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THE GUINEA PIG 'CAVIA PORCELLUS' AS A MODEL FOR ISONIAZID-INDUC--ETC(U)
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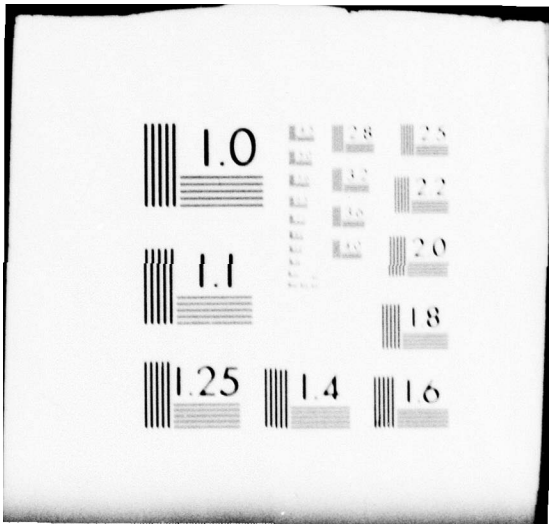
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The Guinea Pig (*Cavia porcellus*) as a Model for Isoniazid-Induced Reactions[1,2]

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Master's thesis

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Running head: ISONIAZID IN GUINEA PIGS

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Daily injections of isoniazid were given to evaluate the guinea pig as a model in the study of such human side effects as coagulopathy syndrome, pyridoxine deficiency and hepatic lesions. Mild hepatitis was demonstrated by significant increases in SGOT and SDH levels. Hepatic lesions ranged from focal areas of inflammatory cell infiltrate to necrosis. Cutaneous testing demonstrated that the hepatitis was probably a hypersensitivity reaction to both isoniazid and isonicotinic acid. Significant eosinophilia and eosinophils present in hepatic

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also suggested a hypersensitivity reaction.

Significant increases in prothrombin time, partial thromoboplastin time and fibrin split products as well as significant decreases in fibrinogen and thrombocytes indicated that a coagulopathy syndrome has been produced. Specifically, a disseminated intravascular coagulation-like syndrome was shown to occur in significant numbers of treated animals. Isoniazid treatment also induced a pyridoxine deficiency which was demonstrated by significant decreases in serum pyridoxine concentrations. This deficiency was associated with demyelination of the sciatic nerve in treated guinea pigs. Because the abnormalities found in these guinea pigs are similar to those reported in man, this species appears to be a good model for studying isoniazid-induced hypersensensitivity hepatitis and its possible linkage to the syndrome of disseminated intravascular coagulation.

SUMMARY

Daily injections of isoniazid were given to evaluate the guinea pig as a model in the study of such human side effects as coagulopathy syndrome, pyridoxine deficiency and hepatic lesions. Mild hepatitis was demonstrated by significant increases in SGOT and SDH levels. Hepatic lesions ranged from focal areas of inflammatory cell infiltrate to necrosis. Cutaneous testing demonstrated that the hepatitis was probably a hypersensitivity reaction to both isoniazid and isonicotinic acid. Significant eosinophilia and eosinophils present in hepatic lesions also suggested a hypersensitivity reaction.

Significant increases in prothrombin time, partial thromboplastin time and fibrin split products as well as significant decreases in fibrinogen and thrombocytes indicated that a coagulopathy syndrome had been produced. Specifically, a disseminated intravascular coagulation-like syndrome was shown to occur in significant numbers of treated animals. Isoniazid treatment also induced a pyridoxine deficiency which was demonstrated by significant decreases in serum pyridoxine concentrations. This deficiency was associated with demyelination of the sciatic nerve in treated guinea pigs. Because the abnormalities found in these guinea pigs are similar to those reported in man, this species appears to be a good model for studying isoniazid-induced hypersensitivity hepatitis and its possible linkage to the syndrome of disseminated intravascular coagulation.

Key Words: Isoniazid, hepatitis, hypersensitivity, coagulopathy syndrome, demyelination, guinea pig.

Introduction

Isoniazid is considered by many to be the most effective drug in the control and treatment of tuberculosis (1); however, it does have the potential of producing some serious side effects. The most prominent of these in man includes decreased serum pyridoxine concentrations (2), hepatic toxicity (3-5), and possibly a coagulopathy syndrome (6).

