Acute toxicity of oral pyronaridine in Sprague-Dawley rats

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Abstract

Only partial information exists on the toxicology of pyronaridine, a synthetic antimalarial developed in China in the 1970-80's. The present study was designed to complement available data and obtain information on its toxic effects in fewer animals than that usually used to determine the value of the LD_{50} (often judged marginally informative, toxicologically inadequate, and misleading, especially when derived from single-dose studies). The observations carried out included change in bodyweight, onset of mortality, overt toxicity signs and recovery, and gross pathological changes in target organs. Male rats were more susceptible than female rats to the toxic effect of pyronaridine (LD_{50} was 1491.93 ± 103.50 mg/kg for female and 1139.70 ± 110.30 for male rats). The LD_{50} determined by the moving average method was similar to that determined by probit analysis. The mean time to death of female rats was dose-dependant. The acute toxicity signs observed in treated animals included decrease in spontaneous motor activities, somnolence, watery stools, catalepsy, tremor and convulsion. Animals that died developed tremor, then catalepsy followed by convulsion before death. The single administration of high dose (1000 mg/kg – 2000 mg/kg) pyronaridine resulted in the shutdown of the central nervous system.

Key words: Pyronaridine; LD_{50}, moving average, probit analysis, central nervous system

Introduction

Malaria continues relentlessly to challenge both the health systems and scientists with the emergence of drug-resistant parasite strains. For example, chloroquine the mainstay of antimalarial chemotherapy for the last 50 years is no longer effective against *Plasmodium falciparum*, the most dangerous malaria parasite, in nearly all parts of the world. Second-line drugs have become ineffective too in some malarious areas, and multi-drug resistance exists in areas of SE Asia (White, 1998). In 1970, a new antimalarial drug, pyronaridine was synthesised in China. Pyronaridine is effective against the erythrocytic stage of several species of malaria parasites. Promising results were obtained in clinical trials in the treatment of *Plasmodium vivax* and *P. falciparum* (including chloroquine-resistant cases) infections, and cerebral malaria, using oral and intramuscular or intravenous drug administration (Chang & Xianyu, 1992; Olliaro, 2000). In addition, apart from being effective against multidrug-resistant *P. falciparum*, pyronaridine does not seem to exhibit much cross-resistance with chloroquine (Olliaro, 2000). However, it is not widely used in *P. falciparum* endemic areas of the world. This poor usage is largely due to limited formulation and pharmacokinetic data; its toxicological profile has not been established to standards acceptable in the USA or the European Union (Winstanley, 1996). This paper presents the first study of ongoing toxicology research on pyronaridine carried out by the USM Centre for Drug Research which seeks to develop a toxicological profile of pyronaridine that is acceptable internationally.

Materials and Methods

Animals

A total of 100 male and female Sprague-Dawley rats aged between 4 and 5 weeks (weighing about 132 g for males and 103 g for females) were acclimatised for 7 days (before the start of dosing) in experimental room at 22 ± 3°C and relative humidity of 30-70% with 12 hours light and 12 hours dark cycles (Auletta, 1995).

Towards the end of the acclimatisation period the animals were physically examined to confirm their suitability for experimental use. Unsuitable animals were discarded and 50 rats (25 male and 25 female) were randomly assigned to 10 groups (4 treatment and 1 control each for male and female rats). Allocation was performed by means of a stratified randomisation based on body weight (Chan & Hayes, 1994).

The animals were housed in groups of 5, and given tap water and pelleted commercial diet (Gold Coin®) for rodents *ad libitum* throughout the period studied (Chan & Hayes, 1994; Auletta, 1995).

Dose levels and dosages of test substance

Four dose levels were selected based on the results of a pilot study. The four dose levels (with equal logarithmic intervals between each dose level) selected for female rats were different from that of male rats. The dose levels selected for female rats were 488 mg/kg, 781 mg/kg, 1250 mg/kg, and 2000 mg/kg and those selected for male rats were 867 mg/kg, 1041 mg/kg, 1250 mg/kg, and 1500 mg/kg.

Pyronaridine salt was formulated for dosing as a solution in deionised water. Separate formulations were prepared for each dose level. The weighed quantity of pyronaridine was dissolved in the appropriate quantity of deionised water. The solution was then sonicated for approximately 15 minutes. Formulations were used within 5 minutes of preparation.

