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MEDROXYPROGESTERONE ACETATE IMPROVEMENT OF THE HEPATIC DRUG-METABOLIZING ENZYME SYSTEM IN RATS AFTER CHEMICAL LIVER INJURY

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Abstract—Medroxyprogesterone acetate (MPA) has an inducing effect on the hepatic drug-metabolizing enzyme system in the rat. The effect of MPA on the liver metabolism was further evaluated here by investigating the restoration of hepatic function after chemical liver injury in female rats. The hepatic injury was induced by pretreating the animals with CCl_4 and dimethylnitrosamine for 4 weeks, after which rats treated with MPA for a week were compared with rats showing spontaneous regeneration upon treatment with the MPA vehicle only. Changes in various parameters of the drug-metabolizing enzyme system were used as indices of hepatic function together with liver protein content. The results showed that MPA therapy increased the cytochrome P-450 content and the activity of NADPH–cytochrome *c* reductase, the monooxygenase enzymes benzo[*a*]pyrene hydroxylase and aminopyrine *N*-demethylase, epoxide hydrolase and glutathione *S*-transferase. MPA increased the relative values in the rats with liver injury almost equally to, or even more than, that seen in the intact animals in comparison to the corresponding vehicle-treated rats. MPA seemed to enhance protein synthesis during liver regeneration, as indicated by changes in total liver protein and in the gel electrophoresis pattern of the microsomal proteins. The hepatic enzyme induction and enhancement of protein synthesis achieved by MPA after liver injury may be of value in the treatment of liver diseases.

The liver may be damaged by environmental chemicals or their reactive metabolites [1, 2], in which case histological examination will reveal mild to severe morphological changes such as fat accumulation, necrosis and fibrosis while the biochemical data often reflect impaired protein synthesis. The activation of hepatocellular protein synthesis may therefore have beneficial effects on subjects with liver injury. Treatment with an inducing drug such as medroxyprogesterone acetate (MPA) improves the clinical condition of patients with various liver diseases, such as alcoholic cirrhosis or primary biliary cirrhosis [3, 4]. MPA induces the activity of the hepatic drug-metabolizing enzyme system [5, 6], a phenomenon associated with increased protein synthesis [7, 8]. The exact mechanism by which the improvement in liver function is brought about is still unknown.

This study was undertaken to evaluate the effect of MPA therapy on the restoration of hepatic function after chemical injury in an animal model. Liver damage was induced by the administration of CCl_4 and dimethylnitrosamine (DMN) to female rats, after which the effects of MPA were compared with the changes occurring during spontaneous regeneration. Various parameters of the xenobiotic-metabolizing enzyme system were used as indices of hepatic function, together with measurement of the liver protein content. MPA was also given simultaneously with CCl_4 to test its effect on the liver during the development of injury.

MATERIALS AND METHODS

Chemicals. MPA (Lutopolar®) and Lutopolar vehicle (V) were kindly supplied by Medipolar Ltd (Oulu, Finland). [7-^{3}H]Styrene oxide (sp. act. 61.9 μCi/mmole) was a generous gift from Dr Max Parkki.† All the other chemicals were of the highest grade commercially available.

Animals. Female Wistar rats (161 ± 17 g) were used. The animals were allowed water and standard rodent chow *ad lib.* and kept in plastic cages under controlled light and temp conditions.

Treatment of animals. The 54 rats were divided into nine groups of six rats each. Groups CCl_4, CCl_4–V and CCl_4–MPA received CCl_4 [1 ml/kg body wt (in corn oil 1:1)] s.c. on the first and third day of each week over a 4-week period, and groups DMN, DMN–V and DMN–MPA were given DMN [10 μl/kg body wt (diluted 1:100 with 0.15 M NaCl)] i.p. on the first three days of each week over a 4-week period. Groups C, V and MPA received no treatment during that time.

