Comparative Toxicology of Monensin Sodium in Laboratory Animals

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COMPARATIVE TOXICOLOGY OF MONENSIN SODIUM IN LABORATORY ANIMALS

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Summary
The toxicology of monensin has been studied in several laboratory animal species. There was considerable species variation in acute oral LD₅₀ values. The consistent signs of acute toxicity were: anorexia, hypoactivity, skeletal muscle weakness, ataxia, diarrhea, decreased weight gain and delayed deaths. The 3-mo study in rats fed diets containing 0, 50, 150 or 500 ppm monensin resulted in no effects at the lowest dose level, slight reduction of body weight gain in the middle-dose group and severe depression in body weight gain, skeletal and cardiac lesions, and deaths in the highest dose group. The 3-mo study in dogs given daily oral doses of 0, 5, 15 or 50 mg/kg monensin resulted in no effects at the lowest dose level. Dogs in the 15 and 50 mg/kg groups developed, during test wk 1 to 4, anorexia, weakness, ataxia, labored respiration, body weight loss, increased serum muscle enzyme values, severe skeletal muscle degeneration and necrosis with less severe heart lesions and deaths. Mice fed diets containing 0, 37.5, 75, 150 or 300 ppm monensin for 3 mo had reduced body weight gain in all test groups but no other physical signs. Serum creatine phosphokinase (CPK) values were increased in mice in the two highest dose groups and minimal heart lesions were found in the highest dose group. Dogs given daily oral doses of 0, 1.25, 2.5, 5 or 7.5 mg/kg monensin for 1 yr survived with no evidence of toxicity in the two lowest dose groups. Dogs in the two highest dose groups had transient signs of anorexia, hypoactivity, and increased alanine transaminase (SGPT) and CPK values. There was a decrease in body weight in the highest dose group but no pathologic lesions. Four generations of rats were continuously maintained on diets containing 0, 33, 50 or 80 ppm monensin. Except for a decrease in body weight gain, there were no compound-related effects on reproduction and no teratogenic effects. Groups of rats were fed diets containing 0, 33, 50 or 80 ppm monensin for 2 yr with no increase in chronic lesions or neoplasms. The primary target organs affected by toxic doses of monensin were skeletal and cardiac muscles.
(Key Words: Monensin, Ionophores, Toxicity, Myopathy, Laboratory Animals.)

Introduction
Before a new chemical compound is introduced for use in agriculture or medicine, it must be shown to be safe and useful. Experimental animal toxicity studies are performed as a predictive system for estimating the safety for man or target animals. The progression of toxicity studies included acute, subchronic, chronic, reproduction and oncogenicity. These studies, when appropriately designed, usually lead to a stepwise understanding and characterization of the toxicity/safety profile.

Much of the initial understanding of the toxicity of monensin was gained from a former ionophore compound, A204 (Worth et al., 1971). Skeletal muscles and, to a lesser degree, cardiac muscles were the primary target organs affected with toxic doses of A204 (Todd et al., 1971; Griffing et al., 1971). The mechanism of action was proposed to be related to a depletion of intracellular potassium (Meyers et al., 1971).

Toxicity studies with monensin have been performed over the past 20 yr in our research laboratories and studies continue today.
COMPARATIVE TOXICITY OF MONENSIN

The lial and crystalline forms of monensin have been tested in laboratory animals. The kind and degree of toxicity were similar with both forms. Mycelial monensin sodium was used in the following studies and the doses used were based on the analytical monensin activity. The oral route of administration was selected because it represents the usual route of exposure to monensin.

Experimental Procedure

Acute Toxicity. Monensin was administered in single oral doses to groups of several experimental animal species. The animals were observed for signs of toxicity for 14 d and, when possible, a median dose (LD$_{50}$) was calculated by the method of Bliss (1938).

Three-Month Study in Rats. Three groups of 15 male and 15 female Wistar rats, 4 to 5 wk old, were maintained on diets containing 50, 150 or 500 ppm monensin. A similar group was given untreated feed and served as the control group. All animals were examined daily for physical signs of toxicity. Individual animal body weight and food consumption were measured weekly. Before terminating the study, a blood sample was collected from each animal for hematologic and blood biochemistry evaluations. The animals were necropsied and pathologic examinations were made of all major organs and tissues.

Three-Month Study in Dogs. Three groups of two male and two female Beagle dogs were given daily oral doses of 5, 15 or 50 mg/kg monensin by gelatin capsules (approximately equivalent to dietary concentrations of 200, 600 or 2,000 ppm). A similar group was maintained untreated and served as the control group. The physical condition of each animal was assessed daily by observation and body weights were measured weekly. Hematologic and blood biochemistry values were obtained on all animals before and five times during the test period. All animals were necropsied and pathologic evaluations were made of all major organs and tissues.

Three-Month Study in Mice. Four groups of 15 male and 15 female B6C3F$_1$ mice were fed diets containing 37.5, 75, 150 or 300 ppm monensin. A similar group was given untreated diet and served as the control group. Hematologic and blood biochemistry values were obtained before necropsy. Pathologic examinations were made of all major organs and tissues.