Decreased serum pyridoxine values occur rather frequently in human patients receiving isoniazid, and are probably due to the drug's competition with pyridoxal phosphate for the enzyme apotryptophanase (7). One of the signs of a pyridoxine deficiency in man is peripheral neuropathy which, initially, is sensory and later may progress to motor involvement (8). A similar peripheral neuropathy is seen in approximately 17% of the patients receiving 6 mg/kg of isoniazid daily with increasing incidences occurring at higher doses (9). Histologically, isoniazid-induced peripheral neuropathy is characterized by fragmentation of the myelin sheath of the small and medium sized sensory and mixed nerve fibers to the muscle spindles (10). In addition, there is concomitant remyelination and collateral sprouting of motor axons. Numerous studies on humans and rats have shown that isoniazid-induced peripheral neuropathy can be prevented or reversed by the administration of pyridoxine (2, 11).

The frequency of isoniazid-induced liver disease is highly variable in humans, ranging from 8.7% to 22.9% (3, 5). Changes in liver function tests have been demonstrated by increased alkaline phosphatase activity, marked elevation of serum glutamic oxaloacetic

transaminase (SGOT) activities and increases in serum glutamic pyruvic transaminase (SGPT) in symptomatic as well as asymptomatic hepatitis patients (12).

The duration of isoniazid therapy before the onset of hepatic disease varies considerably, but nearly one-half of the patients with or without tuberculosis lesions developed evidence of hepatic disease within the first 2 months of isoniazid therapy, with more hepatic involvement occurring during the second month than at any other time (5). However, hepatitis can develop at any time during the first year of isoniazid treatment.

The cause of isoniazid-induced hepatic injury is uncertain, but it is believed that its pathogenesis represents a hypersensitivity reaction to isoniazid or its metabolites (4, 13-19). Presumably, the drug attaches to a liver cell protein in such a way as to make it immunogenic (20).

A secondary effect of isoniazid-induced hepatic damage may be a coagulopathy syndrome. Approximately 85% of the patients with liver disease have at least one abnormality in tests of clotting function (21). Disseminated intravascular coagulation (DIC) has been described in various types of liver disease, including isoniazid-induced liver damage (6). Theoretically, DIC develops as a consequence of liver disease because thromboplastic substances from damaged hepatic cells are released into the blood stream and initiate systemic coagulation. Some of the clotting defects seen in DIC could be a result of decreased hepatic synthesis as well as an increased utilization of

clotting factors. However, in fibrinogen turnover studies in patients with diseased livers (22), it appears that increased utilization is the mechanism involved rather than decreased synthesis of fibrinogen.

The purpose of this study was to demonstrate isoniazid toxicity in guinea pigs and to determine if the syndromes were similar to those which occurred in man. Guinea pigs were chosen for this study primarily because they are excellent models for the study of immunologic disease and often exhibit similarities in their immune responses to those of man (23, 24).

Materials and Methods

Animals. One hundred and fifty female, Dla:(DH) guinea pigs (Dutchland Laboratory Animals, Inc., Denver, Pa.) weighing 300-500 g were randomly assigned into 5 groups of 30 guinea pigs each. Each group was given twice-daily intramuscular (i.m.) injections of isoniazid (Nydrazid, Squibb and Sons, Princeton, N.J.) in divided doses for 11 weeks. Groups 1-4 received isoniazid at doses of 10, 30, 60 and 100 mg/kg/day respectively; group 5 served as untreated controls and received 2 ml sterile saline per day. The guinea pigs were weighed weekly and the quantity of isoniazid was adjusted according to the weight gain or loss.

Evaluation of hypersensitivity reaction. The guinea pigs were tested for a cutaneous delayed-type hypersensitivity reaction to isoniazid (INH) (Sigma Chemical Co., St. Louis, Mo.) and its metabolite, isonicotinic acid (INA) (Sigma), using a modification of Mathieu's technique (25). One week after the start of treatment all guinea pigs were immunized with conjugates of INH and INA, human serum albumin (HA) (Sigma) and guinea pig serum albumin (GPA) (Sigma) (26). Ten milligrams of each of these conjugates in complete Freund's adjuvant (Dolbey Scientific, Inc., Philadelphia, Pa.) were injected intradermally (i.d.) in the flank of each guinea pig. The guinea pigs were re-immunized at weeks 4 and 7.