After 4 weeks the animals in groups C, CCl_4 and DMN were killed by decapitation. Groups MPA, CCl_4–MPA and DMN–MPA received MPA [100 mg/kg body wt (in Lutopolar V)] i.p. daily for 7 days. During the same period MPA V alone was given to groups V, CCl_4–V and DMN–V. The last

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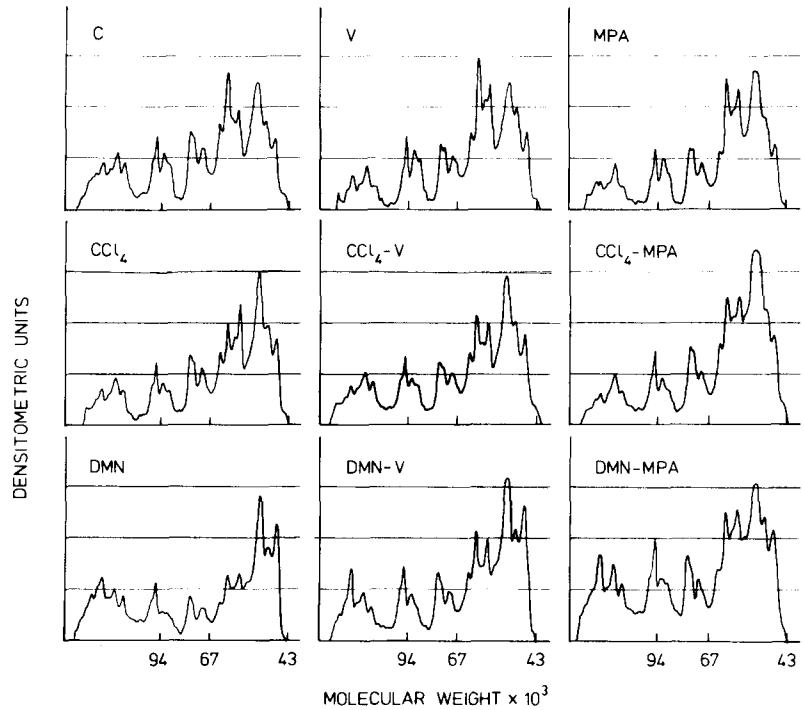

Fig. 1. Densitometric scans of SDS–polyacrylamide gel electrophoresis of the hepatic microsomal proteins stained with Coomassie brilliant blue in the various groups of rats. The rats were pretreated with CCl_4 and DMN for 4 weeks to produce the liver injury and then given MPA or its vehicle (V) for a week as depicted in Materials and Methods. Hepatic microsomal fractions were pooled from six animals, except in the DMN–MPA group, where there were five rats. Thirty micrograms of the sample protein was applied to each well in the slabs. The slabs were photographed after staining and the scans made from the photographic slides. Phosphorylase b (mol. wt 94,000), bovine albumin (mol. wt 67,000) and ovalbumin (mol. wt 43,000) were used as mol. wt standards.

doses were given 24 hr before the rats were killed.*

In a second experiment 16 rats were divided into four groups of four rats each. CCl_4 [0.75 ml/kg body wt (in corn oil 2:3) s.c.] and MPA [100 mg/kg body wt (in Lutopolar V) i.p.] were given separately or simultaneously daily for 7 days. The control group received corresponding amounts of V. The rats were killed by decapitation 24 hr after the last dose.

Preparation of microsomes. After decapitation the liver was quickly removed and homogenized in 4 vol of 0.1 M phosphate buffer (pH 7.4) in a glass Potter–Elvehjem homogenizer. The homogenate was centrifuged for 20 min at 10,000 *g*. Part of the supernatant fraction was stored at −40° for enzymatic determinations, and the remaining part was centrifuged at 105,000 *g* for 60 min. The pellet obtained was resuspended in 30% glycerol in 0.25 M phosphate buffer (pH 7.25), so that 1 ml of suspension contained the microsomes from 1 g of the liver. The suspension was stored at −40°.

Assays of the microsomal fraction. Cytochrome P-450 (Cyt. P-450) was assayed by the method of Omura and Sato [9]. NADPH–cytochrome *c* (NADPH–cyt. *c*) reductase activity was measured as described by Masters *et al.* [10]. Epoxide hydrolase (EH) activity was determined by a modification of the procedure described by Oesch *et al.* [11] in a reaction mixture containing 0.425 ml 0.125 M Tris–HCl buffer (pH 9.0) and 0.025% Tween 80, 50 μl microsomal suspension and 25 μl 40 mM [^{3}H]styrene oxide. After 15 min incubation at 37°, 5 ml petroleum ether was added and the reaction vessel put in an ice bath. The reaction mixture was extracted twice with 10 ml petroleum ether and the organic phase was discarded. Styrene glycol was measured in the aqueous phase.

