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# Induction of hepatic microsomal drug metabolizing enzyme system by levamisole in male mice

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**Abstract**—To examine the effect of levamisole on the hepatic drug metabolizing enzyme system of mice, levamisole at a dose of 20 mg $kg^{-1}day^{-1}$ was administered i.p. for 5 days. Compared with the control values, the levamisole treatment significantly increased the amount of cytochrome P450 and cytochrome b5 and the in-vitro activities of aminopyrine *N*-demethylase, benzphetamine *N*-demethylase and aniline hydroxylase. In contrast, there was no change in microsomal NADPH-cytochrome c reductase activity in-vitro or in the relative liver weight and microsomal protein content compared with the corresponding values for control mice. Furthermore, in-vivo induction of drug metabolism was demonstrated by decreased pentobarbitone sleeping times after levamisole pretreatment. These results indicate that certain hepatic microsomal mixed function oxidases of mice are induced by levamisole, the drug that is frequently used as an anthelmintic in veterinary medicine and as an immunostimulant drug in human medicine.

Levamisole, an anthelmintic drug effective against most nematodes of animals and man has been extensively used in veterinary and human medicine (Symoens & Rosenthal 1977; Plante et al 1981). It is rapidly biotransformed by the liver in the rat to metabolic products essentially through oxidative reactions such as sulfoxidation and hydroxylation (Galtier et al 1983; Kouassi et al 1986; Engelmann & Richardson 1986). Another major metabolite has been found to be a thiol known as OMPI [2-oxo-3-(2-mercaptoethyl)-5-phenylimidazolidine] produced by hydrolysis of the triazolidine ring of levamisole, which may have a role in the immunomodulating property of levamisole (Van Belle & Janssen 1979). On the other hand, levamisole does not seem to be biotransformed to these metabolites in the dog (Plante et al 1981), suggesting some species difference in its metabolism. Engelmann & Richardson (1986) incubated levamisole with cultured hepatocytes from rat and found that it stimulated alkaline phosphatase activity and elevated microsomal cytochrome P450 content after 48 h incubation. Similarly, other workers have reported a modest increase in the liver cytochrome b5 content and the aminopyrine *N*-demethylase activity after chronic treatment of rats (Reinke et al 1976). The present study was undertaken to investigate the effect of levamisole on several liver microsomal mixed function oxidases of mice as another species.

## Materials and methods

Adult, male albino ICR mice, 30–40 g purchased from Harlan Sprague-Dawley Inc., Indianapolis, IN, were housed in plastic cages on hardwood chip bedding and were given free access to feed (Purina chow) and water.

Levamisole hydrochloride was purchased from Sigma Co., St. Louis, MO. Mice (12 per group) were injected intraperitoneally (i.p.) once daily for 5 consecutive days with a 20 mg $kg^{-1}$ dose of the drug dissolved in distilled water (3 mL $kg^{-1}$). The control group of 12 animals received equivalent volumes of distilled water only. Twenty-four h after the final dose, six animals from each group were given 50 mg $kg^{-1}$ of pentobarbitone sodium i.p. to determine the sleeping time. The remaining 6 animals from each group were killed by cervical dislocation, and livers promptly removed, weighed, and perfused with ice-cold 1·15% KCl solution containing 0·5 mM EDTA. Three perfused livers were pooled and homogenized with 3 volumes of ice-cold 0·25 M sucrose solution. The homogenates were centrifuged at 9000 *g* for 20 min in a refrigerated Sorvall centrifuge. Microsomes from the supernatant fraction were isolated by the procedure described by Cinti et al (1972). The microsomal pellets were washed once with the KCl solution and immediately resuspended in an appropriate buffer for determining the following components of the mixed function oxidase system.

The levels of microsomal cytochromes P450 and b5 were measured following the procedures reported by Omura & Sato (1964). NADPH-cytochrome c reductase was determined by slightly modifying the method of Masters et al (1967). To a small test tube were added the following solutions: 5 mL of phosphate buffer (0·1 M, pH 7·4), 0·5 mL of cytochrome c solution (7·36 mg $mL^{-1}$ of water), and 0·1 mL of microsomal enzyme suspension containing 1·2 mg of protein. The resultant solution was mixed well and incubated at 37°C for 5 min. Following the preincubation period, 2·8 mL of the mixture was transferred to each of two cuvettes, a reference and a sample. These were placed in the compartment of a recording spectrophotometer, 0·2 mL of the buffer was added to the reference and 0·2 mL NADPH-generating system to the sample cuvette and the change in optical density at 550 nm was recorded every 30 s for 4 min. The amount of cytochrome c reduced $min^{-1}$ $mg^{-1}$ of microsomal protein was calculated using the molar extinction coefficient of 19·1 $mM^{-1}$ $cm^{-1}$ (Masters et al 1967). Microsomal benzphetamine *N*-demethylase and aniline hydroxylase activities were determined as described by Dalvi et al (1987). Aminopyrine *N*-demethylase activity in the microsomes was assayed following the procedure of Schenkman et al (1967) modified to include Hepes buffer (0·05 M, pH 7·8) and NADPH-generating system to contain 1·2 mM NADP, 8 mM G-6-P, and 2 units of G-6-PD.

