Study on toxicities of 10β-[(2'β-hydroxy-3'-imidazol) propyl] deoxo-artemisinin (32) in reproductive and developmental progresses of mice

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SUMMARY

***Objective**: To test effects of 10β-[(2'β-hydroxy-3'-imidazol) propyl] deoxo-artemisinin (32) on mice's reproductive and developmental processes.*

***Subjects and Methods**: OECD guidelines were applied. Mice were divided into 7 groups consisting of 30 females and 10 males each. In which, the control group received the solvent, only females or males in 4 other groups were taken the testing samples (32), and both females and males of 2 remain groups were given those samples. Mice were given oral samples at a dose of 288 or 576 mg/kg/day × 7 consecutive days depending on individual group. Then, 3 females and a male were grafted in a cage. The compound (32)'s effects on reproductive and developmental processes of mice in 3 generations of P, F1 and F2 were monitored and evaluated by the Bateman technique.*

***Results**: Conception rates, numbers of eggs nested, numbers of pups born, average weights of pups, numbers of days needed to raise pups to adulthood between the test and control groups differed insignificantly (p > 0.05). The pups born from generations P, F1 and F2 all grew and developed normally.*

***Conclusion**: The compound (32) was not mutagenic in mice at oral dose regimens of 288 and 576 mg/kg/day × 7 consecutive days.*

***Key words**: 10β-[(2'β -hydroxy-3'-imidazol) propyl] deoxo-artemisinin, reproduction, development.*

INTRODUCTION

In recent years, the phenomenon of drug-resistant malaria parasites is increasing and spreading in many parts of the world. A number of medicines have become resistant to parasites, including artemisinin's derivatives which are considered effective in killing parasites and quickly reducing fever. This is a major public health concern, forcing the World Health Organization (WHO) to recommend that countries should use combinations of antimalarial drugs with different mechanisms. In addition, it is necessary for countries to research and develop new compounds into antimalarial drugs that have their ability to fight parasites' resistance.[1]. The compound 10β-[(2' β -hydroxy-3'-imidazole) propyl] deoxo-artemisinin being coded (32) was synthesized and purified at the Institute of Natural Products Chemistry and tested for acute toxicity on mice. The results showed that compound (32) did not cause acute toxicity in mice and no mice died even though an oral dose of 5500 mg/kg was given. Furthermore, sub-chronic toxicity in rabbits was also tested, showing (32) to be very safe for rabbits' liver, kidney,

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and hematology functions at oral doses of 72 and 216 mg/kg/day × 28 consecutive days [2],[3],[4]. Also, the compound (32) showed good potency in killing both Plasmodium falciparum (in vitro) as well as P. berghei in mice (in vivo) [2],[3]. With the goal of developing the compound (32) for malaria treatment, this study was conducted to evaluate the toxicity of (32) on reproduction and development in 3 generations of mice.

MATERIALS AND METHODS

Time and location

This study was carried out between November 2020 and April 2021 at National Institute of Malariology, Parasitology and Entomology (Hanoi, Vietnam).

Sample

The compound 10β-[(2'β-hydroxy-3'-imidazol) propyl] deoxo-artemisinin (32) was provided by the Institute of Natural Products Chemistry with its purity of 99.98%.

Experimental animals

A total of 280 white mice (Mus musculus L.) including 210 females and 70 males, Swiss strains, selected for the study were provided by the National Institute of Hygiene and Epidemiology. These mice (the parental generation) had their reproduction and development evaluated to determine whether they were affected by the study sample or not. There are a number of criteria for selecting mice consisting of 4 - 5 weeks old, weight 20 ± 2 g, maturity and good health. Moreover, females must be non-pregnant, non-lactating and have never given birth. All mice were raised stably in experimental conditions for 10 - 14 days until reaching 29 - 32 g before being included in the study. Then, baby mice born from the P, F1 and F2 generations were used to evaluate the effects of the study sample on the reproduction and development of the F1 and F2 generations.