SDS–polyacrylamide gel electrophoresis. The electrophoresis of proteins in the microsomal fraction was performed according to Laemmli [12] using a 3.8% acrylamide stacking gel and 12.6% running gel. The gels were 82 × 82 × 4.9 mm slabs, accommodating 12 samples per gel. The protein bands were visualized using Coomassie brilliant blue staining. Densitometric chromatograms were obtained from the photographic slides of the slabs.

Assays of the 10,000 g *supernatant fraction.* The activities of benzo[*a*]pyrene hydroxylase (BP-hydroxylase) and aminopyrine *N*-demethylase (AMP-demethylase) were determined by the methods described by Nebert and Gelboin [13] and Nash [14] respectively. The activity of BP-hydroxylase was expressed in 3-hydroxybenzo[*a*]pyrene equivalents.

Glutathione S-transferase (GST). Liver samples of

* The 24-hr urine and blood samples withdrawn from the ophthalmic venous plexus with a capillary tube under diethyl ether anesthesia were collected for other experiments.

10 mg were homogenized in 300 μl 0.25 M sucrose with a glass Potter–Elvehjem homogenizer. The activity of GST was measured in a reaction mixture containing 1 ml 0.4 M phosphate buffer (pH 6.5), 0.5 ml 3 mM reduced glutathione, 1.45 ml water, 50 μl 30 mM 1-chloro-2,4-dinitrobenzene in dimethylsulfoxide and 10 μl liver homogenate. The absorbance change at 340 nm was measured from the linear part of the curve over 5 min. The extinction coefficient was 9.6 mM^{-1} cm^{-1} [15].

Protein determination. Protein content was determined by the method of Lowry *et al.* [16] with bovine serum albumin as the standard.

Statistical analysis. Differences between two groups of data were evaluated by the Student's *t*-test. Differences between three groups of data were examined by using the Bonferroni *t*-statistic [17].

RESULTS

Effect of MPA on intact animals

MPA treatment enhanced liver function in the intact rats. An increase in liver wt was noted, associated with a parallel change in the protein content. The amount of Cyt. P-450 and the activity of NADPH–cyt. *c* reductase, BP-hydroxylase, AMP-demethylase, EH and GST all increased significantly (Tables 1 and 2).

CCl_4- *and DMN-produced liver injury*

Treatment with CCl_4 and DMN for 4 weeks produced liver damage in the rats, CCl_4 producing weaker changes than DMN with the doses used. The amount of Cyt. P-450 and the activity of NADPH–cyt. *c* reductase decreased significantly in the CCl_4 group while all the changes except for total and relative liver protein, relative microsomal protein and the activity of GST were significant in the DMN rats (Tables 1 and 2). DMN and CCl_4 also induced alterations in the gel electrophoresis pattern of the microsomal proteins (Fig. 1).

Simultaneous administration of CCl_4 *and MPA*

The administration of MPA together with CCl_4 for 7 days partly prevented CCl_4-caused damage. This was seen here as an increase in liver wt and Cyt. P-450 content. The activity of BP-hydroxylase also showed an increasing trend. The relative microsomal protein content did not change upon MPA treatment (Table 3).

Spontaneous regeneration after chemical injury

The rats given MPA V after the cessation of CCl_4 and DMN treatment showed improved liver function associated with liver regeneration. The changes were greater following DMN treatment than after CCl_4. Significant changes were obtained in relative liver protein and relative microsomal protein content, Cyt. P-450 content and the activity of EH in the CCl_4 rats, and in liver wt, total liver protein and Cyt. P-450 content and the activity of NADPH–cyt. *c* reductase in the DMN animals (Tables 4 and 5). A slight improvement was visible in the gel electrophoresis pattern for the microsomal proteins in the DMN rats (Fig. 1).

Effect of MPA after chemical injury

Therapy with MPA accelerated liver restoration in the CCl_4- and DMN-treated rats as compared to the animals showing spontaneous regeneration. Only the relative liver protein remained unchanged in the CCl_4 rats, but the relative microsomal protein content decreased to the level for the intact rats upon administration of MPA (Tables 4 and 5). Similar changes were noted in the DMN-treated rats, although statistical significance was not reached in every case (Tables 4 and 5). The gel electrophoresis pattern for the microsomal proteins showed an increase in some protein bands as compared with those for the rats showing spontaneous regeneration (Fig. 1).

DISCUSSION

Both the present and previous data [5] show that MPA treatment clearly induces hepatic enzyme activities in intact rats, and these present results further demonstrate a clear induction of various enzyme activities by MPA after chemical injury to the liver. The wt of the regenerative liver increased in parallel with the change in total liver protein content. Simultaneous administration of MPA with CCl_4 reduced the extent of liver injury.