Cutaneous sensitivity test was measured on the abdomen of each guinea pig 72 h before the animal was euthanized. Each guinea pig was tested i.d. with 10.0 mg of each of the following soluble

antigens in a saline solution: INH-HA, INA-HA, INH-GPA, INA-GPA, INH, INA, HA, GPA and tuberculin (Jensen-Salsbery Laboratories, Kansas City, Mo.). At the time of euthanasia, the injection sites were examined for erythema, edema and/or necrosis. The cutaneous lesions were scored on a range of 0 to 7, depending on the type of reaction and to which antigen the reaction had occurred.

Hematologic determinations. Five guinea pigs from each group were exsanguinated on weeks 1, 3, 5, 7, 9, and 11 of the study by cardiac puncture following anesthesia with 1% halothane (Fluothane, Ayerst Laboratories, New York, N.Y.).

Blood collected in the EDTA tubes was used to determine the thrombocyte count by using the manual method (Unopette, Beckton Dickinson Co., Waltham, Mass.) and an automated counter (model ZB16, Coulter Electronics Corp., Hialeah, Fla.) The presence or absence of eosinophilia was determined by a complete blood count (CBC). The plasma obtained from the blood collected in the sodium citrate tubes was used to assay for fibrinogin, prothrombin time, and activated partial thromboplastin time (APTT) (27-29). Blood collected in the fibrin split products tubes was used to detect the presence of FSP utilizing the hemagglutination inhibition immunoassay test (30). Pyridoxine was assayed for in a spectrophotofluorometer (Model MPF-4, Perkin-Elmer Corp., Norwalk, Conn.) (31). The serum obtained from the blood collected in dry tubes was separated into two fractions. One fraction of 1 ml was used for the isoniazid assay (32) and the remainder of the serum was assayed for SGPT, SGOT and SDH.

Histopathology. After collection of the blood samples, a complete necropsy was done on each guinea pig. Tissue samples from the heart, kidney, liver, brain and sciatic nerve were fixed in 10% neutral buffered formalin. Paraffin imbedded sections 5 μ thick were made from each tissue and stained with hematoxylin and eosin. In addition, sections of the sciatic nerve were stained with Luxol's fast blue to demonstrate myelin, and sections of the liver were post-fixed in 1% osmium tetroxide to demonstrate fatty changes. The histopathological evaluation of the liver and nerve were scored on ranges of 0 to 6 and 0 to 5, respectively, depending on the severity of the lesions.

Statistical analysis. A two-way analysis of variance was done to determine if an overall significance ($P < 0.05$) did exist between the guinea pig groups for weight, fibrinogen, APTT, PT, SGPT, SGOT, SDH, pyridoxine, eosinophil count and thrombocyte count at each sampling period and between the weeks of the study. Next a one-way analysis of variance was done on each parameter for each week of the study to determine if a significant difference ($P < 0.05$) existed between the groups. The least-squares difference test (33) was used to compare each treated group mean to the control group mean for each of the 6 weeks of the project. The mean score values for FSP, skin sensitivity, and nerve and liver histopathology, were analyzed nonparametrically using the Kruskal Wallis test (34). The statistically significant presence of a DIC-like syndrome was calculated using Fisher's Exact test (35). A value of $P < 0.05$ was considered statistically significant.

Results

Clinical chemistry: evaluation of hepatic damage. SGPT activities were similar in the control and isoniazid-treated guinea pigs throughout the experimental period. SDH and SGOT values of the isoniazid-treated guinea pigs were increased beginning in week 3 when compared to the controls (tables 1 and 2, respectively). Significant increases ($P < 0.05$) in the SDH values of the isoniazid-treated guinea pigs occurred throughout the project except in weeks 5 and 7 when the control SDH values were also elevated (table 1). In the 10 mg/kg group the SDH activities were significantly increased ($P < 0.05$) in weeks 7 and 11; in the 30 and the 100 mg/kg groups significant increases ($P < 0.05$) existed in weeks 3, 7, 9, and 11; and the 60 mg/kg group demonstrated significant increases ($P < 0.05$) in weeks 3, 7, and 9. These results indicate that the three higher dose groups developed early liver damage which continued to the end of the study. The most severe liver damage occurred in week 9 in the 60 and 100 mg/kg groups when the mean activities values were 241.3 and 250.9 Sigma units/ml, respectively.

A significant increase ($P < 0.001$) in the SGOT activity was first demonstrated in week 3 in the 100 mg/kg group and in week 5 in the 60 mg/kg group (table 2). In week 7, the 10 and the 100 mg/kg groups were significantly increased ($P < 0.01$). By week 9 significant increases ($P < 0.05$) in the SGOT activities were found in the 30, 60 and 100 mg/kg groups and this elevation persisted in week 11.

