SUPPRESSIVE EFFECT OF INTERFERON INDUCER, POLYRIBOINO-
SINIC ACID-POLYRIBOCYTIDYLIC ACID ON INDUCTION OF URI-
DINE DIPHOSPHATE-GLUCURONYLTRANSFERASES AND MONO-
OXYGENASES IN LIVER MICROSOMES OF RATS

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The effect of polyriboinosinic acid-polyribocytidylic acid [poly(I)-poly(C)] on glucuronyltransferase activities toward 4-nitrophenol and 4-hydroxybiphenyl in liver microsomes of Wistar rats was examined by its single or co-administration with 3-methylcholanthrene and phenobarbital. The increased 4-nitrophenol glucuronyltransferase activity by treatment with 3-methylcholanthrene was significantly suppressed following the co-administration with poly(I)-poly(C), although the activity was not affected by the treatment with poly(I)-poly(C) alone. In addition, 4-hydroxybiphenyl glucuronyltransferase activity decreased or tended to decrease by the treatment with poly(I)-poly(C) alone, and the activity induced by phenobarbital was strikingly decreased following the co-administration with poly(I)-poly(C). This result suggested that poly(I)-poly(C) comprehensively decrease the induction of glucuronyltransferases regardless of their multiple forms. Furthermore, contents of cytochromes P-450 and b5 were also decreased by the treatment with poly(I)-poly(C) alone or the co-administration with the inducers. Concomitantly, arylhydrocarbon hydroxylase and benzphetamine N-demethylase activities were significantly decreased by the treatment alone or the co-administration with the inducers. These findings supported a view that the suppressive effect of poly(I)-poly(C) may be derived from the prevention of de novo synthesis of the apoprotein of the enzymes and/or the increased degradation.

Keywords — uridine diphosphate-glucuronyltransferase; 4-nitrophenol glucu-
ronyltransferase; 4-hydroxybiphenyl glucuronyltransferase; polyriboino-
sinic acid-polyribocytidylic acid [poly(I)-poly(C)]; suppression; arylhydrocarbon hydroxylase; benzphetamine N-demethylase; cytochrome P-450; cytochrome b5; heme oxygenase

INTRODUCTION

Glucuronidation is the most widespread form of conjugation in mammalian metabolism, which is best known as a detoxication reaction. Uridine diphosphate (UDP)-glucuronyltransferase (GT) activity rises characteristically following administration of various chemicals in analogy with monoxygenase activity.1,2) On the other hand, the drug metabolizing ability is conversely decreased by specific substances through varied mechanisms. 2,2-Diethylaminoethyl-2,2-diphe-
nylvalerate HCl (SKF-525A) and 2,4-dichlorophenylphenoxyethylamine (DPEA) HCl inhibit monoxygenase activity competitively.3,4) Suicide substrates inactivate their own metabolism by binding to cytochrome P-450.5–7) Furthermore, several agents that stimulate the immune system and that induce the formation of interferon are also known to decrease the monoox-
genase activity and content of cytochrome P-450.8,9) Singh and Renton indicated that interferon decreased hepatic drug biotransformation and explained that the depression of drug elimination which occurred following the administration of interferon inducers was likely due to the interferon induced.10) The agents of the last category were especially interesting because of their possible effect of prevention of de novo synthesis11) or the degradation of cytochrome P-450 apoprotein.12)

In addition to monoxygenases, GT activity is known to be decreased by these agents. Corynebacterium parvum vaccine, an immunostimulator, for example, was reported to reduce 2-aminophenol glucuronide formation and to suppress its increased formation by phenobarbi-
tal (PB)-treatment in mice, without a significant effect on the induced cytochrome P-450 content.

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and O-demethylase activity following administration of PB. 13 An endotoxin which is a potent interferon inducer was found to decrease bilirubin GT activity. 14 Thus, the suppressive effect of immunostimulators on GT activity has been studied, although, their effect on heterogenous GTs is not well understood.

In the present study, the suppressive effect of an interferon inducer, synthetic double stranded polyribosinosinic acid-polyriboctydyllic acid [poly(I)-poly(C)] on the activities of 4-nitrophenol (4-NP) GT and 4-hydroxybiphenyl (4-OHBP) GT was investigated following the administration of 3-methylcholanthrene (MC) and PB in rats. Moreover, its effect was compared with that on the contents of cytochromes P-450 and b$_5$ and monoxygenase activities.