MPA is known to increase many hepatic enzyme activities in the rat, including NADPH–cyt. *c* reductase, BP-hydroxylase, 2,5-diphenyloxazole

Table 1. Body wt, liver wt, total and relative liver protein and relative microsomal protein content in female rats treated with CCl_4, DMN or MPA

Parameter	Animal group				
	C*	CCl_4	DMN	V*	MPA
Body wt (g)	224 ± 21	196 ± 34	190 ± 26	213 ± 21	195 ± 10
Liver wt (g)	8.82 ± 0.65	9.18 ± 1.26	6.52 ± 0.98†	8.67 ± 0.79	9.69 ± 0.85
Total liver protein (g)	1.43 ± 0.16	1.44 ± 0.22	1.15 ± 0.20	1.43 ± 0.15	1.56 ± 0.14
Liver protein (mg × g $liver^{-1}$)	162 ± 14	157 ± 3	176 ± 10	165 ± 8	162 ± 11
Microsomal protein (mg × g $liver^{-1}$)	26.1 ± 1.5	25.4 ± 0.9	26.2 ± 2.6	25.0 ± 0.8	23.6 ± 2.1

CCl_4 or DMN were given to rats for 4 weeks to produce liver injury and the other animals were given MPA or its vehicle (V) for a week as described in Materials and Methods.

Each value represents the mean ± S.D. from six animals.

* Reference value.

† $P < 0.01$ as compared to the reference value.

Table 2. Cytochrome P-450 content and the activity of NADPH–cytochrome *c* reductase, benzo[*a*]pyrene hydroxylase, aminopyrine *N*-demethylase, epoxide hydrolase and glutathione *S*-transferase in the livers of female rats treated with CCl_4, DMN or MPA

Parameter	Animal group				
	C*	CCl_4	DMN	V*	MPA
Cytochrome P-450 (nmoles × mg^{-1})	0.47 ± 0.03	0.40 ± 0.04‡	0.27 ± 0.06§	0.46 ± 0.05	0.55 ± 0.06†
NADPH–cytochrome *c* reductase (nmoles × mg^{-1} × min^{-1})	23 ± 4	18 ± 4†	11 ± 3§	23 ± 2	55 ± 14§
Benzo[*a*]pyrene hydroxylase (pmoles × mg^{-1} × min^{-1})	60 ± 16	55 ± 17	14 ± 8§	56 ± 8	116 ± 7§
Aminopyrine *N*-demethylase (nmoles × mg^{-1} × min^{-1})	0.47 ± 0.15	0.46 ± 0.12	0.18 ± 0.02§	0.54 ± 0.10	1.11 ± 0.30‡
Epoxide hydrolase (nmoles × mg^{-1} × min^{-1})	5.1 ± 0.9	7.4 ± 1.4	18.3 ± 2.4§	3.5 ± 1.2	7.1 ± 2.3‡
Glutathione *S*-transferase (μmoles × mg^{-1} × min^{-1})	0.42 ± 0.07	0.43 ± 0.07	0.37 ± 0.06	0.42 ± 0.07	0.64 ± 0.16†

CCl_4 or DMN were given to rats for 4 weeks to produce liver injury and the other animals were given MPA or its vehicle (V) for a week, as described in Materials and Methods.

Each value represents the mean ± S.D. from six animals.

* Reference value.

† $P < 0.05$, ‡ $P < 0.01$ and § $P < 0.001$ as compared to the reference value.

Table 3. Comparison of the effect of simultaneous administration of MPA and CCl_4 with that produced by CCl_4 alone on hepatic parameters in female rats

Parameter	Animal group				
	C*	MPA	C	CCl_4*	CCL_4 + MPA
Liver wt (g)	5.82 ± 0.54	8.23 ± 0.50‡	5.82 ± 0.54	6.93 ± 1.02	9.78 ± 1.09‡
Microsomal protein (mg × g $liver^{-1}$)	23.8 ± 1.2	23.6 ± 2.2	23.8 ± 1.2§	16.9 ± 1.2	16.2 ± 0.7
Cytochrome P-450 (nmoles × mg^{-1})	0.445 ± 0.029	0.694 ± 0.067§	0.445 ± 0.029§	0.218 ± 0.039	0.315 ± 0.061†
NADPH–cytochrome *c* reductase (nmoles × mg^{-1} × min^{-1})	44.7 ± 4.1	88.3 ± 4.0§	44.7 ± 4.1	42.9 ± 5.8	90.5 ± 7.7§
Benzo[*a*]pyrene hydroxylase (pmoles × mg^{-1} × min^{-1})	60.6 ± 15.1	88.4 ± 8.1‡	60.6 ± 15.1§	21.1 ± 5.1	30.4 ± 11.4