Hematology: evaluation of hypersensitivity reaction. The control guinea pigs had a slight eosinophilia at the beginning of the study.

By week 3, however, all the isoniazid-treated groups had significantly elevated ($P < 0.05$) eosinophil counts which continued through week 11. From week 3, the eosinophil counts for the control group and the isoniazid-treated groups ranged from 45.6 to 125.4/mm^3 and 152.2 to 586.1/mm^3, respectively. No other abnormalities were found in the WBC counts.

Hematology: evaluation of a DIC syndrome. Fibrinogen concentrations of isoniazid-treated guinea pigs were significantly decreased ($P < 0.001$) each week at every dose when compared to the controls (table 3). The only exception to this was that the 10 mg/kg group was not significantly decreased in week 5.

The fibrinogen concentrations remained constant for the control groups. However, they were decreased for all treatment groups by the end of the first week of the study. The guinea pigs treated with the lowest dosage (10 mg/kg) generally had higher values of fibrinogen than the other treatment groups until the seventh week of the study, when these were not significantly higher ($P < 0.05$) than those of the other isoniazid-treated groups.

The prothrombin times for the isoniazid-treated guinea pigs were significantly increased ($P < 0.05$) each week at every dose when compared with the control group values (table 4). In addition, the 100 mg/kg group had longer prothrombin times than the other treated groups; these were significantly longer ($P < 0.05$) at weeks 7 and 11.

The APTT times for the isoniazid-treated guinea pigs were consistently prolonged when compared with the control guinea pigs. However, the results were variable and only the higher isoniazid doses,

30, 60 and 100 mg/kg, produced significantly prolonged times ($P < 0.05$) in half or more of the sampling times (table 5).

Fibrin split products were not present in any guinea pig in any group through the first 7 weeks of the study. By week 9, however, 4 guinea pigs in the 30 mg/kg group and 2 each in the 60 and 100 mg/kg groups had FSP. FSP continued to be present in these groups through week 11. The 60 and the 100 mg/kg groups had 4 guinea pigs each with FSP and the 30 mg/kg group had one.

The thrombocyte count did not vary significantly between the isoniazid-treated guinea pigs and the control guinea pigs through the first 7 weeks of treatment. The 60 and the 100 mg/kg groups had significantly decreased thrombocyte counts in week 9 ($P < 0.01$) and week 11 ($P < 0.05$) as compared to the controls. The mean thrombocyte counts in week 9 in the 60 and the 100 mg/kg groups were 231,000 and 272,000, respectively, compared with a control mean of 614,000. In week 11, the mean thrombocyte counts were 252,000, 373,000, and 464,000 for the 60 mg/kg, the 100 mg/kg and the control groups, respectively.

The criteria for a DIC-like syndrome, including thrombocytopenia, presence of FSP, decreased fibrinogen, increased PT, and increased APTT were met in individual guinea pigs in isoniazid-treated groups beginning in week 7. A DIC-like syndrome was found in significant numbers of guinea pigs in the 60 mg/kg group in weeks 9 and 11 and in the 100 mg/kg group in week 11.

Evaluation of a pyridoxine deficiency. Pyridoxine concentrations of the isoniazid-treated guinea pigs were consistently lower than the controls throughout the experimental period (table 6). Each isoniazid

group had significantly decreased ($P < 0.05$) pyridoxine concentrations in half or more of the testing periods. The 100 mg/kg group had significantly decreased ($P < 0.05$) pyridoxine concentrations each week except week 9.

Cutaneous sensitivity tests: evaluation of hypersensitivity reactions. The control guinea pigs developed a reaction to the tuberculin when first tested at week 3. This reaction consisted of edema, inflammation and necrosis at the site of the injection. None of these guinea pigs demonstrated any reaction to the other agents. By week 5, two of the controls had, in addition to a necrotic reaction to the tuberculin, an inflammatory reaction to the INH conjugates. The control guinea pigs gradually showed increased reactivity to the conjugates: by week 7, some guinea pigs had necrotic reactions to the INH conjugates; and by week 11, two of the five controls had necrotic reactions to both INH and INA conjugates.

