al., 1984).

Based on preclinical studies, however, NSAIDs like aspirin and indomethacin have also been reported to diminish the antiviral effects of IFN. This partly occurs through the inhibition of fatty acid cyclooxygenase (Pottathil et al., 1981) and suppression of IFN-mediated induction of 2,3-indoleamine dioxygenase (2,3-IDO) which may be important in establishing the antiviral state (Sayama et al., 1981; Carlin et al., 1989). Other reports suggest NSAIDs have no such effect (Tovey et al., 1982). Glucocorticoids also suppress IFN-mediated induction of 2,3-IDO (Sayama et al., 1981) in addition to inhibition of IFN-induced lymphocyte migration and gene expression (Issekutz, 1989; Gronemeyer, 1992).

We sought to assess the effects of concurrently and continuously dosed aspirin, acetaminophen, and prednisone on the antiviral and clinical effect of IFN in healthy human subjects. To quantify the antiviral effect induced by IFN, we measured the magnitude and duration of: (1) resistance of peripheral blood mononuclear cells (PBMC) to infection with vesicular stomatitis virus (VSV); and (2) the intracellular PBMC activity of 2′-5′-oligoadenylate synthetase (2,5-AS), an interferon-induced enzyme important in the establishment of the antiviral state protective against some viruses (Baglioni et al., 1979; Rubin and Gupta, 1980). These markers have been used to establish the antiviral dose–response characteristics of interferon in healthy volunteers (Merritt et al., 1986; Barouki et al., 1987; Witter et al., 1987). Additionally, it has been demonstrated that after 18 million units (m.u.) of IFN, neither aspirin, acetaminophen, nor prednisone reduce the side effects of IFN in a clinically meaningful manner. Only prednisone diminished the 2,5-AS response to IFN, and none of the concomitant

medications reduced the duration or intensity of the VSV yield reduction assay (Witter et al., 1988). We designed this study to assess the effects of these concomitant medications at a lower dose of IFN.

2. Materials and methods

2.1. Subjects

Healthy adult volunteers enrolled in the study after giving written informed consent. The subjects were determined to be healthy based upon a thorough history, physical examination, and laboratory screening studies. Volunteers with a history of a viral illness within 2 weeks prior to screening were excluded. Three of a total of 29 subjects participated in more than one treatment arm of this study and 5 others had previously participated in other interferon studies; participation was separated by at least 3 months and none of these 8 subjects had interferon antibodies at the beginning of this study. Control subjects were enrolled from February through May 1985. Subjects in the combination arms were enrolled between March 1988 and June 1989.

2.2. Dosing

Subjects were sequentially recruited into 4 treatment regimens with 8 subjects each: (1) IFN alone (control); (2) IFN plus aspirin 650 mg p.o. every 4 h; (3) IFN plus acetaminophen 650 mg p.o. every 4 h; or (4) IFN plus prednisone 40 mg daily. We previously reported the control group results (Witter et al., 1987). The morning after admission to the Inpatient Clinical Research Center at The Johns Hopkins Hospital, the combination arm subjects began taking their designated medication at the dose and interval specified for 8 days. A single interferon dose was given 72 h after the initiation of the aspirin, acetaminophen, or prednisone in the concomitant therapy groups, or the morning after admission for the interferon alone group. After an overnight fast, all subjects received a 3 m.u. gluteal i.m. injection of recombinant human interferon-α (rHuIFN-α_{2a}; Roferon). All interferon doses were administered between 07.30 and 08.30 h. Blood for VSV and 2,5-AS assays was drawn at 0, 1, 2, 6, 12, 24, 32, 48, 56, 72, 96, 104, 120, and 144 h after the IFN injection. The concomitant medications continued at the same dose for 5 more days (8 days in total), until the last dose was taken 104 h after the IFN dose. Patients were confined to the Clinical Research Center and monitored for body temperature and grading symptoms at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h after IFN dosing and every 24 h thereafter until the study end at 144 h. White blood count with differential, and aspartate aminotransferase levels were monitored at 0, 48, and 96 h after IFN dosing.

2.3. Interferon

rHuIFN-α_{2a} was provided by Hoffmann-LaRoche, Inc. (Nutley, NJ). The dose in units can be converted to picograms by multiplying by a factor of 6.0 as determined by the National Institutes of Health reference standard.