The animals were treated with CCl_4 [0.75 ml/kg body wt in corn oil (2:3) s.c.] (CCl_4) and MPA (100 mg/kg body wt in MPA vehicle i.p.) (MPA) daily for 7 days or simultaneously (CCl_4 + MPA). The control rats (C) were given both vehicles.

Each value represents the mean ± S.D. from four rats.

* Reference value.

† $P < 0.05$, ‡ $P < 0.01$ and § $P < 0.001$ as compared to the reference value.

Table 4. The effect of MPA on body wt, liver wt, total and relative liver protein and relative microsomal protein content in female rats pretreated with CCl_4 or DMN

Parameter	Animal group					
	CCl_4	CCl_4–V*	CCl_4–MPA	DMN	DMN–V*	DMN–MPA
Body wt (g)	196 ± 34	202 ± 8	210 ± 11	190 ± 26	218 ± 19	198 ± 21
Liver wt (g)	9.18 ± 1.26	7.65 ± 0.68	10.51 ± 1.78‡	6.52 ± 0.98†	8.86 ± 1.13	10.19 ± 1.95
Total liver protein (g)	1.44 ± 0.22	1.46 ± 0.16	2.06 ± 0.36‡	1.15 ± 0.20†	1.60 ± 0.16	1.86 ± 0.38
Liver protein (mg × g liver^{-1})	157 ± 3§	190 ± 7	195 ± 6	176 ± 10	181 ± 9	182 ± 6
Microsomal protein (mg × g liver^{-1})	25.4 ± 0.9‡	29.4 ± 2.5	24.9 ± 1.9‡	26.2 ± 2.6	23.8 ± 3.5	26.0 ± 1.2

The animals were pretreated with CCl_4 or DMN for 4 weeks to produce liver injury after which MPA or its vehicle (V) were given for a week, as described in Materials and Methods.

Each value represents the mean ± S.D. from six animals, except for the DMN–MPA group, where there were five rats.

* Reference value.

† $P < 0.05$, ‡ $P < 0.01$ and § $P < 0.001$ as compared to the reference value.

Table 5. The effect of MPA on cytochrome P-450 content and the activity of NHDPH–cytochrome *c* reductase, benzo[*a*]pyrene hydroxylase, aminopyrine *N*-demethylase, epoxide hydrolase and glutathione *S*-transferase in the livers of female rats pretreated with CCl_4 or DMN

Parameter	Animal group					
	CCl_4	CCl_4–V*	CCl_4–MPA	DMN	DMN–V*	DMN–MPA
Cytochrome P-450 (nmoles × mg^{-1})	0.40 ± 0.04†	0.47 ± 0.03	0.64 ± 0.04§	0.27 ± 0.06	0.38 ± 0.08	0.42 ± 0.08
NADPH–cytochrome *c* reductase (nmoles × mg^{-1} × min^{-1})	18 ± 4	19 ± 5	56 ± 8§	11 ± 3†	20 ± 5	47 ± 10§
Benzo[*a*]pyrene hydroxylase (pmoles × mg^{-1} × min^{-1})	55 ± 17	59 ± 8	111 ± 31§	14 ± 8	34 ± 25	78 ± 30†
Aminopyrine *N*-demethylase (nmoles × mg^{-1} × min^{-1})	0.46 ± 0.12	0.55 ± 0.08	0.89 ± 0.24‡	0.18 ± 0.02	0.20 ± 0.04	0.51 ± 0.16§
Epoxide hydrolase (nmoles × mg^{-1} × min^{-1})	7.4 ± 1.4†	5.1 ± 1.0	7.7 ± 1.8†	18.3 ± 2.4	17.2 ± 1.4	12.8 ± 2.6
Glutathione *S*-transferase (μmoles × mg^{-1} × min^{-1})	0.43 ± 0.07	0.40 ± 0.04	0.54 ± 0.09‡	0.37 ± 0.06	0.35 ± 0.07	0.63 ± 0.05§

The animals were pretreated with CCl_4 or DMN for 4 weeks to produce liver injury, after which MPA or its vehicle (V) were given for a week, as described in Materials and Methods.

