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RELATIONSHIP BETWEEN THE ANTIVIRAL EFFECTS OF INTERFERONS AND THEIR ABILITIES TO DEPRESS CYTOCHROME P-450

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Abstract—Several hybrid human interferons have now been constructed by recombinant DNA techniques. Two of these hybrid interferons, IFN-αAD(Bgl) and IFN-αAD(Pvu) differ by only three amino acids, but IFN-αAD(Bgl) was fifteen times more potent than IFN-αAD(Pvu) in antiviral activity towards infection of mouse L-929 cells by vesicular stomatitis virus. Only the hybrid with the greater antiviral activity in the mouse depressed cytochrome P-450, aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase in the liver. These experiments demonstrate that minor changes in amino acid structure not only have a major effect on the antiviral properties of interferon but also influence the ability of interferon to depress cytochrome P-450 in the liver.

In both animals and man, cytochrome P-450 and its related drug biotransformation in the liver can be depressed during viral infections or following the administration of interferon-inducing agents [1–4]. Using mouse strains differing in a defined genetic locus controlling interferon production, Singh and Renton [5] demonstrated that the loss of drug biotransformation is related to the production of interferon. More recently the first direct evidence was provided that highly purified interferons depress hepatic cytochrome P-450 and that this effect is related to the antiviral effect in a particular species [6, 7]. The relationship between antiviral activity and the depressant effects on cytochrome P-450 by interferons and the structural basis for these effects may be investigated using structurally defined interferons of high purity which have different antiviral potency in a given species. In the present study, we utilized two hybrid human interferons which differ by only three amino acids to determine the relationship between effects of interferon on drug metabolism and antiviral activity in mice.

The human leucocyte, or IFN-α interferons are a family of related proteins of 165 or 166 amino acid residues [8], and each subtype has been found to have distinct antiviral properties [9]. The presence of common restriction enzyme sites in the genes for human IFN-α has allowed construction of hybrid human interferons. The Bgl 11 and Pvu 11 restriction sites in the genes for the IFN-αA and IFN-αD subtypes have been used to construct hybrid interferons containing either the first 61 or 91 amino acid residues of IFN-αA with the remaining portion of the interferon derived from IFN-αD [10]. These two interferons are designated IFN-αAD(Bgl) and IFN-αAD(Pvu) and, unlike other human IFN-α interferons, they have pronounced antiviral activity in mouse cells [10] and in mice [11, 12]. IFN-αAD(Bgl) and IFN-αAD(Pvu) differ only in three amino acid residues but IFN-αAD(Bgl) has 15 times greater antiviral activity when titrated in mouse cells and about 25 times greater activity when titrated in human cells [12].

MATERIALS AND METHODS

Materials. Highly purified human interferon hybrids, IFN-αAD(Bgl) and IFN-αAD(Pvu), were prepared, as described previously, in *Escherichia coli* 294 containing the appropriate plasmids [10, 11]. The protein sequence of the two hybrid interferons formed between two leukocyte subtypes differed by only three amino acids in positions 68, 79, and 85 numbered from the N-terminus [12]. These materials were purified to homogeneity, as assayed by polyacrylamide gel electrophoresis. Antiviral activity was determined by plaque reduction using vesicular stomatitis virus in mouse L929 cells [12] titrated against the NIH standard for interferon from the mouse (G-002-904-511). The specific activity for IFN-αAD(Bgl) was 3.8×10^8 units/mg protein and 2.5×10^7 units/mg protein for IFN-αAD(Pvu). The double-stranded polyribonucleic acid (poly rI · rC) of molecular weight greater than 100,000 was obtained from the Sigma Chemical Co., St. Louis, MO.

Animals. Inbred mice of the C57BL/6J strain were obtained from Jackson Laboratories, Bar Harbor,

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ME. Animals were acclimatised for at least 1 week in our own facility before use and were provided water and standard diet *ad lib*. Treated animals received i.p. injections of the interferon preparations or poly rI·rC diluted in sterile saline 24 hr before they were killed. Control animals received equivalent volumes of sterile saline given at the same time.

Microsomes. Hepatic microsomes were prepared as described by el Defrawy el Masry *et al.* [13] and were used the day they were prepared. Microsomal protein levels were determined by the method of Lowry *et al.* [14] using bovine serum albumin as a standard. Cytochrome P-450 and cytochrome b_5 levels in microsomes were determined by the method of Omura and Sato [15]. Microsomal aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase were determined by the method of Sladek and Mannering [16] and Nebert and Gelboin [17] respectively.

Statistics. Results were compared using the Newman–Keuls test for multiple comparisons [18].