Guinea pigs receiving isoniazid at all dosages developed cutaneous reactions to the conjugates when first tested at week 3. The cutaneous reactions to the conjugates were significantly more severe ($P < 0.1$) in the isoniazid-treated guinea pigs during weeks 3 through 9. No significant differences in cutaneous reactions were seen during week 11.

When cutaneously tested at week 3, the isoniazid-treated guinea pigs showed more severe reactions to the INH conjugates than to the INA conjugates. At this first cutaneous test, all but one guinea pig developed a necrotic reaction to one or both of the INH conjugates, while only one demonstrated a necrotic response to the INA

conjugates, in addition to the INH conjugates. By week 9, all but 3 of the guinea pigs receiving isoniazid developed necrotic reactions to both the INH and INA conjugates. However, the severity of the necrotic reactions to both the INH conjugates diminished somewhat during weeks 9 and 11, while the severity of the necrotic reactions to the INA conjugates reached a peak during those two weeks.

The sensitivity that the isoniazid-treated guinea pigs showed for the conjugates of INH and INA generally was more severe than that for tuberculin. The control guinea pigs also demonstrated increased reactivity to the INH and INA conjugates, but not until weeks 7, 9 and 11.

Pathology. The only gross lesions seen in any of the organs were yellowish-brown discolorations of the livers of some of the guinea pigs in the 60 and 100 mg/kg dosage groups in weeks 9 and 11. No histologic lesions were seen in the heart, kidneys or brain of any guinea pigs.

Three control guinea pigs had hepatic lesions; one had a small focal area of hepatic necrosis with no inflammatory cell involvement, while the other two each had a small focal area of necrosis with some inflammatory cell involvement. The mean liver evaluation score for the control guinea pigs was 0 each week except weeks 7 and 9, when it was 0.4 and 1.2, respectively.

The isoniazid-treated guinea pigs had statistically significant ($P < 0.1$) hepatic damage when compared with the controls. The hepatic damage occurred primarily in the 30, 60 and 100 mg/kg groups where the mean liver evaluation score ranged from 1.0 in the 30 mg/kg group in the first week to a high of 5.2 in the 100 mg/kg group in week 7.

A statistically significant ($P < 0.01$) amount of hepatic damage was seen in the 10 mg/kg group during week 5 when the mean liver evaluation score was 2.6. Consistent statistically significant ($P < 0.05$) hepatic damage in the higher isoniazid doses began in week 7 and continued through to the end of the study.

The hepatic changes seen in the isoniazid-treated guinea pigs included focal aggregation of inflammatory cells, focal areas of necrosis occasionally associated with inflammatory cells, and diffuse areas of hepatocyte degeneration and regeneration. Focal and diffuse areas of fatty degeneration among hepatocytes, was demonstrated by osmium tetroxide post-fixation.

None of the control guinea pigs had sciatic nerve lesions. Demyelination of the sciatic nerve was first found in week 3 in two isoniazid-treated guinea pigs, one each in the 60 and 100 mg/kg groups. The incidence of demyelination continued to be low in week 5, occurring in only 2 animals in the 60 mg/kg group. In week 7, 3 of 5 guinea pigs in each of the 60 and the 100 mg/kg groups had demyelination of the sciatic nerve. The incidence of demyelination increased greatly in week 9 involving all guinea pigs at the higher dosages as well as some guinea pigs in the 10 and 30 mg/kg groups. A similar incidence was found in week 11, except that no lesions were found in the former group. The occurrence of nerve lesions was statistically significant ($P < 0.05$) in the 60 mg/kg group in week 9 and in the 100 mg/kg group ($P < 0.01$) in weeks 9 and 11.

The nerve lesions seen in the isoniazid-treated guinea pigs became progressively more severe as the treatment continued and

appeared to be dose-related. Infiltration of the sciatic nerve with inflammatory cells was characterized by eosinophils, neutrophils and macrophages. Soon after the appearance of the inflammatory cells, small eosinophilic-appearing granules were formed among the fibers and in vacuole-like spaces. Other abnormalities seen during this time, or shortly thereafter, included swelling of the myelin sheaths adjacent to the groups of inflammatory cells and intimal proliferation of nearby arteries. These lesions progressed to large areas containing many inflammatory cells and few or no axons in the 60 and 100 mg/kg groups.

