EFFECT OF DIETARY SENECIO JACOBAEA AND INJECTED SENECIO ALKALOIDS AND MONOCROTALINE ON GUINEA PIGS

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Summary

The chronic toxicity of 10% dietary Senecio jacobaea (SJ) in guinea pigs was studied during a 365-d feeding trial. The SJ plant contains hepatic toxic pyrrolizidine alkaloids (PA). The chronic lethal dose (LD_{100}) of SJ for guinea pigs was 1,264 g/kg initial body weight or 526% of initial body weight with an average survival time of 279 d. No mortality was observed in the controls. The LD_{100} and survival time were slightly, but not significantly, increased when 1% cysteine was included in the SJ diet. Histopathological examination of liver tissue from SJ-intoxicated guinea pigs revealed extensive megalocytosis, cytoplasmic and nuclear vacuolization, biliary hyperplasia and periportal fibrosis. Centrilobular areas appeared spared from necrotic lesions. The toxicity of acutely administered (ip) PA was determined in another experiment. The PA monocrotaline was non-toxic to guinea pigs at doses up to 1,000 mg/kg body weight, whereas jacobine and mixed SJ PA were highly toxic at doses ranging from 100 to 150 mg/kg body weight. These results indicate that guinea pigs are resistant to dietary SJ and vary in response to single ip doses of isolated PA.

(Key Words: Senecio, Pyrrolizidine Alkaloids, Guinea Pigs.)

Introduction

Plants containing pyrrolizidine alkaloids (PA) are widely distributed botanically and geographically. Contamination of feedstuffs with these plants represents an important economic loss to livestock producers. In the U.S. Pacific Northwest, Senecio jacobaea (SJ), a PA-containing plant, infests pasture land and is often found in hay. Ingestion of PA causes liver damage mainly characterized by centrilobular necrosis, biliary hyperplasia and megalocytosis (McLean, 1970). The pattern of toxicity varies between animal species, individual PA and dosage. In susceptible species, PA are metabolized to highly toxic, tissue damaging pyrrole metabolites by the mixed function oxidase (MFO) enzyme system present in liver endoplasmic reticulum (McLean, 1970). Among the livestock species, cattle and horses are notably susceptible while sheep (Bull et al., 1968), goats (Goeger et al., 1982) and rabbits (Pierson et al., 1977) are resistant to ingested PA. The rate of pyrrole formation in vitro by MFO is a good indication of resistance or susceptibility of an animal to PA (Shull et al., 1976). Other factors including intestinal absorption, rumen detoxification and urinary excretion may also be important with regard to resistance. Because guinea pigs have also been identified as PA-resistant animals (Chen et al., 1935; Carlton, 1967; Chesney and Allen, 1973; White et al., 1973) based mainly on their response to monocrotaline and retrorsine, it was of interest to further characterize their toxic response to Senecio PA. The object of this experiment was to determine the effect of long term feeding of diets containing SJ to guinea pigs and to compare toxicity of single doses of the PA monocrotaline, jacobine and mixed SJ PA. The possible protective effect of cysteine, previously observed in rats (Buckmaster et al., 1976) was also examined.
TABLE 1. PERCENTAGE DIET COMPOSITION

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>SJ</th>
<th>SJ, Cys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn meal</td>
<td>58.5</td>
<td>48.5</td>
<td>47.5</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Sucrose</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Senecio jacobea(a)</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mix(b)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin mix(c)</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
</tr>
</tbody>
</table>

\(a\) Dried, ground whole plant, excluding root.

\(b\) Jones and Foster (1942).

\(c\) Cheeke et al. (1977), in addition ascorbic acid was supplied at 2 g/liter in the drinking water.

Materials and Methods

Fifteen male guinea pigs of 250 to 300 g initial body weight were randomly assigned to three treatment groups as follows: 1) control; 2) 10% SJ; 3) 10% SJ plus 1% cysteine. The SJ whole plant, excluding roots, was collected near Corvallis, Oregon, forced-air dried (45 C for 24 h) and finely ground for incorporation into diets. Guinea pigs were fed the pelleted diets listed in table 1 and watered ad libitum for 365 d. The animals were housed individually in meshed floor, stainless steel cages under controlled light, temperature and humidity. Feed intake, weight gain and survival were recorded for each animal. The chronic oral lethal dose (LD\(_{100}\)) was calculated using the method of Litchfield and Wilcoxon (1948). Upon death or at termination, animals were necropsied and tissues fixed in formalin (10%). Selected tissues were prepared for histopathological examination after staining with hematoxylin and eosin.

In a second experiment, seven guinea pigs of about 500 g body weight were injected ip with either monocrotaline\(^6\), jacobine or mixed SJ PA. Mixed alkaloids were extracted from dried SJ flowers by the method of Campbell (1956). Jacobine was prepared from the mixed SJ alkaloids by successive recrystallization from cold methanol until a single peak was observed after gas-liquid chromatographic analysis. Data were analyzed statistically by one-way analysis of variance and when significance was detected, means were compared by the Student-

\(^6\) Trans World Chemical Co., Washington, DC.
Results and Discussion

The chronic lethal oral dose (LD_{100}) of SJ for guinea pigs was 1,264 g/kg initial weight or 526% of initial body weight with an average survival time of 279 d (table 2). No mortality was observed in control guinea pigs. In contrast, the chronic LD_{100} of SJ for rats consuming the identical diet was 58% of initial body weight with an average survival of 50 d (Swick et al., 1979). In cattle, consumption of SJ equivalent to 5 to 20% of body weight is lethal (Bull et al., 1968). Thus, it is apparent that although guinea pigs are able to consume large amounts of SJ, they are not totally resistant to PA intoxication.