One-Year Study in Dogs. Four groups of four male and four female Beagle dogs were given daily oral doses of 1.25, 2.5, 5 or 7.5 mg/kg monensin. A similar group was maintained untreated and served as the control group. The animals were examined daily. Body weights, hematologic and blood biochemistry values were determined at specified intervals before and during the test period. Ophthalmoscopic and electrocardiographic (EKG) evaluations were performed before, during and at the end of the study. All dogs were necropsied and all major organs and tissues were examined and evaluated histopathologically. Very critical evaluations were made of the heart, diaphragm and skeletal muscles because these tissues are known to be affected when toxic doses of monensin are administered.

Multigeneration Reproduction Study in Rats. Three groups of 25 to 30 male and female Wistar rats were continuously maintained on diets containing 33, 50 or 80 ppm monensin for four generations. A similar group was given untreated diet and served as the controls (figure 1). The reproductive capability of parents and the health status of offspring were evaluated. The $f_{1a}$ offspring were assigned to the 2-yr chronic and oncogenic study. The progeny of the $F_2$ generation were evaluated for teratogenic defects by macroscopic and microscopic examinations for visceral or skeletal abnormalities.

Chronic and Oncogenic Study in Rats. Three groups of 100 male and 100 female Wister rats derived from parents given diets containing monensin were fed 33, 50 or 80 ppm monensin in the diet for 2 yr. A similar group was given untreated diet and served as the control group. The animals were examined daily for physical signs of toxicity. Body weights, hematologic and blood biochemistry values were determined at intervals during the test period. Animals dying during the study and those surviving to the end of the study were necropsied. Special attention was directed to chronic and neoplastic lesions during the pathologic evaluation.

Results

Acute Toxicity. The comparative LD$_{50}$ values for various species are presented in table 1. Female rats and dogs tolerated less monensin than males. The consistent signs of acute toxicity appeared in the following order:
Dose Groups: 0, 33, 50 and 80 ppm Monensin in Diet

Figure 1. Treatment and testing protocol for multigeneration reproduction study of monensin in Wistar rats. *Gross internal examination of one sex -1 litter -1 .

anorexia, hypoactivity, skeletal muscle weakness, ataxia, diarrhea, decreased weight gain and delayed death. The condition of surviving animals improved during the observation period.

Three-Month Study in Rats. The results for the low-dose group of animals were similar to the control group. A slight effect on body weight gain was the only sign of toxicity in the middle-dose group. Major signs of toxicity evident in the high-dose group were: severe depression in body weight gain, 17 deaths during the first 3 wk of the study, moderate skeletal muscle degeneration and necrosis, and slight cardiac muscle degeneration. There were no significant alterations in the hematologic or blood biochemistry values.

Three-Month Study in Dogs. The low dose of 3 mg/kg was tolerated with no important signs of toxicity. However, the 15 and 50 mg/kg doses produced anorexia, weakness, ataxia, labored respiration and body weight loss. These signs occurred during test wk 1 to 4. Aspartate transaminase (SGOT) values were greatly elevated during wk 1 to 4 in six of eight dogs of the two highest dose groups (table 2). Afterwards these values returned to normal ranges for the remainder of the study. The elevation of this enzyme is an important indicator of muscle injury. Two dogs died and one was killed in a moribund condition during the first 2 wk with severe skeletal muscle lesions and minimal cardiac lesions. Only minimal skeletal muscle damage was found in dogs of the top two dose groups at the termination of the study. The histopathologic spectrum of degenerative changes in skeletal muscles consisted of swollen eosinophilic homogenous staining cytoplasm with loss of cross-striations, fragmentation of fibers and vacuolization (figure 2). Dogs surviving the acute phase of skeletal muscle degeneration had histologic evidence of repair consisting of the infiltration of macrophages to remove cellular debris,
Species  

<table>
<thead>
<tr>
<th>Species</th>
<th>Male</th>
<th>Female</th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td>Mouse</td>
<td>70.0 ± 9.0</td>
<td>96.0 ± 12.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>40.1 ± 3.0</td>
<td>28.6 ± 3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>&gt;20.0</td>
<td>&gt;10.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>41.7 ± 3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>&gt;160.0</td>
<td></td>
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<tr>
<td>Chicken</td>
<td>200.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>26.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>11.9 ± 1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>26.4 ± 4.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>16.7 ± 3.57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse</td>
<td>2 to 3 (estimated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trout</td>
<td>&gt;1,000</td>
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</tr>
</tbody>
</table>

increased sacrolemma nuclei and regeneration of muscle fibers. There were no important changes in the hematologic values.

Three-Month Study in Mice. A dose-related decrease in body weight gain (P<.05) occurred in all dose groups, but there were no other physical signs of toxicity. Serum creatine phosphokinase (CPK) values were slightly increased in several animals in the two highest dose groups. The source of the CPK was considered to be from either skeletal or heart muscles. Minimal heart lesions were found on microscopic examination in the highest dose group. No other toxicologic effects were observed.