2.4. Clinical parameters

Occurrence of symptoms, for determination of quantitative grading, was solicited in a non-directed manner at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h after IFN dosing and every 24 h thereafter throughout the study. The subjects graded the symptoms as none (grade 0), mild (grade 1), moderate (grade 2), or severe (grade 3) without further definition. These symptom grades were recorded on standardized case report forms which include 18 categories of symptoms (paresthesias, feverishness, chills, nausea, vomiting, headache, neck stiffness, photophobia, myalgia, fatigue, confusion, concentration difficulty, malaise, anorexia, abdominal pain, diarrhea, constipation, and other) at the time points listed above. Previous experience (Barouki et al., 1987; Witter et al., 1987) has indicated that these forms allow complete, time-dependent collection of all symptomatic data in a quantitative manner. The 16-h symptom score was calculated as the sum of the graded scores (1, 2, or 3) for all symptoms in the first 16 h after IFN dosing. This time cut-off was chosen because only two subjects reported any symptoms beyond the 16-h time point, and the assessment points were equally spaced for most of the first 16-h interval. This method incorporates number, severity, and duration of qualitative symptoms into a quantitative symptom score for comparative analysis. It has been developed by our group for this and a previously published study (Hendrix et al., 1993). Maximum temperatures (T_{max}) and hours with temperature greater than or equal to 38°C (HRS > 38) were also calculated.

2.5. PBMC preparation

PBMC were isolated from 20 ml heparinized venous blood by means of Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation. Of the total PBMC sample collected, one and a half million cells were used to test for in vitro protection from VSV infection. The remaining cells were resuspended at 10^7 cell/ml in a lysis buffer containing 20 mmol/l HEPES buffer (pH 7.5), 5 mmol/l $MgCl_2$, 120 mmol/l KCl, 7 mmol/l dithiothreitol, 10% glycerol by volume, and 0.5% Nonidet P40 by volume. After centrifugation for 10 min at 8000 g, the supernatant fluid extracts were stored at −70°C until the enzyme assay could be performed simultaneously on all of the samples from a given subject for each experiment.

2.6. 2,5-AS assay

The 2,5-AS activity was assayed according to the method of Schattner et al. (1981) with the following modifications: aliquots (10 μl) of extracts were mixed with 160 μl poly(rI)–(rC) agarose beads suspended in 40 μl of the same buffer as the cells; the beads were washed only once after 15 min of incubation with the cell extracts at 30°C. The incubation time was shortened to 2 h. Under the conditions of this assay, the accumulation of 2,5-AS in vitro was linear with respect to both time and enzyme concentration. Enzyme activity is expressed as picomoles of 2,5-AS synthesized per 10^5 cells per hour. Intra-assay variation was less than 10% and interassay variation was less than 20%. Area under the 2,5-AS-versus-time curve (2,5-AS AUC) for the first 96 h

after IFN dosing was calculated by the trapezoidal rule. This time interval included the time during which the concomitant medications were taken and for which samples were available in all subjects. For statistical analysis, the net increase in 2,5-AS 96-h AUC over baseline was used to compare treatments.

2.7. VSV yield inhibition assay

One hundred microliters of RPMI-1640 (GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (GIBCO) containing 10^5 tissue culture infectious doses of VSV was added to sterile tubes containing either 2.5×10^6 Ficoll-Paque-separated PBMC from study subjects (experimental) or no cells (control for the residual amount of virus present in the inoculum). Both tubes were incubated for 45 min at 37°C to allow for virus absorption to the cells in the experimental tubes. Following this, 1 ml RPMI-1640/5% fetal bovine serum was added to each tube before continued incubation for 48 h at 37°C. Tubes were then frozen at −70°C for subsequent titration. In the final virus titration step, all samples for each patient were run on the same microtiter plate. The amount of VSV present in the experimental tubes with cells (test for susceptibility of cells to VSV infection) and in the control tubes was assayed by incubating the thawed supernatant fluid in serial 10-fold dilutions on L-929 cells. The cytopathic effect was read at 48 h. Data are presented as VSV yield inhibition. The VSV yield is the highest dilution of supernatant fluid producing cytopathic effect minus the residual amount as determined from the control tube for any given sample. The VSV yield inhibition is defined by the following equation: VSV yield inhibition = $\log_{10}$ (baseline VSV yield/experimental VSV yield). Area under the VSV yield inhibition-versus-time curve (VSV AUC) was calculated by the trapezoidal rule. Due to a laboratory accident, VSV yield data are available on only 5 acetaminophen subjects and 4 subjects in each of the prednisone and aspirin groups.