Cysteine has been reported to be protective against PA intoxication as determined by survival time. Cysteine has been shown to be protective against PA intoxication in rats. Hayashi and Lalich (1968) observed a significant increase in survival of rats injected with the PA monocrotaline after pretreatment with cysteine. Buckmaster et al. (1976) observed significantly increased survival in rats consuming SJ when cysteine was incorporated into the diet. These researchers hypothesized that the sulfhydryl group of cysteine reacts with electrophilic pyrrolic metabolites, thus preventing their attack on essential cellular components.

Histopathological examination of the livers of guinea pigs consuming SJ revealed extensive megalocytosis and severe cytoplasmic vacuolization with biliary hyperplasia and fibrosis occurring primarily in the perportal areas (figure 1). The centrilobular and midzonal areas appeared to be somewhat spared with the worst lesions occurring in the portal areas (figure 2). Administration of PA to animals such as rats produces necrosis most often in the centrilobular areas (McLean et al., 1964). Because hepatocytes in this region are particularly high in MFO activity (Plaa, 1975), pyrrole formation and subsequent necrosis in this area would be expected. The perportal location of the lesions observed in the chronically intoxicated guinea pigs might be explained several ways. Pyrrole-producing enzymes other than the microsomal MFO could have high activity in this area in guinea pigs, or the lesions observed (biliary hyperplasia and fibrosis) might be due to the parent alkaloids themselves.

Severe nuclear vacuolization in addition to the cytoplasmic vacuolization was observed (figure 3). In addition, nuclear size was irregular with chromatin clumped around the inside periphery of the nuclear membrane. Kidney sections (figure 4) had hyaline or proteinacious
casts inside the tubular lumens. Although abnormal, this was not specific enough to be ascribed to PA.

Shown in table 3 are the responses of guinea pigs to injected (ip) PA. Interestingly, monocrotaline was nontoxic at doses up to 1,000 mg/kg body weight, whereas jacobine and the mixed alkaloids from SJ were lethal at much lower dose levels. According to Culvenor et al. (1976), monocrotaline has two features con-

Figure 2. Liver section centered on portal triad showing periportal fibrosis, midzonal megalocytosis, with little centrilobular damage (H and E X 80).

Figure 3. Liver section (midzonal area) showing nuclear and cytoplasmic vacuolization, hepatocyte nuclear variation and clumping of chromatin (H and E X 125).
Figure 4. Kidney section centered near cortio-medullary junction showing hyaline or protein casts in tubules and collecting ducts (H and E X 80).

Reducive to reduced toxicity: a low lipid/water partition coefficient and a facile cleavage of the secondary ester linkage once the primary ester is hydrolyzed, thus suggesting monocrotaline may behave similar to monoesters in biological systems. It should be noted, however, that White et al. (1973) could not determine the lethal dose of retrorsine in guinea pigs because the animals were resistant to doses above 800 mg/kg. In their study, phenobarbital pretreatment increased liver microsome conversion of retrorsine to pyrroles, the level of liver-bound pyrrole and the susceptibility of guinea pigs to the PA. Retrorsine is considered more toxic than monocrotaline (Culvenor et al., 1976). Others have also reported guinea pigs resistant to PA. Chen et al. (1935) reported the acute LD₅₀ of retrorsine for mice and rats and found guinea pigs could tolerate at least four times this dose without exhibiting pathologic effects. McLean (1970) indicated that they are resistant to senecionine. Carlton (1967) reported the resistance of these animals to seeds of Crota laria, containing monocrotaline. Chesney and Allen (1973) also demonstrated that injected monocrotaline at levels four times the LD₅₀ in rats, produced no pathologic effects in guinea pigs. These researchers demonstrated a lack of conversion of PA to pyrrole by guinea pig microsomes in vitro. The present findings and those of others indicate a resistance of guinea pigs to PA intoxication. The fact that the level of resistance is different for individual PA is interesting and worthy of further study. Determining the factors responsible for detoxification of monocrotaline and retrorsine in the guinea pig could aid in the development of protective agents that might prevent PA poisoning in susceptible livestock.

### Table 3. Toxicity of Intraperitoneal-Administered Pyrrolizidine Alkaloids in Guinea Pigs

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Dose, mg/kg</th>
<th>Survival time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocrotaline</td>
<td>500</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>nm</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>nm</td>
</tr>
<tr>
<td>Jacobine</td>
<td>100</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>66</td>
</tr>
<tr>
<td>Mixed SJ</td>
<td>100</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>78</td>
</tr>
</tbody>
</table>

a No mortality within 23-d period.
b Contains jacobine, seneciphylline, jacoine, jacoboline, senecionine (Ramsdell and Buhler, 1981).
Literature Cited


