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SELECTIVE DEPRESSION OF HEPATIC CYTOCHROME P-450 HEMOPROTEIN BY INTERFERON INDUCERS

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Summary: The interferon inducing agents, polyriboinosinic: polyribocytidylic acid and tilorone, and Freund's complete adjuvant cause a marked depression of several components of the hepatic mixed-function oxidase system. Separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and quantitation by fluorescence gel scanning of different molecular weight species of cytochrome P-450 indicate that the depressant effect of these agents on the apoprotein moieties of cytochrome P-450 is of a specific nature.

INTRODUCTION

The administration of a wide variety of interferon inducing agents, including poly rI:rC and tilorone, cause a significant depression of the MFO system in rats (1). A marked depression of hepatic microsomal enzyme is also seen in developing and established adjuvant disease (2). The mechanism underlying this depression which occurs following the administration of interferon inducers of Freund's adjuvant is yet unknown, but may be initially associated with the interferon induction process itself. This study was undertaken to ascertain whether these

Abbreviations: Poly rI:rC, polyriboinosinic polyribocytidylic acid; MFO, mixed-function oxidase. Freund's adjuvant, FA; SDS, sodium dodecyl sulfate.

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agents depressed the various cytochrome P-450 hemoproteins equally or whether selective depression of only certain molecular weight species was evident.

MATERIALS AND METHODS

Chemicals: Tilorone (2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one dihydrochloride) was a gift from Richardson-Merril, Inc., Cincinnati, OH. Poly rI:rC was purchased from Sigma Chemical Co., St. Louis, MO as the disodium salt. Mycobacterium butyricum for the Freund's adjuvant preparation was obtained from Difco Laboratories, Detroit, MI and fluorescamine from Roche Diagnostics, Nutley, NJ.

Animals: Male Albino rats (Holtzman, Madison, WI) weighing 200-250 g were fed lab chow 'ad libitum'. The following materials were administered for three days (per kg of body weight); saline (2.0 ml, i.p. or p.o.); tilorone (50 mg in deionized water, p.o.); poly rI:rC (2.5 mg in deionized water, i.p.). Rats were killed 24 hr after the last administration of an agent. The Freund's adjuvant preparation, consisting of 0.5 mg of heat killed Mycobacterium butyricum suspended in 0.1 ml of mineral oil, was injected once subdermally in the distal one-third of the tail. Rats administered adjuvant or the same volume of mineral oil (controls) were killed 72 hr after treatment. Following decapitation of rats, livers were removed and hepatic microsomes prepared as described previously (3). Protein content of fresh microsomal preparations was determined by the Lowry method (4) and microsomes were stabilized with glycerol and solubilized with sodium deoxycholate as described previously (3).

Assays: Solubilized microsomal samples of predetermined protein concentration were labelled with fluorescamine and prepared for SDS-polyacrylamide gel electrophoresis by the addition of sodium dodecyl sulfate and 2-mercaptoethanol, as described by Ragland et al. (5). Electrophoresis was performed for approximately 3 hr at 1.5 ma per gel tube using a discontinuous gel system described by Neville (6). Gels were scanned in the electrophoresis tubes and the intensity of fluorescence was measured using a Gilford Linear Transport, Model 2410-S, equipped with a Model 2515-S Fluorescence gel accessory (sensitivity scale factor setting 100, scan speed 2 cm/min, chart speed 0.5 min/inch). Molecular weights for separated protein fractions were determined using a standard curve prepared from the electrophoretic mobilities in this gel system of known molecular weight proteins. The protein fractions estimated to have molecular weights within the range reported for cytochrome P-450 (43,000 to 54,000 daltons) were quantitated.

RESULTS

Fig. 1 shows two representative scans of fluorescamine-labelled and electrophoretically separated microsomes from tilorone treated and saline control animals. Molecular weight estimations of fractions 1-7, designated by decreasing