2.8. Statistical analysis

Normality of the data was assessed using visual inspection of quantile–quantile plots and the Shapiro–Wilks test statistic. Levene's test statistic was used to assess homogeneity in variance across groups. For normally distributed parameters with equal variance across groups (2,5-AS AUC, VSV AUC, T_{max}), we summarized group data using mean and standard deviation; for comparisons among and between regimens, respectively, we used analysis of variance (ANOVA) and the modified Bonferroni correction for multiple comparisons. Non-normally distributed parameters (symptom scores and HRS > 38) were described by median and interquartile range; the Kruskal–Wallis and Mann–Whitney *U*-tests were used to assess for differences among and between groups for these parameters. The Spearman-rank correlation test was used to test the significance of correlations between parameters. Two-tailed *P*-values of less than 5% were considered statistically significant. A median polish was used to determine the effect of interferon on 2,5-AS and VSV yield inhibition over time (t_i), distinct from individual patient effects (p_j), overall median (μ), and residual or unexplained effects (e_{ij}) (Tukey, 1977). Each single observation (y_{ij}) can be expressed as follows: $y_{ij} = \mu + t_i + p_j + e_{ij}$. The

sample size used, based on 5% type I error and 20% type II error (80% power), should have allowed the detection of the following differences compared to controls in each of the 3 concomitant medication arms, a priori: 2,5-AS, 25% below controls; VSV infectivity, 31% below controls; symptom score, 52% of controls; maximum temperature below 38°C compared to 38.4°C for controls; 2.1 h less time above 38°C compared to controls.

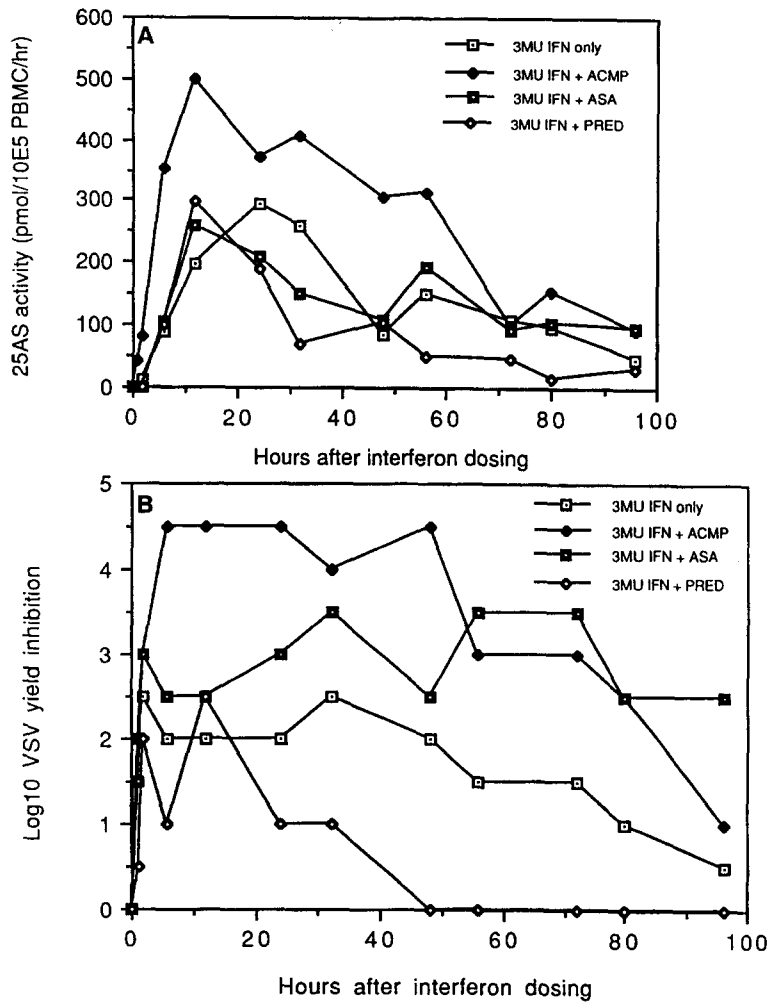

Fig. 1. Effects over time, based on median polish, are shown for: (A) 2′,5′-oligoadenylate synthetase activity (25AS) in peripheral blood mononuclear cells (PBMC); and (B) resistance of PBMC to vesicular stomatitis virus (VSV) infection expressed as $\log_{10}$ VSV yield reduction. Results are for the first 96 h following intramuscular administration of 3 million units of α-interferon (IFN) and are grouped by treatment arm (IFN alone and IFN plus acetaminophen, aspirin, or prednisone).