MATERIALS AND METHODS

Chemicals — Poly(I)-poly(C) was purchased from Yamasa Shoyu Co., Ltd., Chiba. 3-Hydroxybenzo[a]pyrene and benzphetamine HCl were kindly donated by Dr. N. Kinoshita, School of Health Sciences, Kyushu University, Fukuoka, and by Dr. R. A. Neal, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn., respectively.

Animal Treatment — Adult male Wistar rats (7 weeks old, weighing approximately 240 g) were used in all experiments and housed in stainless steel cages with water and a standard laboratory chow ad libitum. PB (100 mg/kg in saline) and MC (40 mg/kg in corn oil) were administered i.p. at a single dose. Poly(I)-poly(C) (10 mg/kg in saline) was injected i.p. 2 h before and every 24 h for 2 d after the injection of PB and MC. Control groups were injected with the vehicle alone at an equivalent volume to that of the treated groups. Following fasting for 22 h, the animals (3—4 rats per group) were killed by decapitation 2 d after the injection of PB and MC.

Assay of Enzyme Activities — Liver microsomes were prepared following the method reported previously. 15 4-NP GT activity was determined spectrophotometrically following the method of Isselbacher et al. 10 by incubating the microsomes in 100 mM potassium phosphate buffer, pH 7.4 containing 0.05% Brij 58, with 10 mM MgCl$_2$, 2 mM UDP-glucuronic acid and 0.2 mM 4-NP in a total volume of 0.5 ml. 4-OHBP GT activity was assayed fluorometrically by incubating 4-OHBP in the same medium as above, following the method of Bock et al. 17

![Graph showing the effect of Poly(I)-Poly(C) on the induction of 4-Nitrophenol Glucurononyltransferase (4-NPGT) and 4-Hydroxybiphenyl Glucurononyltransferase (4-OHBP GT) by PB and MC-Treatment.](image)

Data are expressed as relative activities (mean ± S.E.) to those of the control group. The activities (mean ± S.E.) in the control of PB-(A) and MC-treated group (B) were determined, respectively, as follows: 4-NPGT, (A) 14.1 ± 0.7 and (B) 17.2 ± 1.3 nmol of 4-NP glucuronide formed/mg protein/min, and 4-OHBP GT, (A) 1.87 ± 0.21 and (B) 1.73 ± 0.10 nmol of 4-OHBP glucuronide formed/mg protein/min. Each group consisted of 4 rats.

a) Significantly different from the control group (p < 0.05). b) Significantly different from the inducer treated group (p < 0.05).
hydrocarbon hydroxylase (AHH) activity in microsomes was determined as described by Nebert and Gelboin.\textsuperscript{18} Benzphetamine N-demethylase activity was determined in the incubation medium described previously,\textsuperscript{15} measuring the amount of formaldehyde formed by the method of Nash.\textsuperscript{19} Content of cytochromes P-450 and $b_5$ in the microsomes was determined, according to the methods of Omura and Sato, and Omura and Takesue, respectively.\textsuperscript{20,21} Microsomal heme oxygenase activity was assayed by the method of Maines and Kappas.\textsuperscript{22} Protein was determined by the method of Lowry \textit{et al.} using bovine serum albumin as a standard.\textsuperscript{23}

RESULTS

\textbf{Effect on UDP-Glucuronyltransferase Activities}

The effect of treatment with poly(I)-poly(C) alone or co-treatment with inducers on GT activities was studied. As shown in Fig. 1, 4-NP GT activity was not affected by poly(I)-poly(C) alone; however, the 2.6-fold increase in activity, following MC-treatment, was reduced almost to the control level by the co-treatment with poly(I)-poly(C). PB-treatment caused a slight increase in this activity, which was also suppressed by co-treatment with poly(I)-poly(C). In addition, 4-OHBP GT activity decreased or tended to decrease by treatment with poly(I)-poly(C) alone, and the induction of this GT activity by either PB or MC was strikingly suppressed below the control level by co-treatment with poly(I)-poly(C). Thus, poly(I)-poly(C) decreased significantly the increased GT activities by inducers more than these activities in the control animals.

\textbf{Effect on Contents of Cytochromes P-450 and $b_5$, and Monoxygenases Activities}

The suppressive effect of poly(I)-poly(C) on contents of cytochromes P-450 and $b_5$ and on activities of monoxygenases was compared with that on GT activities. Fig. 2 shows the effect on contents of the cytochromes. Cytochrome P-450 content was significantly decreased to 70–80% of the control level by treatment with poly(I)-poly(C) alone. The induction of cytochrome P-450 by PB and MC was also significantly suppressed to the control level by co-administration of poly(I)-poly(C). Furthermore, cytochrome $b_5$ content was also decreased following treatment with poly(I)-poly(C) alone or co-treatment with inducers.