RESULTS

Effect of interferon hybrids on cytochrome P-450. Twenty-four hours after the administration of 5×10^4 units (0.13 μg) of IFN-αAD(Bgl), the level of cytochrome P-450 in hepatic microsomes was depressed significantly by 37% compared to levels in saline-treated control mice (Fig. 1). Cytochrome b_5 levels in both groups of animals were identical. In contrast, the administration of 5×10^4 units (2.0 μg) of IFN-αAD(Pvu) had no effect on the levels of cytochrome P-450 or cytochrome b_5 in hepatic microsomes. Even when 10-fold greater amounts of IFN-αAD(Pvu) were utilized, cytochrome P-450 was unaffected. Cytochrome P-450 levels in hepatic microsomes were depressed significantly by 45% in animals treated with the interferon inducer poly rI·rC (10 mg/kg).

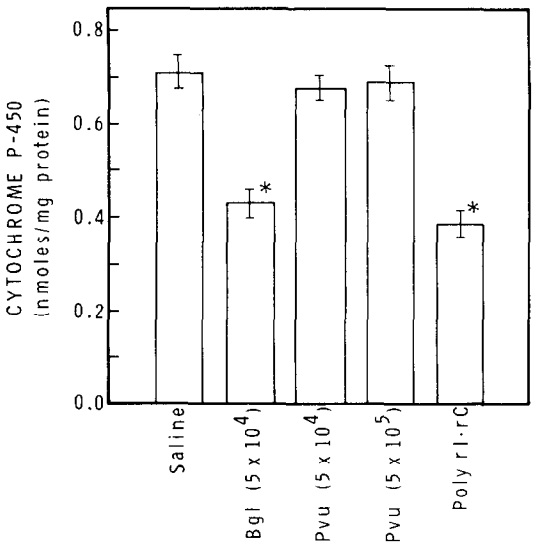

Fig. 1. Effect of hybrid interferons and poly rI·rC on cytochrome P-450 levels in hepatic microsomes. The IFN-αAD hybrid interferons are indicated by the restriction enzyme sites (Bgl and Pvu) used in their construction (see Materials and Methods). Animals were killed 24 hr after the administration of interferon. Each value is the mean ± standard error, N = 4. Key: (*) significantly different from saline-treated control, $P < 0.05$.

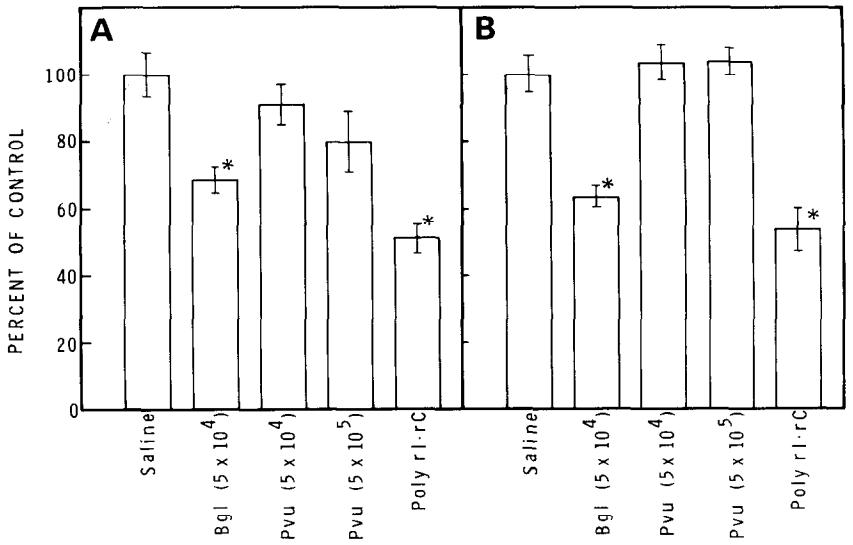

Fig. 2. Effect of hybrid interferons and poly rI·rC on aminopyrine *N*-demethylation (A) and benzo[*a*]pyrene hydroxylase (B) in hepatic microsomes. The IFN-αAD hybrid interferons are indicated by the restriction enzyme sites (Bgl and Pvu) used in their construction (see Materials and Methods). Animals were killed 24 hr after the administration of interferon. The results are expressed as a percentage of control values determined from saline-treated control animals killed at the same time. The control value for aminopyrine *N*-demethylase was 521 ± 33 μmoles HCHO formed/mg protein/hr and the control value for benzo[*a*]pyrene hydroxylase was 4.7 ± 0.4 nmoles 3-OH benzo[*a*]pyrene formed/mg protein/hr. Each value is the mean ± standard error, N = 4. Key: (*) significantly different from control, $P < 0.05$.