3. Results

After a single 3 m.u. i.m. dose of rHuIFN-α_{2a} at time 0, the 2,5-AS activity began to rise from baseline at 1–6 h in all treatment groups. The 2,5-AS activity then peaked between 12 and 24 h, and gradually returned toward, but did not reach, baseline by 96 h (Fig. 1). The median 2,5-AS increase decayed to below one-half the peak level at 72 h after IFN dosing in all groups, except prednisone which did so at 36 h. The net increase in the 96-h 2,5-AS AUC after IFN dosing was different among the 4 groups (ANOVA, $P = 0.003$). The acetaminophen arm was 1.70 times the control group value (modified Bonferroni, $P < 0.05$), while the prednisone and aspirin groups were not statistically different from controls (Table 1).

The median VSV yield inhibition had risen above baseline within the first hour after IFN dosing in all treatment groups, then reached a plateau in 1–6 h, before returning toward baseline. The $\log_{10}$ decay of VSV reached one-half the peak value beyond 72 h for all groups except prednisone which reached baseline by 48 h. The net increase in VSV yield inhibition was also statistically significant among the treatment groups (ANOVA, $P = 0.03$). The median VSV 96-h AUC for the acetaminophen group was 2.1 times greater than the control group mean (modified Bonferroni, $P < 0.05$), while the other two groups were not different from controls (Table 1).

Symptoms first occurred between 3 and 6 h after IFN injection among the treatment groups and subsided in all but two subjects by 16 h after interferon dosing. As a group, the subjects receiving concomitant medications had a 47% reduction in symptom score (Mann–Whitney U-test, $P = 0.03$) compared to controls; the median acetaminophen and prednisone group symptom scores were 43% ($P = 0.04$) and 42% ($P = 0.06$), respectively, below the value of the control group (Table 1); the aspirin group was not different from the controls ($P = 0.28$). When adjusting for multiple comparisons, however, these individual comparisons were not significant. The prednisone group also

Table 1
Summary of antiviral and clinical effects after a 3 m.u. IFN dose grouped by drug regimen

Regimen	2,5-AS AUC [a]	VSV AUC [b]	Symptom score	T_{max} (°C)	Hours > 38°C
IFN alone	12,996 (4,613) [c]	138 (60)	11.5 (8.5, 19.0)	38.4 (0.6)	3 (1, 6)
IFN + acetaminophen	23,491 (10,126) [d]	285 (129) [e]	5.5 (4.0, 8.0) [f]	38.0 (0.7)	1 (0, 4)
IFN + aspirin	12,899 (4,389)	244 (154) [g]	3.5 (1.5, 14.0)	38.2 (0.6)	2 (0, 4)
IFN + prednisone	10,246 (6,638)	97 (68)	5.0 (1.0, 7.5) [h]	38.0 (0.7)	0 (0, 2) [h]

[a] Area under the 2′,5′-oligoadenylate synthetase activity time curve (pmol/10^5 PBMC/h × 96 h).
[b] Area under the $\log_{10}$ vesicular stomatitis virus yield reduction time curve (96 h); for VSV AUC, $n = 5$ (ACMP), $n = 4$ (ASA, PRED), and $n = 8$ (control); $n = 8$ for all other variables.
[c] Mean (standard deviation) for 2,5-AS, VSV, T_{max}; median (lower, upper quartile) for symptom score, hours ⩾ 38°C.
[d] $P < 0.05$ compared to IFN alone, aspirin, and prednisone (modified Bonferroni correction).
[e] $P < 0.05$ compared to IFN alone and prednisone (modified Bonferroni correction).
[f] $P = 0.04$ compared to IFN alone (Mann–Whitney U-test).
[g] $P < 0.05$ compared to prednisone (modified Bonferroni correction).
[h] $P = 0.06$ compared to IFN alone (Mann–Whitney U-test).

experienced fewer hours of fever, a median of 0 h, compared to 3 h for controls ($P < 0.05$) when accounting for multiple comparisons (Table 1). The mean T_{max} was not different among groups (ANOVA, $P > 0.05$) (Table 1).

