Chronic Effects of Monocrotaline Pyrrole on Hepatic Mitosis and DNA Synthesis[1] (37192)

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Pyrrolizidine alkaloids (PA), present in a variety of plants throughout the world, have been shown to produce quite diverse effects on the tissues of man and lower animals (1, 2). Within a 2–3 wk period following the administration of these alkaloids to rats there is a decided inhibition of liver cell division. However, many of the affected cells continue to synthesize DNA, thereby giving rise to megalohepatocytes (3, 4). Chronicity of these hepatic changes is associated with a gradual decrease in the hepatic cell population and eventual death of the affected animals. Seemingly in direct contrast to the inhibition of mitosis is the development of hepatomas in rats that have been exposed to the alkaloids (5, 6). It is possible that different metabolites of the PA are responsible for these diverse effects. Butler, Mattocks and Barnes (7) observed liver necrosis, venous thrombi and hyperplastic nodules in the liver of rats given metabolic pyrroles. In the present study it was shown that the pyrrole metabolite of the PA, monocrotaline, caused megalohepatocytosis, inhibited liver cell mitosis and retarded hepatic DNA synthesis.

Materials and Methods. Male, Sprague-Dawley rats, weighing 200 ± 15 g were housed in a well-ventilated, windowless room which was illuminated from 6:00 AM to 6:00 PM daily. A commercial diet[3] and water were supplied *ad libitum* throughout the study. Ninety-six animals were randomly divided among 4 groups of 24 rats each. Two groups received either 0.5 or 2.0 mg/kg body weight of a freshly prepared monocrotaline pyrrole solution (8). A laparotomy was performed on each rat and the monocrotaline pyrrole, dissolved in 0.05 ml of dimethylformamide (DMF), was slowly injected into a mesenteric vein. A third group of 24 animals was injected in a similar manner with 2 mg/kg body weight of monocrotaline[4] dissolved in DMF. The control group received an injection of only DMF solvent.

Thirty days following injection, a partial hepatectomy (9) was performed on all rats at 8:00 AM ± 30 min. Liver removed during surgery was fixed in buffered 10% formalin and subsequently treated to yield paraffin wax light microscopic slides which were stained with hematoxylin and eosin.

Each of the four groups of animals were further divided in half and treated in a manner to permit the evaluation of either hepatic mitotic indices or DNA synthesis. Mitotic indices were determined separately for each lobe of regenerating liver from each rat. Six hours prior to sacrifice the rats received 1 mg/kg body weight of colchicine. Liver lobes were placed in Carnoy's fixative and subsequently subjected to an acetocarmine squash procedure (10). Three thousand hepatocytes from each liver lobe were observed in successive high power microscopic fields in order to determine the number of colchicine arrested metaphases present per 1000 hepatocyte nuclei. DNA synthesis was also determined separately for each lobe of regenerating liver from each rat. Two hours prior to sacrifice rats received 500 μCi/kg body

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[3] Rockland Rat Diet, Teklad, Inc., Monmouth, IL.

[4] Monocrotaline obtained from S. B. Penick & Co., 100 Church Street, New York, NY.

weight of tritiated thymidine[5]. Liver lobes were individually homogenized and centrifuged, and DNA was determined by the method of Munro and Fleck (11). The radioactivity of the purified DNA extract was measured in a Packard Tri-Carb liquid scintillation spectrometer using Scintisol-Complete[6] as the counting solution.

All rats were sacrificed by decapitation either 38 or 50 hr following partial hepatectomy. Analysis of variance was used to determine overall statistical significance and Fischer's LSD test was subsequently employed to determine differences between the means (12).

Results. Grossly and light microscopically, the livers of all animals regardless of treatment were free of obvious lesions. However, even though the normal architectural pattern of the hepatic lobules was maintained, some enlarged hepatocytes were present in the periportal region of rats injected with the larger dose of monocrotaline pyrrole. These cells were darkly stained, contained a moderately enlarged nucleus and exhibited a marked increase in cytoplasmic material. The presence of these megalohepatocytes was less conspicuous in liver from the 0.5 mg/kg pyrrole and monocrotaline groups, and absent in the control group.

The use of colchicine permitted determination of the number of hepatocytes entering mitosis in the 6 hr period immediately prior to sacrifice. The data depicted in Table I demonstrate an 80% decrease in the mitotic index of the 2.0 mg/kg monocrotaline pyrrole group which were sacrified 38 hr after partial hepatectomy. With the exception of this group there is no significant difference in mitotic indices among groups regardless of the time of sacrifice or material injected.

The rate of DNA synthesis was estimated by measuring the tritiated thymidine incorporation into DNA over a 2 hr period. The difference in mean values between animals sacrificed at 38 versus 50 hr following partial hepatectomy is not statistically significant. However, there is a significant effect between treatment groups as shown in Table II. Monocrotaline and 2.0 mg/kg pyrrole groups are both significantly different from the control animals.

Mitotic index and DNA synthesis were determined separately for each lobe of regenerating liver from each rat. No significant difference was observed among the lobes from the same liver; therefore, the individual results were pooled to derive one value for each determination from each rat liver.

Discussion. The presently reported data indicates that the antimitotic effect of the PA, monocrotaline, is due to its pyrrole metabolite. In previous studies conducted by Peterson (13) and Downing and Peterson (14) it was demonstrated that other PA and their *N*-oxides were also capable of producing antimitotic effects in regenerating livers. In these

TABLE I. Inhibition of Cell Division in Regenerating Liver of Monocrotaline Pyrrole-Injected Rats.