Effect of interferon hybrids on aminopyrine N-demethylase and benzo[a]pyrene hydroxylase activities. The administration of 5×10^4 units of IFN-αAD(Bgl) significantly depressed the level of aminopyrine *N*-demethylation and benzo[*a*]pyrene hydroxylation by 47 and 41%, respectively, compared to controls (Fig. 2). The administration of IFN-αAD(Pvu) had no effect on the biotransformation of either aminopyrine or benzo[*a*]pyrene. Poly rI·rC significantly depressed aminopyrine *N*-demethylase and benzo[*a*]pyrene hydroxylase by 66 and 49% respectively. In all of the experiments, the liver weight and total microsomal protein levels were unaffected.

DISCUSSION

The construction of a number of recombinant genes coding for human leucocyte interferons has resulted in the synthesis of hybrid interferons in *E. coli* [10]. These hybrid interferons have antiviral properties which are distinct from the parent interferons and vary widely in their abilities to inhibit plaque reduction following infection of cells with a variety of viruses [10]. The two hybrids used in these experiments were 15-fold different in their antiviral activities towards vesicular stomatitis virus in mouse L-929 cells even though they differed in only three amino acids at positions 68, 79, and 85. The greater activity of IFN-αAD(Bgl) in murine cell cultures was also reflected in its *in vivo* ability to protect mice against encephalomyocarditis virus infections [12]. The hybrid interferon with the greatest antiviral activity in the mouse, IFN-αAD(Bgl), depressed cytochrome P-450 and its related drug biotransformation in the liver, in contrast with IFn-αAD(Pvu) which had no effect. Even 10-fold higher doses of IFN-αAD(Pvu) by units had no depressant effects on cytochrome P-450 and this represents 150 times more material on a weight basis. In previous experiments using both crude and pure human interferons, only the preparations which had antiviral activity in the mouse were found to depress cytochrome P-450 [6]. It was suggested that the depressive effects of interferon on drug metabolism may be a property inseparable from the antiviral or antitumor activity of interferons. This idea was supported by Parkinson *et al.* [7] who found a correlation between the antiviral effects of three human interferons in murine cell lines and their abilities to depress cytochrome P-450 in the mouse liver. At the present time it is unknown if this effect of interferon is confined to human interferons which have antiviral activity in the mouse or if the effect would also occur with purified homologous mouse interferons tested in the mouse.

The properties of interferons are critically dependent on their structures, and in human fibroblast interferon a single amino acid change has been shown to abolish completely the antiviral effect in human cells [19]. The two highly purified hybrid interferons used in the present experiments differed by three amino acids and demonstrated that minor changes in amino acid structure not only affect the antiviral activity of interferons but have a major influence on the ability of interferon to depress cytochrome P-450. The potency of an interferon as an antiviral agent, however, does not correlate directly with its ability to depress cytochrome P-450. In the present studies, 15 times greater amounts of IFN-αAD(Pvu) compared to IFN-αAD(Bgl) were administered in order to achieve a comparable antiviral dose on a unit basis, but this dose had no effect on cytochrome P-450. Even 10 times greater doses of IFN-αAD(Pvu) on a unit basis were used without detectable effects on cytochrome P-450-dependent metabolism. The absence of effects of IFN-α-AD(Pvu) on cytochrome P-450 in the present experiments does not mean that this interferon is completely devoid of such effects. In recent experiments, Lee *et al.* [12] demonstrated that IFN-αAD(Pvu) at 2×10^4 units per mouse has a small but significant effect in preventing the toxicity caused by acetaminophen but does not prolong hexobarbital sleeping time. Both of these pharmacological responses which are mediated by cytochrome P-450 are profoundly affected by IFN-αAD(Bgl).

Despite an apparent overall relationship between the antiviral activities of interferons and their abilities to suppress cytochrome P-450-dependent metabolism, these effects can be separated: compared with IFN-αAD(Bgl), IFN-αAD(Pvu) had no effect on cytochrome P-450-dependent metabolism at doses that were 10 times greater on a unit basis or 150 times greater on a weight basis. The separation of the antiviral and the cytochrome P-450-depressant effects of interferons is important as it demonstrates that, using recombinant DNA methods, it is possible to produce interferons with desired properties without effects on the cytochrome P-450-mediated metabolism of drugs. The use of such interferons will decrease the possible occurrence of drug interactions when interferons are used concomitantly with other drugs during antiviral or antitumor therapy.

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