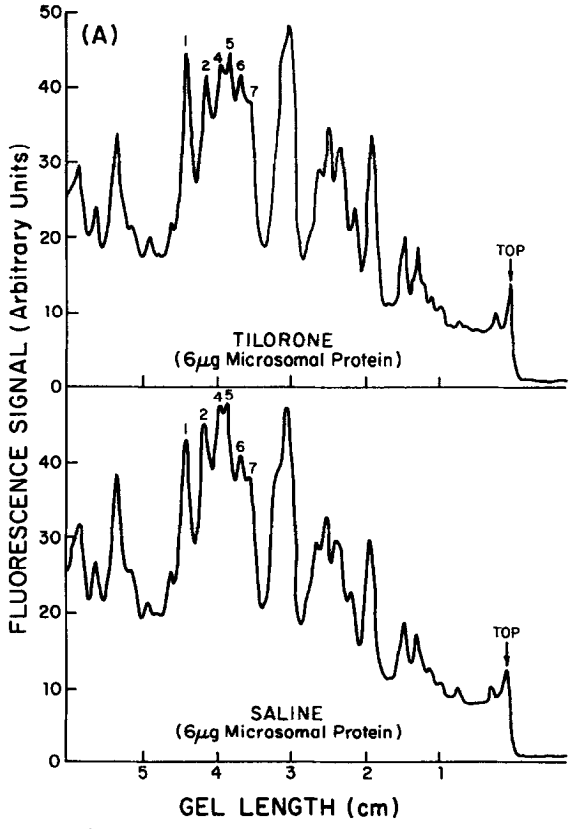

Fig. 1: Effect of tilorone on different molecular weight species of cytochrome P-450 hemoproteins. A) Fluorescence gel scans of 6 μg of microsomal protein from rats given saline, p.o. (50 mg/kg/day) for 3 days. Fractions 1-7 represent different molecular weight species within the range reported for cytochrome P-450.

electrophoretic mobility were 43,000; 45,500; 46,500; 48,000; 49,500; 51,500; 54,000 daltons, respectively. Fraction 3, which appears in scans of microsomes from phenobarbital treated rats (7), was absent from rats treated with interferon inducers, Freund's adjuvant, saline or mineral oil. The estimates are consistent with the molecular weight ranges of the multiple forms of cytochrome P-450 as reported by other investigators. Quantitation of the 6 molecular weight species present in microsomes from tilorone treated and saline control rats indicated no significant decrease in any cytochrome P-450 hemoprotein.