Discussion

The experimental injection of guinea pigs (C. porcellus) with isoniazid appeared to produce clinical, laboratory and pathologic changes similar to those reported for isoniazid-induced hepatic damage in humans (20, 30, 36). There were significant increases in the SGOT and SDH activities as well as significant differences in liver lesions between isoniazid-treated guinea pigs and controls. The delayed hypersensitivity demonstrated in this study indicates an allergic rather than a toxic reaction to the drug. The study also provides a possible linkage between isoniazid-induced liver damage and a DIC-like syndrome as well as a parallel pyridoxine deficiency.

The elevated SDH activities for the isoniazid-treated guinea pigs suggest that while some liver damage, including necrosis, has occurred in the treated guinea pigs, the damage is not massive and involves only a portion of the hepatic tissue. Further evidences of a mild hepatitis are the elevations seen in the SGOT and SGPT activities. The isoniazid-treated guinea pigs had only a moderate increase in SGOT and almost no increase in SGPT. If massive hepatitis had occurred in the treated guinea pigs, many hepatocytes would be damaged, thus releasing large quantities of these enzymes into the circulation resulting in much higher values.

The liver histopathology also indicated that mild hepatitis had occurred in the isoniazid-treated guinea pigs. The areas of inflammatory cell infiltrate and/or necrosis were focal and involved only a small portion of the liver. These changes appeared to be due to a hypersensitivity reaction to isoniazid and/or isonicotinic acid.

The fatty changes, seen primarily in weeks 9 and 11, are not indicative of a hypersensitivity reaction. However, focal areas of mononuclear cell infiltrates could be seen in areas unaffected by the fatty change indicating that a hypersensitivity-type reaction also occurred in these livers.

The results of this study demonstrate induction of a specific delayed-type hypersensitivity to isoniazid and its metabolite, isonicotinic acid, when they were conjugated to a protein, serum albumin. By demonstrating a hypersensitivity reaction to both isoniazid and isonicotinic acid, it appears that the guinea pig is similar to man (37) and the rhesus monkey (38) in the liver's ability to metabolize a portion of the isoniazid to isonicotinic acid.

Both the control and the isoniazid-treated guinea pigs demonstrated that a time difference was required to develop a sensitivity to isoniazid and isonicotinic acid. This indicates that the guinea pigs first became sensitized to isoniazid and later to isonicotinic acid. The hypersensitivity that developed was a specific reaction to isoniazid and isonicotinic acid and not to the protein to which they were conjugated, since no reaction occurred to either the human albumin or to the guinea pig albumin when they were tested separately. All guinea pigs reacted to the tuberculin because they had been sensitized with complete Freund's adjuvant. This reaction was expected and thus was used as an indicator to ensure that the sensitivity test was done properly. The increased severity and the decreased time involved to develop the cutaneous reactions in the isoniazid-treated guinea pigs indicates that these guinea pigs had a higher antibody titer to isoniazid

and isonicotinic acid. This coincided with the development of hepatic lesions in the isoniazid-treated guinea pigs and would seem to indicate that liver lesions are similar to those seen with a hypersensitivity reaction rather than a direct toxicity.

The occurrence of eosinophilia in the isoniazid-treated guinea pigs beginning in week 3 also suggests a hypersensitivity reaction. Eosinophilia is common to antigen-antibody reactions including instances of parasitism and in disease leading to degeneration of body protein (39). The cutaneous sensitivity reaction seen in all guinea pigs could account for some, but not all, of the eosinophil increase. This tends to indicate that a hypersensitivity reaction is occurring elsewhere in the isoniazid-treated guinea pigs; the most likely site appears to be the liver. The presence of necrosis and eosinophils in hepatic lesions supports the theory that the liver is the site of a hypersensitivity reaction.

The diagnosis of a DIC-like syndrome in the isoniazid-treated guinea pigs was suggested by the development of thrombocytopenia, decreased fibrinogen, prolonged APTT and PT and elevated FSP. However, the presence of FSP was inconsistent, as was the decrease in thrombocytes. A possible explanation for the failure to detect FSP earlier and in higher titers is that the test, while sensitive for human FSP, may not be sufficiently sensitive for the guinea pig; some other test, such as thrombin times, may be a more sensitive indicator of guinea pig FSP.

The cause of the coagulopathy syndrome demonstrated in this study is probably isoniazid-induced liver damage because the liver is the

primary site for the synthesis of many of the clotting factors. When liver damage occurs, there is either decreased synthesis or synthesis of abnormally-structured clotting factors. Another possible cause of the coagulopathy is a vasculitis which can occur as a result of a hypersensitivity reaction. This was probably not involved, however, in the pathogenesis of the coagulopathy syndrome because few lesions resembling vasculitis were seen histopathologically. Some minor vasculitis did occur around the cutaneous test sites, but was probably not sufficiently extensive to produce a coagulopathy syndrome.