Without respect for the treatment group, there was a significant correlation ($P < 0.001$) between VSV and 2,5-AS 96-h AUC ($r = 0.72$), and between the 16-h symptom score and both T_{max} ($r = 0.62$) and HRS > 38 ($r = 0.58$). There was no significant correlation between clinical and antiviral parameters ($r < 0.21$, $P < 0.30$). No toxicities were noted in hematologic or aspartate aminotransferase levels in any group (data not shown).

4. Discussion

Concomitant use of acetaminophen enhanced the antiviral effect, measured using the VSV and 2,5-AS assays, following a single 3 m.u. i.m. IFN dose in these healthy volunteers. Prednisone and aspirin did not consistently alter the antiviral effect. The magnitude of this acetaminophen effect may be clinically significant. The antiviral effects of the acetaminophen group in this 3 m.u. IFN study are similar to values measured after an 18 m.u. IFN dose (Barouki et al., 1987). At higher IFN doses, however, we have shown that the addition of acetaminophen does not have this same enhancing effect (Witter et al., 1988). In that study, prednisone did cause a statistically, but not clinically, significant reduction in the 2,5-AS activity.

These results may partly be explained by the narrower range of acetaminophen's theoretically interferon-enhancing immunologic effect compared to aspirin and prednisone which have broader mixed immunologic effects, based on in vitro studies. PGE, which is inhibited by all 3 of the concomitant drugs used in this study, reduces lymphokine function (Kunkel et al., 1986), decreases antigen- and mitogen-induced lymphocyte proliferation (Leung and Mihich, 1980) and also decreases class Ia and class II MHC expression in vitro (Papiernik et al., 1986; Tripp et al., 1986). Arachidonic acid inhibition, which results in PGE inhibition among other effects, enhances IFN-dependent DNA binding factors and increases 2,5-AS activity after IFN administration in vitro (Hannigan and Williams, 1991). Were these effects mediated through PGE inhibition, drugs that inhibit PGE could enhance either the IFN-induced VSV yield reduction or 2,5-AS activity. Aspirin and prednisone, unlike acetaminophen, both inhibit induction of interferon-induced 2,3-IDO (Sayama et al., 1981) which is important in the development of the antiviral state (Carlin et al., 1989). Prednisone also acts post-transcriptionally to diminish IFN-induced effects. These IFN-inhibiting effects of aspirin and prednisone may counter their potentially beneficial antiviral effects mediated by PGE inhibition.

Whatever the mechanism of the acetaminophen-induced enhancement of IFN's antiviral effect at low IFN doses, any enhancement is inconsequential at high 18 m.u. IFN doses as previously reported (Witter et al., 1988). Our findings suggest that acetaminophen co-administration results in greater antiviral effect at lower doses. The similar effects of acetaminophen plus 3 m.u. IFN and 18 m.u. IFN alone may represent maximal antiviral doses, as measured by the VSV and 2,5-AS assays. We do not have observations of VSV and 2,5-AS at doses higher than 18 m.u. to confirm this possibility.

Acetaminophen and prednisone reduced symptoms by nearly half in our study, although these effects were not statistically significant using the rigorous criterion for multiple testing. Aspirin greatly reduced symptoms in some subjects, but its overall effects were erratic. All combinations tended to reduce the duration of fever compared to controls, but this was only significant in the prednisone group. The study size was sufficiently large to confidently exclude (power greater than 80%) a normalization of maximum temperature to below 38°C and a 2.1-h decrease in time spent above 38°C compared to the control group.

The unblinded aspect of the study design may have introduced bias in the measurement of clinical symptoms, although laboratory measurements should be minimally affected. Generalizations based on this study should be made cautiously given the single form of interferon studied, α_{2a}, and the health of our volunteers who may have a different tolerance for side effects compared to persons with viral infection.

We have shown that clinical toxicity and antiviral effects can be dissociated, both in magnitude and duration of effects. This finding may be explained by different IFN-induced mediators of acute clinical toxicity and antiviral effects, although some of these mechanisms may also be linked (Yaron et al., 1977; Baglioni et al., 1979; Rubin and Gupta, 1980; Dinarello et al., 1984; Carlin et al., 1989). These differences are not so clear at higher IFN doses where the influence of medications used to reduce side effects are apparently overwhelmed (Witter et al., 1988). At this low dose of interferon, however, it appears that one may take advantage of these differences in antiviral effect and toxicity by the use of concomitant medications to alter the therapeutic index of IFN. Controlling for these medications should also be considered in the design of clinical trials at similarly low doses of IFN.

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