Appliances

Aquatron water stills (Bibby sterilin company, UK), Sauter scale with accuracy d = 0.1 mg, Precisa XB 320C digital scale with accuracy d = 1 mg, Jaw-head needles, graduated glass beakers, 1 ml syringes (divided in 100 lines), magnifying glass; surgical scissors, pank, and scalpel.

Chemicals

Double distilled water; Pharmaceutical gum arabic (Thailand).

Method

Testing was conducted according to OECD guidelines [5]. The effects of compound (32) on the reproduction and development of mice in three generations P, F1 and F2 were monitored and evaluated using the Bateman technique (Dominant lethal test) [6].

Parent mice:

Mice were marked and randomly divided into 7 groups comprising 30 females and 10 males each.

Group 1: females were drunk (32) of 288 mg/kg/day × 7 days but males (CTDK-1).

Group 2: both females and males were drunk (32) of 288 mg/kg/day × 7 days (CTDT-2).

Group 3: males were drunk (32) of 288 mg/kg/day × 7 days but females (CKDT-3).

Group 4: females were drunk (32) of 576 mg/kg/day × 7 days but males (CTDK-4).

Group 5: both females and males were drunk (32) of 576 mg/kg/day × 7 days (CTDT-5).

Group 6: males were drunk (32) of 576 mg/kg/day × 7 days but females (CKDT-6).

Group 7: both female and males were drunk 1% gum arabic solvent (CKDK-7, the control group). Mice were given the solvent or sample with a jaw-head needle once a day in the morning, with a volume of 0.1ml/10g of body weight. Next, 3 females and a male were grafted in a cage in 7 - 10 days. Then, the development of mouse fetuses was monitored. On the 13th - 14th day of pregnancy, 30 - 40% of pregnant mice were randomly selected and operated on to detect numbers of normally developed fetuses, early died fetuses (at weeks 1-2 of pregnancy term) or late died ones (the 3rd week of pregnancy), if any. Besides, the remaining pregnant mice were raised until giving birth to observe the number of offspring without or with birth defects (if any) and the average weight of offspring (grams) in each litter.

Baby mice (F1, F2, F3 generations):

F1 generation mouse pups were raised until adulthood, reaching an average weight of 20 g/mouse. After that, males and females were separated and raised until they reached weights of 30 - 33 g. Mice were given the test sample and paired F1 female and male mice in the same way as done with the P generation. The steps and observations were made similarly until the F2 and F3 generations were born. Finally, the research ended when F3 generation mouse pups were monitored and observed until they reached adulthood and had an average weight of 18 - 20 g/mouse.

Evaluation criteria:

Research indicators included the percentage of mice impregnated in each experimental group, the numbers of fetuses of each pregnant mother after cesarean sections, the numbers of early or late fetal deaths, fetal malformations, number of offsprings per litter, average weight of a mouse, the number of days required for the pups to reach a weight of about 20 g and birth defects (if any).

Data processing

Data expressed as mean $\bar{X}$ ± SD were processed by Excel program (Microsoft XP) according to the method of medical statistics, using Student's t-test and Fisher's exact test to compare the data before, during and after the test. Also, those data were compared among the control and treated arms. The difference was statistically significant when $p<0.05$.

Research ethics

The study complied with ethical regulations in biomedical research. Animals were handled properly after the end of the experiment.

RESULTS

The parameters of mice's reproductive processes in 3 generations are shown in table 1 - 3.