An obvious hemorrhagic disorder, including subcutaneous hemorrhage and microthrombi, was not seen in any of the isoniazid-treated guinea pigs. This suggests that severe thrombocytopenia had not occurred, even though significant thrombocyte decreases at the two highest dosages were found. It is possible, however, that the thrombocytopenia would have progressed and that a hemorrhagic syndrome would have been demonstrated had the study continued.

The isoniazid-induced pyridoxine deficiency in the treated guinea pigs appeared to have produced peripheral nerve lesions similar to those reported in other laboratory animals as well as man (8-10, 40). The nerve lesions associated with isoniazid therapy are similar to those described for Wallerian degeneration of the axon (43), with the additional component of inflammatory cells being present. In this degeneration, the myelin sheath is converted into an irregular series of ovoids which contain some plasma and fragments of axon. Inflammatory cells are probably attracted by the degeneration of the myelin sheath.

These macrophages appear to phagocytize the myelin sheath and produce the eosinophilic-appearing granules seen histologically.

The nerve lesions did not involve the entire nerve. In most of the guinea pigs the lesions were confined to one or more areas with normal appearing axons and myelin sheaths in the other areas. As a result of limited nerve involvement, the lesions did not produce any clinically evident motor deficit.

The results of this study indicate that isoniazid, when given to guinea pigs at high dosage levels for a protracted period of time, produces some changes, including hepatitis, peripheral neuropathy, and a DIC-like syndrome. The hepatitis appears to be due to a hypersensitivity reaction caused by isoniazid and/or its metabolite, isonicotinic acid. Because the isoniazid-induced hepatic lesions found in the guinea pigs in this study are very similar to those reported in man and other animals, it can be postulated that a hypersensitivity-type reaction is also responsible for the hepatitis reported in the other species. The guinea pig is thus a valuable potential model in the study of side-effects of isoniazid therapy, and may be used to improve evaluation, understanding and prediction of these detrimental reactions.

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TABLE 1

COMPARISON OF THE MEAN SORBITOL DEHYDROGENASE LEVELS BETWEEN CONTROL AND ISONIAZID-TREATED GROUPS

Treatment	SDH by Weeks of Treatment (Sigma Units/ml)					
	1	3	5	7	9	11
Control	76.9 ± 53.7	72.4 ± 19.5	132.6 ± 21.5	139.2 ± 12.5	68.4 ± 13.2	121.8 ± 20.9
10 mg/kg	132.8 ± 50.7	102.8 ± 37.7	167.9 ± 80.5	178.1 ± 20.1*	128.1 ± 75.4	209.6 ± 54.1*
30 mg/kg	25.1 ± 12.5	200.2 ± 30.9*	151.0 ± 74.7	174.3 ± 36.4*	156.2 ± 24.9*	183.5 ± 7.5*
60 mg/kg	131.6 ± 60.6	188.9 ± 65.5*	193.3 ± 32.8	178.4 ± 11.8*	241.3 ± 27.2*	181.5 ± 71.7
100 mg/kg	98.7 ± 39.6	241.2 ± 46.5*	201.8 ± 42.6	167.8 ± 19.4*	250.9 ± 44.9*	185.0 ± 26.7*

Data are expressed as mean ± SD.

*$P < 0.05$ from to the control group.

TABLE 2

COMPARISON OF THE MEAN SERUM GLUTAMIC OXALOACETIC TRANSAMINASE LEVELS

BETWEEN CONTROL AND ISONIAZID-TREATED GROUPS

Treatment	SGOT by Weeks of Treatment (Reitman-Frankel Units/ml)					
	1	3	5	7	9	11
Control	91.5 ± 33.9	66.4 ± 18.1	80.8 ± 12.8	61.5 ± 17.2	89.2 ± 28.3	69.4 ± 21.2
10 mg/kg	159.5 ± 95.2	118.2 ± 39.6	75.0 ± 14.2	243.0 ± 21.9*	93.0 ± 30.3	83.6 ± 51.8
30 mg/kg	174.0 ± 9.8	53.5 ± 12.2	53.6 ± 28.4	72.0 ± 6.9	191.5 ± 25.6*	155.0 ± 25.6*
60 mg/kg	138.5 ± 96.8	56.7 ± 16.7	152.5 ± 27.4*	87.5 ± 29.6	157.2 ± 45.5*	116.0 ± 31.5*
100 mg/kg	156.4 ± 53.4	146.0 ± 66.6*	73.4 ± 25.1	423.5 ± 256.6*	176.4 ± 45.8*	155.6 ± 17.8*

Data are expressed as mean ± SD.