The animals were fasted overnight before dosing the following day. Pyronaridine solution was administered to the animals at a constant concentration across all dose levels (by varying the dose volume), with a maximum dose volume not exceeding 20 ml/kg body weight (Chan & Hayes, 1994). Administration of pyronaridine solution was by means of a single oral gavage using a 3-inch ball-tipped intubation needle (Biomedical Needles, USA). Control groups were given deionised water through oral gavage.

Observations

Cage side observation of overt toxicity signs and mortality checks were made shortly after dosing; half-hourly over the first 4 hours, and 6 hourly thereafter for the first 24 hours to determine the onset of signs, onset of recovery, and the time to death of each dosed animal. Cage side observation of toxicity signs of surviving animals was done twice daily for 14 days.

Individual body weights were determined shortly before dosing, once weekly, and at death or at termination (Chan & Hayes, 1994). Necropsies were performed on animals that were moribund, found dead or killed at the conclusion of the study (fourteenth day). Animals were killed by means of cervical dislocation. Evidence of gross pathological changes on specific organ(s) were recorded and if necessary tissues from these lesions were preserved in 10% v/v neutral buffered formaldehyde for further study.

Statistical Procedures

Probit analysis and the moving average method were used to determine the LD50 values for both male and female rats. For probit analysis the individual rat data were entered and computer analysed by the Statistical Package for the Social Sciences (SPSS) for Windows Version 9.0 programs (Voelkl & Gerber, 1999). Tables for convenient calculation of LD_{50} reported by Weil (1952) were used for determining LD_{50} by way of the moving average. Probit analysis was conducted concurrently for the purpose of comparison.

The OECD guidelines for acute oral toxicity testing as outlined in Auletta (1995) were followed in the experimental design of the study. The OECD's and FDA's Good Laboratory Practice Regulations were observed and the study protocol was approved by the Animal Ethics Review Committee, University Science Malaysia, Penang, Malaysia.

Results

Mortality

None of the male or female rats of the lowest dose groups died after dosing. One out of five female rats given 781 mg/kg of pyronaridine solution died 9.75 hours after dosing, while 2/5 female rats on 1250 mg/kg and all (5/5) animals of the 2000 mg/kg group died 65.5 ± 39.5 hours and 36.7 ± 12.11 hours after dosing, respectively.

Two out of the five male rats of the 1041 mg/kg group died 15.75 ± 15.25 hours after dosing. Three out of five animals of the 1250 mg/kg group took a shorter time than the 1041 mg/kg group to die (13.86 ± 11.11 hours). The mean time to death of all the animals in the highest dose group (1500 mg/kg) was 36.32 ± 28.00 hours after dosing.

The LD_{50} values for both female and male rats determined by the moving average method (1491.93 ± 103.50 mg/kg, with 95% confidence limits of 885.63 – 1604.16 mg/kg for female rats; 1139.70 ± 110.30 mg/kg, with 95% confidence limits of 1004.66 – 1292.97 mg/kg for male rats) are almost identical to the values determined by probit analysis (1476.50 ± 152.95 mg/kg with 95% confidence interval of 1143.74 – 2083.38 mg/kg, for female rats; 1123.35 ± 126.71 with 95% confidence interval of 839.16 – 2051.20 mg/kg, for male rats).

Mean body weights

The mean body weight of female rats in the control group increased steadily throughout the 14-day observation period. The same pattern was observed in animals given 1250 mg/kg but with the mean body weight lower than the control group throughout the period studied. The mean body weights of animals on 488 mg/kg and 781 mg/kg seemed to be almost constant and lower than that of the control group throughout the observation period (Table 1).

Table 1. Body weight changes and toxicity in rats treated with pyronaridine

Dose (mg/kg)	No.	No. with toxicity signs	Body weight (mean ± SEM) (g)		
			Week 0	Week 1	Week 2
			Female rats		
488	5	2	156.30±6.54	161.40±8.75	164.20± 9.85
781	5	5	156.20±1.98	150.60±3.75	153.80±13.36
1250	5	5	144.44±3.01	156.48±3.75	174.90± 4.37
2000	5	5	152.80±1.32	-	-
Control	5	0	159.20±1.77	166.60±2.48	174.40± 3.78
			Male rats		
867	5	4	199.60±6.44	182.20±9.57	161.60±13.24
1041	5	5	200.40±7.11	190.70±4.82	230.70± 6.17
1250	5	5	195.94±3.85	209.00±12.90	234.08±14.43
1500	5	5	191.80±2.22	181.00	-
Control	5	0	204.00±2.76	217.00±8.36	228.00± 7.80