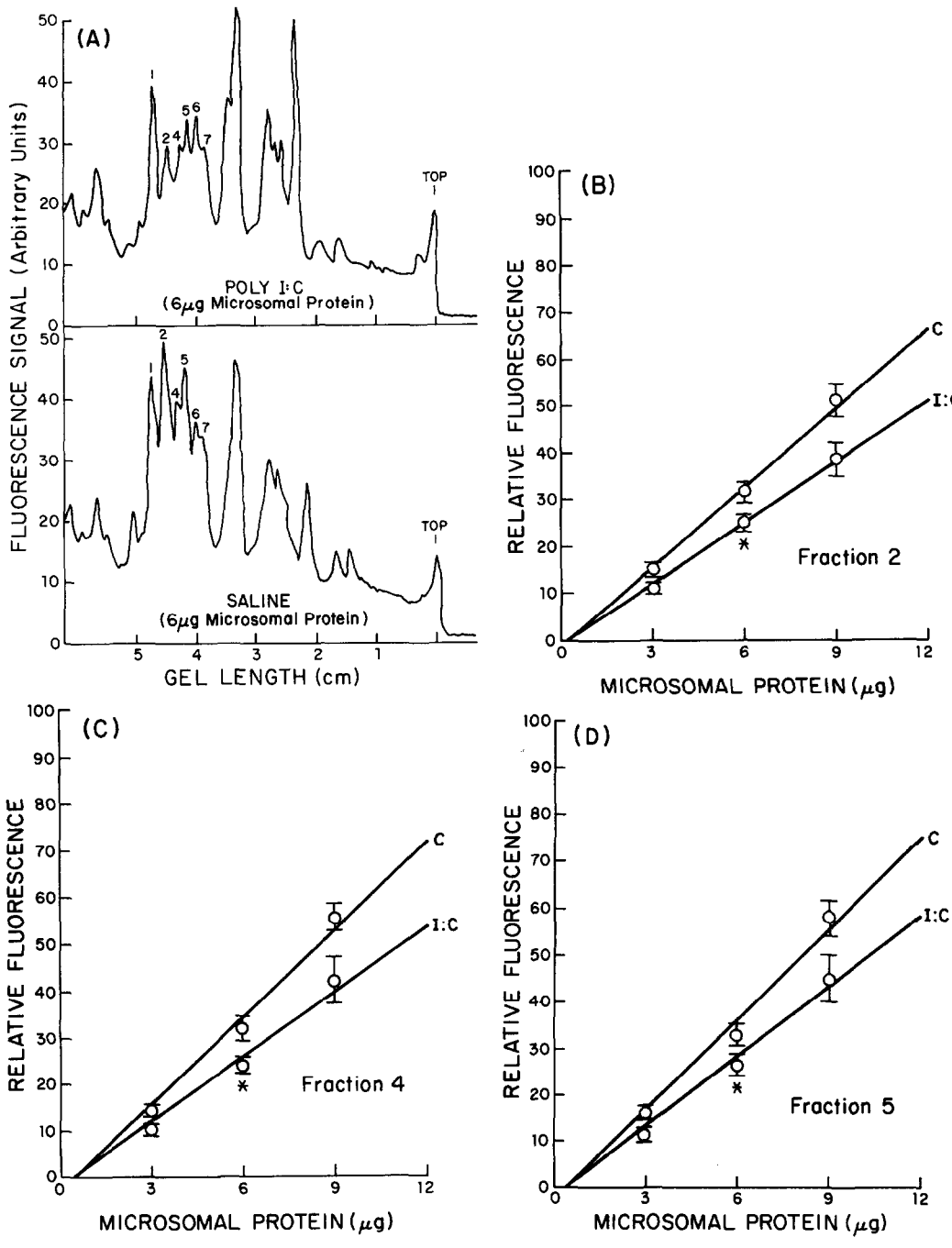

Fig. 2: Effect of poly rI:rC on different molecular weight species of cytochrome P-450 hemoproteins. A) Fluorescence gel scans of 6 μg of microsomal protein from rats given saline, i.p. or poly rI:rC, i.p. (2.5 mg/kg/day) for 3 days. B,C,D) Plots of relative fluorescence vs. microsomal protein concentration for fractions 2, 4 and 5, respectively, from rats given poly rI:rC (I:C) or saline (C). Fluorescence intensity is expressed as mean ± S.E., n = 8. *Denotes treatment significantly ($p<0.05$) reduced the quantity of fractions 2, 4 and 5 by 26.1%, 25.9% and 23.3%, respectively. Fluorescence was linear over the three-fold range of microsomal protein applied to the electrophoresis gel column.

Two fluorescence scans of microsomal protein from rats receiving poly rI:rC or saline are presented in Fig. 2A. The plots of figs. 2B-D illustrate that poly rI:rC treatment significantly reduced the quantity of 3 of the 6 proteins considered to be cytochrome P-450. Fractions 2, 4 and 5 were decreased 26.1% 25.9% and 23.3%, respectively, from corresponding control levels.

Fig. 3A shows representative fluorescence gel scans of microsomal protein from rats administered either FA or mineral oil.