Group	Cumulative mitotic index[a] 38 hr	50 hr
Control	62.0 ± 20.8	61.1 ± 19.2
Monocrotaline	63.3 ± 12.9	55.6 ± 17.2
Pyrrole, 0.5 mg/kg	61.0 ± 22.5	62.7 ± 14.6
2.0 mg/kg	11.0 ± 2.4[b]	60.8 ± 20.3

[a] Number of colchicine arrested mitoses per 1000 hepatocytes when rats sacrificed at either 38 or 50 hr after partial hepatectomy. Data expressed as mean values ± 1 SD.

[b] Significantly different from all other values ($p < .001$).

TABLE II. Effect of Monocrotaline Pyrrole on DNA Synthesis in Regenerating Rat Liver.

Group	DNA sp act[a] (dpm DNA/μg DNA) 38 hr	50 hr
Control	1917 ± 574	1458 ± 252
Monocrotaline	1196 ± 278[b]	1288 ± 100[b]
Pyrrole, 0.5 mg/kg	1776 ± 548	1547 ± 127
2.0 mg/kg	1044 ± 381[b]	1161 ± 443[b]

[a] Two hour cumulative uptake of tritiated thymidine when rats were sacrificed at either 38 or 50 hr after partial hepatectomy. Data expressed as mean values ± 1 SD.

[b] Significant difference with control group ($p < .02$).

[5] New England Nuclear Corp., Boston, MA, sp act 5.7 Ci/mmole.

[6] Isolab, Inc., Drawer 4350, Akron, OH.

in vivo experiments the PA apparently were converted to pyrrolic metabolites by hepatic enzymes, thereby producing the antimitotic effect. Since lasiocarpine *N*-oxide is a relatively nontoxic metabolite of a PA, it is likely that the liver enzymes convert the *N*-oxide back to the parent alkaloid and subsequently metabolize it to a pyrrole derivative. Lasiocarpine pyrrole then produces the antimitotic effects attributed to lasiocarpine *N*-oxide.

In addition to the overall inhibition of cell division, there was a delay in the time of peak mitosis of the regenerating liver cells as was reported for lasiocarpine *N*-oxide (13). Peak mitotic indices were not attained during the 30–38 hr post-partial hepatectomy period as they normally are in control regenerating liver, but probably developed during the subsequent 8 hr. The cumulative mitotic indices obtained during the 44–50 hr period in the 2.0 mg/kg pyrrole group were much greater than those of the 30–38 hr period.

Some of the toxic alkaloids such as lasiocarpine cause a greater inhibition of mitosis in regenerating liver than do other PA, including monocrotaline. Whether these differences are a result of the amount or chemical structure of the pyrrole derivatives produced remains to be clarified. It is also worthy of note that the alkaloids which exert the greatest antimitotic effects are also responsible for the induction of hepatic carcinomas (6, 15). The relationship of the inhibitory effects on hepatic mitosis and DNA synthesis to the carcinogenicity of the PA has not been established.

The inhibition of DNA synthesis by monocrotaline pyrrole further supports the contention that the action of PA is due to their pyrrole derivatives. The data suggest that there is a population of hepatocytes which are injured in such a way as to prevent both DNA synthesis and mitosis from occurring. There is also a population of hepatocytes which is unable to divide yet continues to synthesize DNA. There appears to be a third population of hepatocytes that are apparently unaffected, giving rise to hepatic regenerative nodules that invariably develop in chronically intoxicated animals. When lesions become more chronic, the severely affected liver cells gradually disappear, leaving either enlarged cells that continue to synthesize DNA without dividing or cells that are seemingly unaffected. Due primarily to continued synthesis of DNA there is a gradual increase in the level of this nucleic acid in the affected livers (4).

Pyrrolic metabolites have been identified in rat liver after *in vivo* administration of PA (16) and after *in vitro* incubation of hepatic microsomes with PA (17). It has been suggested that the cytological and biochemical alterations of the liver associated with these pyrrole derivatives are due to their alkylating ability (18). The DNA molecule is susceptible to alkylation, and present evidence indicates that pyrrolic metabolites may act directly on this genetic material (19). However, the exact mechanism of action of these metabolites has not yet been elucidated.

This study clearly shows that monocrotaline pyrrole not only induces antimitotic effects and inhibition of DNA synthesis, as does monocrotaline, but does so to a greater degree than the same amount of the parent alkaloid. This supports the hypothesis that metabolic pyrroles are the proximate toxins in pyrrolizidine intoxication. In addition, it seems highly probable that megalohepatocytosis may also be attributed to the action of the pyrroles; however, it was not within the scope of this study to demonstrate continued DNA synthesis in the absence of mitosis. The presence of enlarged cells observed light microscopically in the affected livers supports this contention.

There is evidence to show that hepatic necrosis, inhibition of cell division, altered DNA synthesis and the development of megalohepatocytosis caused by the PA may be attributed to the metabolic pyrroles. Consideration must now be given to the establishment of the relationship, if any, between these pyrrole-induced hepatocellular alterations and the formation of hepatoma in PA-intoxicated animals.

Summary. Monocrotaline, a pyrrolizidine alkaloid, and its metabolite, monocrotaline pyrrole, caused a significant suppression of DNA synthesis following partial hepatectomy in rats. Rats receiving mesenteric vein injections of monocrotaline pyrrole prior to par-

tial hepatectomy showed a marked inhibition of liver cell division between 32 and 38 hr following surgery. Both the parent alkaloid and its pyrrole derivative induced megalohepatocytosis in rats 4 wk after their administration. The effect of the pyrrolic metabolite was considerably greater than that of the parent compound, supporting the contention that the consequences of pyrrolizidine alkaloid intoxication are due primarily to *in vivo* hepatic conversion of the alkaloid to its pyrrole derivative.

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