One-Year Study in Dogs. All animals survived the test period and there was no evidence of toxicity in the two lowest dose groups. Dogs in the 5 and 7.5 mg/kg dose groups had transient signs of anorexia, hypoactivity and weakness. Six of 16 dogs in the two highest dose groups had elevated alanine transaminase (SGPT) values during test wk 1 and 2. Creatine phosphokinase values were increased in 13 of 16 dogs in the two highest dose groups during test wk 1 to 4. These serum enzyme values returned to normal range during the remainder of the study. This pattern of enzyme alteration is indicative of muscle damage during the first 4 wk with recovery. No abnormal EKG values were found. Except for a decrease in body weight in the highest dose group, there were no other toxicity findings and no pathologic lesions attributable to treatment.

TABLE 2. THE ELEVATION AND RETURN TO NORMAL OF ASPARTATE TRANSAMINASE (SGOT) VALUES (IU/LITER) IN DOGS GIVEN 15 OR 50 MG/KG MONENSIN FOR 3 MO

<table>
<thead>
<tr>
<th>Dose, mg/kg</th>
<th>Animal number</th>
<th>Sex</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
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<tbody>
<tr>
<td>15</td>
<td>120472</td>
<td>M</td>
<td>38</td>
<td>62</td>
<td>5,000</td>
<td>162</td>
<td>58</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>121032</td>
<td>M</td>
<td>35</td>
<td>680</td>
<td>(Killed moribund)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120652</td>
<td>F</td>
<td>34</td>
<td>84</td>
<td>185</td>
<td>98</td>
<td>50</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>120682</td>
<td>F</td>
<td>42</td>
<td>71</td>
<td>3,800</td>
<td>7,600</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>50</td>
<td>121073</td>
<td>M</td>
<td>51</td>
<td>1,150</td>
<td>(Died)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>121313</td>
<td>M</td>
<td>45</td>
<td>3,000</td>
<td>(Died)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>118843</td>
<td>F</td>
<td>49</td>
<td>99</td>
<td>950</td>
<td>1,190</td>
<td>45</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>121563</td>
<td>F</td>
<td>47</td>
<td>150</td>
<td>97</td>
<td>78</td>
<td>53</td>
<td>43</td>
</tr>
</tbody>
</table>


Figure 2. A longitudinal section of skeletal muscle from a dog given monensin (X400). At the top of the photomicrograph is a normal muscle fiber with cross-striations. Most of the other fibers are in stages of degeneration and necrosis, evident by the loss of cross-striations, vacuolization, fragmentation and infiltration of macrophages.

Multigeneration Reproduction Study in Rats. The reproductive capacity through four generations of rats fed monensin, including fertility, litter size, gestation length, parent and progeny survival and sex distribution was unaffected. The only treatment effect was a slight decrease in body weight gain noted in each generation. There was no evidence of teratogenicity in the progeny from treated parents.

Chronic and Oncogenic Study in Rats. Survival was better in the treated groups than in the control group. There was no evidence of toxicity, except for a slight decrease in body weights, in any of the measured determinations. The test compound did not affect the incidence or severity of chronic lesions including those in skeletal and cardiac muscles. The type, latency and prevalence of benign and malignant neoplasms were similar in control and treated rats.

Discussion

The toxicity of monensin has been extensively investigated in laboratory animals. Dose levels were identified which could be administered daily for long periods of time without producing harmful effects. Predictable dose-related toxicity was limited to target organs in several mammalian species. The characterization of the target organ effects and correlation to physical signs has been determined. Animals given toxic doses of monensin developed anorexia, hypoactivity, weakness, ataxia, diarrhea and body weight loss. These signs were usually delayed from one to several days depending on dosage and were reversible even when animals were maintained on test compound.

The target organs damaged by toxic doses of monensin were identified to be skeletal and cardiac muscles. The development and recovery of the muscle damage can be effectively monitored by measuring serum muscle enzymes. The muscles that were most severely and extensively damaged are those that maintain the highest level of activity, such as the diaphragm, abdominal and heart muscles. Although muscle damage is the hallmark of monensin toxicity in animals, it is not pathognomonic of monensin toxicity. The myopathy produced by monensin is very similar to that reported for plasmocid (Price et al., 1962; D'Agostino, 1963) and for A204 (Todd et al., 1971).

The pathogenesis of the muscle lesions induced by monensin is not completely un-
stood. It has been demonstrated that monensin and other polyether monocarboxylic ionophores form cationic (Na⁺, K⁺) complexes that enhance their transport across bimolecular lipid membranes (Pinkerton and Steinrauf, 1970; Wong et al., 1971; Sandeaux et al., 1982). These ionophores produce ultrastructural changes in muscles consisting of dilated sacroplasmic reticulum, misshapened mitochondria and lysis of myofibrils (Griffing et al., 1971). The functional disturbances observed in animals given toxic doses of monensin can be explained by the structural changes in muscles due to the altered transport of cations.

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