Each value represents the mean ± S.D. from six animals, except for the DMN–MPA group, where there were five rats.

* Reference value.

† $P < 0.05$, ‡ $P < 0.01$ and § $P < 0.001$ as compared to the reference value.

hydroxylase and 7-ethoxycoumarin *O*-deethylase [5], ethylmorphine *N*-demethylase [18], testosterone A-ring reductase [19], "malic" enzyme and glycerol-3-phosphate dehydrogenase [20], as well as the metabolism of *p*-nitroanisole, aniline and aminopyrine [21] and the Cyt. P-450 content [5]. The present data demonstrate that it also induces the activity of EH and GST in female rats. Thus MPA resembles phenobarbital (PB) and pregnenolone-16α-carbonitrile (PCN) in the wide range of hepatic enzymes it induces [22].

The liver damage produced by DMN was more severe than that produced by CCl_4 with the doses used here. This was seen in the reduction in enzyme activities and in the total liver protein content in the DMN-treated rats. Consequently the enzyme activities were lower in the DMN than in the CCl_4 rats upon MPA therapy. When we compare MPA-treated animals with the corresponding V-treated rats, it was found, however, that relative induction was almost equal to, or even greater than, that in the intact rats. Corresponding changes are also produced in CCl_4-pretreated rats by PB [23].

MPA increased the total liver protein content more markedly in the damaged than in the intact liver. This was associated with a parallel increase in liver wt and thus the relative liver protein remained unchanged. These results suggest that MPA enhanced liver protein synthesis in a damaged liver. It has been suggested previously that MPA increases amino acid conservation in the rat liver [20]. PB has been shown to enhance in mice the inductive effect of chronic CCl_4 treatment on liver wt, liver DNA synthesis and to a lesser extent on liver protein synthesis [24]. These effects were supposed to result from a summation of the effects of each chemical, although PB alone had only a moderate if any influence on these parameters. When MPA and CCl_4 were given simultaneously there was also no change in the relative microsomal protein content in the liver although the liver wt increased upon MPA treatment. These results are supported also by the findings in intact animals [19].

PCN stimulates liver growth and enhances mitotic activity in intact and regenerative liver [25]. MPA and PCN seem to be quite similar in their ability to induce hepatic drug metabolism [5, 22]. From these data one could suggest that MPA may also have an enhancing effect on liver regeneration. The increase in liver protein synthesis induced by MPA in the damaged livers could be explained by an acceleration in the production of certain cellular components whose synthesis was first stimulated by liver regeneration. The protein patterns in gel electrophoresis also support this assumption, as MPA increased some microsomal proteins in the CCl_4- and DMN-treated animals to a greater extent than in the rats showing spontaneous regeneration.

PB pretreatment enhances the hepatotoxicity of CCl_4 [26], and in chronic experiments cirrhosis develops more rapidly following concomitant administration of CCl_4 and PB [27]. These effects are supposed to be due to induction of the monooxygenase enzyme system by PB, which results in an increase in the reactive metabolites of CCl_4 [26]. 3-Methylcholanthrene has a protective effect against CCl_4 toxicity [28], and PCN protects against DMN hepatotoxicity [29], although they also induce hepatic drug metabolism. In the present study simultaneous MPA administration partly prevented CCl_4 toxicity in rats. MPA either was not able to enhance the metabolism of CCl_4 or the increased toxicity of CCl_4 due to metabolites was compensated by the inducing effect of MPA on the affected parameters. The other explanation might be that MPA eliminates the reactive metabolites of CCl_4. MPA induced the activity of GST, which is able to conjugate electrophile radicals with glutathione, making them less toxic [30]. It has been suggested that by inducing "malic" enzyme and increasing NADPH content in the liver, MPA may supply reducing equivalents for the formation of reduced glutathione [20].

The present results demonstrate that MPA is a wide-spectrum inducer of the liver enzyme systems and its effects are not impaired by the presence of chemical injury. MPA seems to enhance the protein synthesis stimulated by liver regeneration, and if this were to be the case in man the drug could be of considerable benefit to patients with liver disease. An animal model does not always correspond exactly to the human situation, however, although the present results do at least agree with the clinical findings in that the drug-metabolizing enzyme system is induced by MPA in both animals and man.

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