Protein concentrations were measured by the biuret method modified to include 0·1 mL of 1% deoxycholate in each sample.

The data were analysed using Student's *t*-test. Significance of mean differences was based on a $P$ value of 0·05.

## Results and discussion

Pretreatment of mice with levamisole did not produce a significant change in either the relative liver weights or the microsomal protein content of the treated animals as compared to the corresponding control values (Table 1). Although levamisole is predominantly metabolized in the liver, it has not been reported to be harmful to the liver at therapeutic levels. The present results suggest that this compound does not belong to a group of foreign compounds which produce liver enlargement and concomitant increase in the values of above parameters (Dalvi 1987). A similar observation has been also made by Reinke et al (1976) in male and female rats pretreated with levamisole.

As can be seen from the data presented in Table 1, levamisole treatment significantly increased the amount and activity of several components of the hepatic drug metabolizing system.

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Table 1. Effect of levamisole pretreatment on various parameters of hepatic drug metabolizing enzyme system in male mice.

| Parameters | Control | Levamisole |
|---|---|---|
| Relative liver weights (% of body weight) | 4·795 ± 0·523 | 4·775 ± 0·369 |
| Microsomal protein (mg $g^{-1}$ liver) | 27·011 ± 4·010 | 24·394 ± 2·073 |
| Cytochrome P450 (nmol (mg protein)$^{-1}$) | 0·535 ± 0·008 | 0·794 ± 0·078[a] |
| Cytochrome b5 (nmol protein)$^{-1}$) | 0·114 ± 0·002 | 0·169 ± 0·017[a] |
| NADPH-cytochrome c reductase (nmol $min^{-1}$ (mg protein)$^{-1}$) | 156·24 ± 10·29 | 180·05 ± 13·66 |
| Aminopyrine *N*-demethylase (nmol HCHO $min^{-1}$ (mg protein)$^{-1}$) | 4·567 ± 0·091 | 5·725 ± 0·462[a] |
| Benzphetamine *N*-de-methylase (nmol HCHO $min^{-1}$ (mg protein)$^{-1}$) | 2·912 ± 0·118 | 4·014 ± 0·402[a] |
| Aniline hydroxylase (nmol *p*-aminophenol $min^{-1}$ (mg protein)$^{-1}$) | 0·577 ± 0·035 | 0·851 ± 0·093[a] |

Results are expressed as the mean ± s.e.m. [a]Significantly different ($P < 0·05$) from the corresponding control value.

For example, there was almost 50% increase in the amount of microsomal cytochromes P450 and b5 over the corresponding control values. Although increase in the activity of NADPH-cytochrome c reductase was slight, the activities of aminopyrine *N*-demethylase, benzphetamine *N*-demethylase and aniline hydroxylase were significantly higher than those for the respective untreated control animals, suggesting levamisole as an inducer of the hepatic mixed function oxidase system in male mice. In-vivo induction of microsomal drug metabolizing enzymes activity was demonstrated when the mean of pentobarbitone sleeping times of levamisole-pretreated mice was compared with the mean of sleeping times of untreated control mice given the same dose of pentobarbitone (control: 45·75 ± 2·06, treated: 24·75 ± 3.25 min mean ± s.e.m. $P < 0·05$). The results show a significant decrease in the sleeping time of levamisole-pretreated mice, suggesting increased rate of pentobarbitone metabolism by the microsomal enzymes. The results are consistent with those reported for female rats (Reinke et al 1976). These investigations demonstrate that compared with microsomal enzyme induction by the classic inducer phenobarbitone, levamisole is a modest inducer of the microsomal drug metabolizing enzyme system in mice as it is in rats.

Studies on spectral binding of levamisole to mouse liver microsomes revealed that levamisole is a type II compound characterized by a peak at 422 nm and a trough at 394 nm (unpublished data). Unlike most type II compounds, which usually cause inhibition of the hepatic microsomal mixed function oxidases (Peeples & Dalvi 1982), levamisole produced induction of the liver drug metabolizing enzyme system. However, this is not uncommon for a type II compound. For example, metyrapone causes both induction and inhibition, depending on dose and frequency of dosing. The levamisole induction of mixed-function oxidases suggests that it may give rise to drug interactions by enhancing the metabolism of other concomitantly administered drugs, thus reducing their therapeutic efficacy or increasing their toxicity if their metabolic products are more toxic than the parent compounds.

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