Monoxygenase activities in control rats and

\begin{figure}
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Effect of Poly(I)-Poly(C) on the Induction of Cytochromes P-450 and $b_5$ by PB and MC-Treatment}
\textit{Data are expressed as relative contents (mean ± S.E.) to those of the control group. The contents (mean ± S.E.) in the control of PB-(A) and MC-treated group (B) were as follows: cytochrome P-450 (A) 0.60 ± 0.03 and (B) 0.86 ± 0.03 nmol/mg protein, and cytochrome $b_5$ (A) 0.25 ± 0.02 and (B) 0.33 ± 0.01 nmol/mg protein. Each group consisted of 4 rats.}
\textit{a) Significantly different from the control group (p < 0.05). b) Significantly different from the inducer treated group (p < 0.05).}
\end{figure}
pretreated rats with inducers were also suppressed by treatment with poly(I)-poly(C). As seen in Fig. 3, benzphetamine N-demethylase activity which was induced approximately 3.4-fold over the control value by PB-treatment was strikingly suppressed to 50% by co-treatment with poly(I)-poly(C). The induction of AHH activity was similarly suppressed by poly(I)-poly(C). The figure also shows that benzphetamine N-demethylase activity was reduced by the treatment with MC and this decrease was further depressed by co-administration with poly(I)-poly(C). The induction of AHH activity by MC was also significantly suppressed by poly(I)-poly(C), but this activity was decreased no more than approximately 20%.

**Effect on Heme Oxygenase**

Since cytochrome P-450 consists of protoheme and apoprotein, the above suppressive effects of poly(I)-poly(C) on monoxygenase activity must be considered in the following two aspects. One is a wasting effect of this suppressor on heme pool by inducing heme oxygenase. Another is prevention of the *de novo* synthesis of apoprotein or increased degradation, resulting in a decrease of the amount of apoprotein. The effect of poly(I)-poly(C) on the activity of microsomal heme oxygenase is shown in Table 1. The activity was decreased to 62% of the control level by the administration of PB and increased about 3 times the control level by administration of poly(I)-poly(C) alone. Thus, the increased heme oxygenase activity may be correlated with the suppressive effect of poly(I)-poly(C) on the monoxygenase activities.

**DISCUSSION**

The depression of microsomal mixed-function oxidase activity following administration of interferon inducers has been well established. The present study demonstrated also that an interferon inducer, poly(I)-poly(C), reduced cytochrome content and monoxygenase activities in untreated animals and suppressed the increased contents and activities in rats treated with inducers. Poly(I)-poly(C) also suppressed significantly the induction of 4-NP and 4-OHBP GT activities by MC and PB, respectively.

The induction of cytochrome P-450 by MC was suppressed completely by poly(I)-poly(C), but the decrease of AHH activity was no more

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**FIG. 3.** Effect of Poly(I)-Poly(C) on the Induction of AHH and Benzphetamine N-Demethylase by PB- and MC-Treatment

Data are expressed as relative activities (mean ± S.E.) to those of the control group. The activities (mean ± S.E.) in the control of PB-(A) and MC-treated group (B) were determined, respectively, as follows: AHH, (A) 0.14 ± 0.004 and (B) 0.06 ± 0.009 nmol of 3-hydroxybenzo[a]pyrene formed/mg protein/min, and benzphetamine N-demethylase, (A) 6.86 ± 0.76 and (B) 8.33 ± 0.78 nmol of HCHO formed/mg protein/min. Each group consisted of 4 rats.

a) Significantly different from the control group (p < 0.05). b) Significantly different from the inducer treated group (p < 0.05).
TABLE I. Effect of PB and Poly(I)-Poly(C) on the Activity of Microsomal Heme Oxygenase

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heme oxygenase (pmol/mg protein/min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>66.3 ± 9.5</td>
<td>[4]</td>
<td>(1.00)</td>
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<tr>
<td>Poly(I)-poly(C)</td>
<td>182.4 ± 14.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>[3]</td>
<td>(2.75)</td>
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<tr>
<td>PB</td>
<td>41.3 ± 11.4</td>
<td>[5]</td>
<td>(0.62)</td>
</tr>
<tr>
<td>PB + poly(I)-poly(C)</td>
<td>185.5 ± 16.8&lt;sup&gt;a, b&lt;/sup&gt;</td>
<td>[4]</td>
<td>(2.80)</td>
</tr>
</tbody>
</table>

The values represent the mean ± S.E. [numbers of rats]. The values in parentheses represent the relative value to the control.  
<sup>a</sup> Significantly different from the control group (p < 0.05).  
<sup>b</sup> Significantly different from the inducer treated group (p < 0.05).

than approximately 20%. Renton et al. have also remarked upon the difference between suppression of spectral P-450 content and AH activity. The discrepancy of suppression between P-450 content and AH activity in rats co-treated with MC and poly(I.C) remains obscure.