The mean body weights of male animals in all the treated groups were lower than that of the control group. The mean weight of animals of the 1250 mg/kg group increased steadily throughout the period studied. However, the mean weight of animals on 867 mg/kg decreased throughout the 2 weeks observation period. Interestingly, the mean weight of animals of the 1041 mg/kg group decreased significantly during the first week of observation but increased quite rapidly in the second week (Table 1).

Cage side observation and necropsy findings

Two of the five female rats dosed with 488 mg/kg pyronaridine solution showed signs of toxicity within the first 24 hours after dosing. Fourof the five male rats of the lowest dose group (867 mg/kg) suffered mild toxicity during the first 24 hours after dosing, while all animals of the other three groups (both male and female) showed common toxicity signs within 24 to 48 hours after dosing (Table 1).

The acute toxicity signs observed in treated animals were decrease in spontaneous motor activities, somnolence, discharge of watery stools, catalepsy, tremor, and convulsion. Both male and female rats of all groups that survived the 14 days observation period only showed signs of somnolence, decrease spontaneous motor activities and discharge of watery stools. They recovered within 48 hours after dosing.

Animals in higher dose groups that died showed signs of tremor, catalepsy followed by convulsion before death. Onset of catalepsy in animals in the higher dose groups was within 24 hours after dosing. Manifestation of tremor usually occurred 3-5 minutes before the onset of convulsion that occurred within one hour before death. Treatment may thus affect the central nervous system (CNS), autonomic nervous system, and/or neuromuscular system.

Necropsy findings showed that the lungs of the animals (female and male) in the higher dose groups (> 781 mg/kg in female; >867 mg/kg in male) were dark-red. Livers of most animals were either partially or totally black. The liver histopathology of one male rat in the 1041 mg/kg group showed diffuse congestion and extensive focal necrosis. Evidence of similar necrotic patches was seen on livers of 3 male rats in the 1500 mg/kg group. All organs were yellowish in colour.

Discussion

In this study there was no general trend in changes of mean body weight in male and female rats treated with single various doses of pyronaridine. The mean time to death was dose-dependant in female but not in male rats. The effect of treatment on LD_{50} was gender-dependent; the LD_{50} of female rats was 1.31 times greater than the LD_{50} of male rats. The LD_{50} in female rats in the current study (1491.93 mg/kg) is higher than the LD_{50} (1281 mg/kg) reported previously by Shao *et al.* (1989).

The cage side observations suggest that single administration of high dose (1000 mg/kg – 2000 mg/kg) of pyronaridine results in the shut down of the CNS. Treatment effect on sensation may be ruled out since the treated animals did not show evidence of loss of righting reflex and responded well to toe and tail pinching.

Neuromuscular and autonomic adverse effects cannot be ruled out in this study. Necropsy findings showed congestion in various organs and liver damage.

The LD_{50} determined by moving average method was almost identical to that determined by probit analysis. Therefore, we consider the moving average method appropriate for determination of the LD_{50} in an acute toxicity study that uses fewer animals. Furthermore it de-emphasises the apparent precision of LD_{50} that is frequently used to establish the degree of toxicity of a chemical. We conclude that the moving average method is preferred over probit analysis in determining the LD_{50} value particularly in laboratories like ours for the following reasons: (1) it gives a rapid and reasonably accurate estimate of median-effective dose and the estimated standard deviation of its logarithm with a smaller number of animals; (2) it does not assume that a precise fundamental curve is involved but is capable of taking into account more data than any method that uses only data on both sides of the 50 per cent level of effectiveness; (3) it involves simple computations; and (4) it replaces the fitting of complex mathematical curves (Weil, 1952).

In conclusion, the findings of the study suggest that acute toxicity of high dose pyronaridine (given orally) may affect the central nervous system, the lungs and livers of rats. Pyronaridine produced yellow discoloration of the vital organs of rats. However, there was no general pattern of treatment effect on body weight changes. The LD_{50} of pyronaridine was different in female and male rats, this being 1492 ± 103 and 1140 ± 110 mg/kg respectively.

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