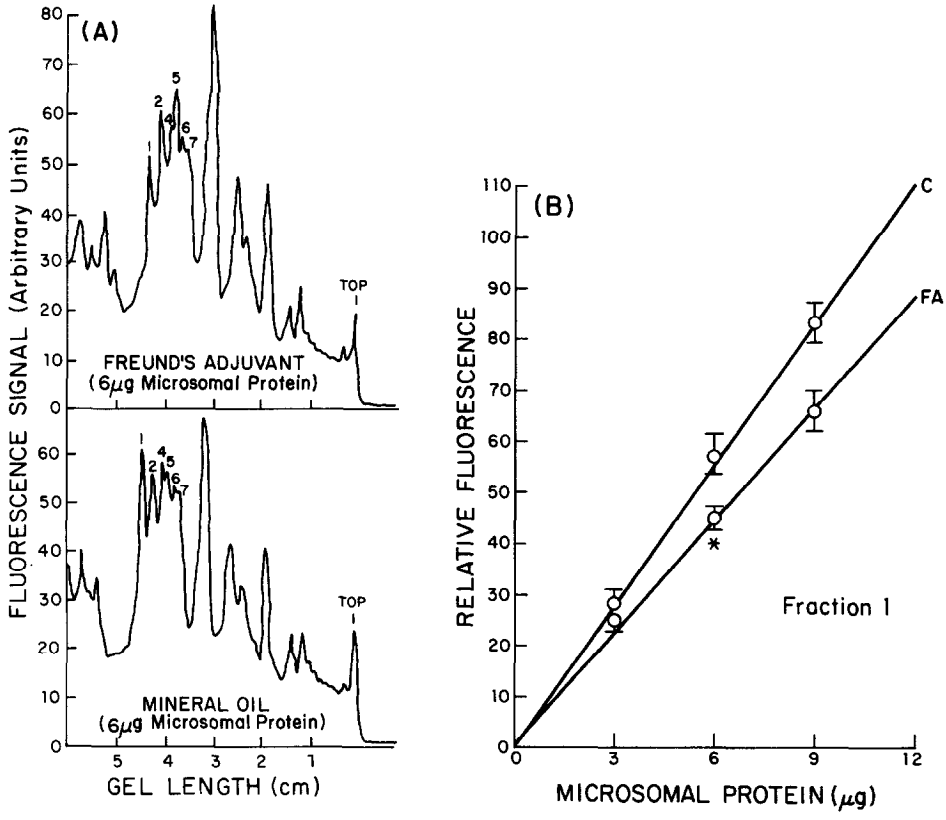

Fig. 3: Effect of Freund's adjuvant on different molecular weight species of cytochrome P-450 hemoproteins. A) Fluorescence gel scans of 6 μg of microsomal protein from rats given mineral oil or Freund's adjuvant in one dose administered subdermally in the tail. B) Plot of relative fluorescence vs. microsomal protein concentrations for fraction 1 from rats given mineral oil (c) or Freund's adjuvant (FA). Fluorescence intensity is expressed as mean ± S.E.M., n = 8. *Denotes treatment significantly ($p<0.05$) reduced the quantity of fraction 1 by 19.9%.

One cytochrome P-450 hemoprotein, the 43,000 molecular weight species, was significantly decreased (19.9%) in rats given FA (Fig. 3B).

The fluorescence signals of the 6 gel bands in the M.W. range of the cytochrome P-450 hemoproteins were linear over a three-fold range of microsomal protein applied to the gels regardless of experimental treatment.

DISCUSSION

In an effort to characterize the cytochrome P-450 present after tilorone, poly rI:rC and FA treatment, the individual molecular weight species of the cytochrome P-450 hemoproteins were quantitated by means of a sensitive fluorescence technique developed for the quantitative assay of proteins in polyacrylamide gels (5). Although it has been demonstrated that cytochrome P-450, as measured by its carbon monoxide - binding spectrum, was decreased following treatment with tilorone, poly rI:rC and FA, it has not been ascertained whether these agents decrease certain species of cytochrome P-450 hemoproteins selectively or if the depressant effect is general in nature. Using the fluorescence gel electrophoresis technique, our data suggest that tilorone administration did not significantly reduce the quantity of any of the separated cytochrome P-450 hemoproteins. Treatment of animals with poly rI:rC, however, changed the cytochrome P-450 profile of rat hepatic microsomes by quantitatively decreasing three of the six putative species of cytochrome P-450. Finally, pretreatment of rats with FA decreased the quantity of yet another molecular weight species (43,000) of cytochrome P-450. These investigations suggest that the depression of cytochrome P-450 levels seen with poly rI:rC and FA is due in part to a decrease in the concentration of cytochrome P-450 apoprotein. These

findings do not rule out the possibility that some qualitative change in the carbon monoxide-binding activity of one or more of the multiple forms of cytochrome P-450 may also be involved. Although tilorone causes a pronounced depression of MFO system function as revealed by the suppression of the CO-binding difference spectrum, the protein concentration of each cytochrome P-450 in the membrane is not altered. Thus, tilorone may have an effect on the molecular configuration or the position in the membrane of one or more of the cytochromes. Cheng, et al., (8) have observed that various lipid diets can affect the reactivity of cytochrome P-450 without affecting total apocytochrome P-450 concentration. It is evident from this investigation that depression of MFO activity by tilorone, poly rI:rC and FA involve different mechanisms. Partial loss of the protein moiety of certain cytochrome P-450 hemoproteins following treatment with poly rI:rC and FA has been shown in this study, but how this reduction is accomplished has not been elucidated. Decreased synthesis or increased degradation by normal pathways of specific P-450 apoproteins are possible explanations and plausible targets for further research.

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