*$P < 0.05$ compared to the control group.

TABLE 3

COMPARISON OF THE MEAN FIBRINOGEN LEVELS BETWEEN CONTROL AND ISONIAZID-TREATED GROUPS

Treatment	Fibrinogen by Weeks of Treatment (mg/dl)					
	1	3	5	7	9	11
Control	192.6 ± 39.9	232.0 ± 14.4	153.8 ± 36.4	270.8 ± 87.6	274.2 ± 95.6	363.2 ± 29.6
10 mg/kg	96.8 ± 34.8*	103.5 ± 1.9*	154.2 ± 41.1	95.4 ± 11.7*	96.4 ± 2.2*	111.0 ± 31.9*
30 mg/kg	44.0 ± 0.7*	48.2 ± 2.0*	59.0 ± 10.3*	54.6 ± 8.9*	62.1 ± 0.8*	86.6 ± 8.8*
60 mg/kg	39.0 ± 3.2*	39.6 ± 1.5*	41.0 ± 2.2*	43.0 ± 1.1*	39.7 ± 2.1*	32.7 ± 23.4*
100 mg/kg	40.6 ± 1.1*	39.4 ± 1.9*	47.8 ± 21.7*	40.4 ± 2.2*	35.8 ± 4.1*	5.25 ± 7.9*

Data are expressed as mean ± SD.

*$P < 0.001$ compared to the control group.

TABLE 4

COMPARISON OF THE MEAN PROTHROMBIN TIME BETWEEN CONTROL AND ISONIAZID-TREATED GROUPS

Treatment	Prothrombin Time by Weeks of Treatment (sec)					
	1	3	5	7	9	11
Control	15.6 ± 4.0	13.2 ± 1.9	16.8 ± 3.0	15.9 ± 3.1	17.6 ± 3.5	16.3 ± 5.3
10 mg/kg	28.2 ± 8.8*	36.5 ± 3.8*	26.9 ± 0.9*	31.4 ± 7.1*	35.8 ± 3.9*	30.2 ± 2.7*
30 mg/kg	24.9 ± 2.1*	35.5 ± 17.4*	32.9 ± 6.8*	33.7 ± 5.4*	33.3 ± 2.1*	33.7 ± 3.8*
60 mg/kg	32.4 ± 0.7*	45.4 ± 15.5*	34.9 ± 9.0*	38.6 ± 4.1*	35.8 ± 6.9*	34.1 ± 3.9*
100 mg/kg	29.6 ± 4.3*	38.5 ± 8.5*	32.8 ± 2.0*	55.6 ± 12.2*	39.5 ± 9.6*	42.3 ± 4.9*

Data are expressed as mean ± SD.

*$P < 0.05$ compared to the control group.

TABLE 5

COMPARISON OF THE MEAN ACTIVATED PARTIAL THROMBOPLASTIN TIME BETWEEN CONTROL AND ISONIAZID-TREATED GROUPS

Treatment	APTT by Weeks of Treatment (sec)					
	1	3	5	7	9	11
Control	28.8 ± 4.6	33.8 ± 2.7	31.0 ± 2.9	32.7 ± 1.6	29.1 ± 5.1	37.8 ± 2.2
10 mg/kg	42.9 ± 3.2*	38.7 ± 9.9	38.5 ± 9.8	44.5 ± 6.7	30.1 ± 2.4	40.2 ± 6.0
30 mg/kg	49.4 ± 3.2*	29.5 ± 2.1	49.4 ± 9.8*	50.7 ± 3.1	46.6 ± 3.2*	45.1 ± 5.4
60 mg/kg	49.6 ± 2.3*	52.1 ± 7.9*	35.0 ± 9.0	43.8 ± 1.9	47.0 ± 3.4*	59.1 ± 26.0*
100 mg/kg	47.0 ± 6.3*	49.2 ± 7.5*	34.6 ± 4.5	109.4 ± 54.2*	54.3 ± 13.5*	48.9 ± 8.3*

Data are expressed as mean ± SD.

*$P < 0.05$ compared to the control group.