In recent years, the functional heterogeneity of GT has been attributed to the multiplicity of the enzyme. Bock et al. has classified GT into two categories; GT<sub>1</sub>, which is inducible by MC and catalyzes the conjugation of flat substrates such as 4-NP, 2-aminophenol, 2-naphthol and 4-methylumbelliferone, and GT<sub>2</sub> which is inducible by PB and conjugates bulky substrates such as 4-OHBP, morphine and bilirubin. Bock et al. isolated MC-inducible and PB-inducible GTs, and Falany and Tephly purified 3 GTs, one of which was MC-inducible. Recently, Mackenzie et al. separated GT into 3 types according to the charge heterogeneity. Thus, both 4-NP and 4-OHBP GT activities determined in this study represent two distinct activities of multiple GTs. The induction of 4-NP and 4-OHBP GTs by MC and PB, respectively, was significantly suppressed by the concomitant administration of poly(I)-poly(C). This result shows that the interferon inducer effects the induction of GT activity regardless of its multiplicity. On the other hand, treatment with poly(I)-poly(C) alone decreased or tended to decrease 4-OHBP GT, but resulted in an insignificant effect on 4-NP GT. The origin of this difference in contrast to the evident decrease in cytochrome contents and monoxygenase activities following the treatment with poly(I)-poly(C) alone remains obscure, but it seems likely that individual GTs have a varied turnover rate and are influenced differently by poly(I)-poly(C).

The present result agreed with previous reports that Escherichia coli endotoxin, an interferon inducer, decreases bilirubin GT<sub>1</sub> although it does not affect 4-NP GT activity. The depressive effect of interferon inducers seems to be greater for GT<sub>2</sub> than for GT<sub>1</sub>. Macnee and Nimmo-Smith reported that C. parvum vaccine decreased activity of 2-aminophenol GT and suppressed induction of the activity by PB, without having a significant effect on the increased cytochrome P-450 content and induced O-demethylation of 4-nitroanisole by the inducer. This discrepancy with our result in cytochrome P-450 content and monoxygenase activity may arise from the different period of treatment. They measured the effect on day 13 after administration of the vaccine. Thus, it seems to take a longer period for the recovery of the decrease of GT activity than that of cytochrome P-450.

Numerous investigators have examined the mechanism of the suppression by interferon inducers. El Azhary et al. found a lowered half life of heme moiety of the cytochrome P-450 and an increased incorporation of heme precursor following poly(I)-poly(C)-treatment. Furthermore, several agents that stimulate immune systems and that induce the formation of interferon have been shown to increase heme oxygenase activity and decrease cytochrome P-450-linked monoxygenase activities. The present results also supported that the decrease of cytochrome contents and monoxygenase activity resulted, at least in part, from the increased heme oxygenase activity. However, the decreased activities of non heme protein, GT, strongly suggested the presence of another mechanism of the suppression. Singh and Renton have recently reported that poly(I)-
poly(C) affect mainly through a depression in the synthesis of the apoprotein of cytochrome P-450 by measuring the incorporation of \( ^{14}C \)-leucine into partially purified cytochrome P-450.\[^{12}\] On the other hand, Zerkle and Wade demonstrated that the incorporation of \( ^{14}C \)-leucine into a fraction containing cytochrome P-450 was increased following poly(I)-poly(C)-treatment, drawing another conclusion that this suppressor lower cytochrome P-450 content by increasing the degradation rather than by depressing the synthesis.\[^{11}\] To resolve these discrepancies, further investigation is necessary.

We reported here that the interferon inducer poly(I)-poly(C) caused a marked suppression of the induction of 4-NP and 4-OHBP GT activities together with the reduction of cytochrome content and monooxygenase activities. This supports the view that the suppressive effect may originate from the prevention of \textit{de novo} synthesis of the apoprotein of the enzymes and/or the increased degradation. Interferon inducers suppress vast drug metabolizing abilities.

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