Table 1. Conception and reproduction processes of parental mice

Group (number of females = 30)	Evaluation indices					
	Conception rates (%)	Number of pregnant mice were operated on (%)	Number of fetuses/ 1 mother mouse (mean $\bar{X}$ ± SD)	Number of early died / late died / defect fetuses	Pregnant mice were raised until giving birth (%)	Number of pups/ litter (mean $\bar{X}$ ± SD)
CTDK-1	21/30 (70.0)	7/21 (33.33)	9.4 ± 1.5	0/0/0	14/21 (66.67)	9.3 ± 1.7
CTDT-2	21/30 (70.0)	7/21 (33.33)	7.7 ± 3.1	0/0/0	14/21 (66.67)	10.4 ± 1.7
CKDT-3	20/30 (66.67)	7/20 (35.00)	7.4 ± 3.8	0/0/0	13/20 (65.00)	9.1 ± 2.1
CTDK-4	22/30 (73.33)	8/22 (36.36)	9.6 ± 1.7	0/0/0	14/22 (63.64)	9.8 ± 2.1
CTDT-5	22/30 (73.33)	8/22 (36.36)	9.5 ± 2.6	0/0/0	14/22 (63.64)	9.6 ± 2.4
CKDT-6	21/30 (70.00)	8/21 (38.10)	9.4 ± 2.4	0/0/0	13/21 (61.90)	9.3 ± 2.1
CKDK-7	21/30 (70.00)	7/21 (33.33)	8.9 ± 2.1	0/1/0	14/21 (66.67)	9.7 ± 3.99
p (i-n)*	> 0.05	> 0.05	> 0.05		> 0.05	> 0.05

(p (1-7), p (2-7), p (3-7), p (4-7), p (5-7), p (6-7), p (1-2), p (1-3), p (1-4), p (1-5), p (1-6), p (2-3), p (2-4), p (2-5), p (2-6), p (3-4), p (3-5), p (3-6), p (4-5), p (4-6), p (5-6))*

The parental mice's conception rates in the test and control groups were 66.67 - 73.33% and did not differ statistically ($p>0.05$). Moreover, all 6 groups receiving oral administration (32) did not have any early or late fetal deaths or fetal malformations. Nevertheless, the mouse, code 187, in the control group carried 11 fetuses, of which 1 fetus was stillborn at the 3rd week. The average numbers of offspring per litter were not statistically different ($p>0.05$). Normally, each mouse gave birth to 7 - 13 offspring/ litter. A few mice gave birth to fewer babies

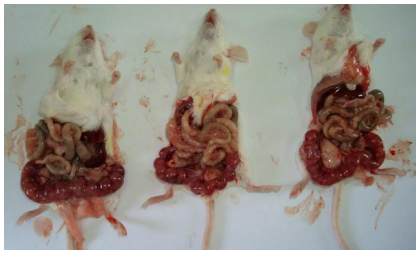

Figure 1. Pregnant mice of the parent generation in group 2 were operated on to observe their fetuses

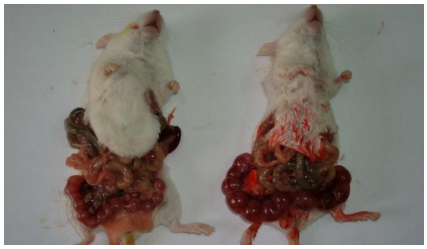

Figure 2. Pregnant mice of the parent generation in the control group were observed their fetuses

Figure 1 shows that the fetuses were all alive, red in color, and had no malformations. On the other hand, the mouse (code 187) in the control group (figure 2) had a stillborn fetus (dark black) while the remaining fetuses developed normally and had no defects.

Table 2. Conception and reproduction processes of F1 mice

Group (number of females = 30)	Evaluation indices					
	Conception rates (%)	Number of pregnant mice were operated on (%)	Number of fetuses/ 1 mother mouse ($\bar{X} \pm SD$)	Number of early died / late died / defect fetuses	Pregnant mice were raised until giving birth (%)	Number of pups/litter ($\bar{X} \pm SD$)
CTDK-1	19/30 (63.33)	7/19 (36.84)	9.4 ± 3.1	0/0/0	12/19 (63.16)	9.7 ± 1.2
CTDT-2	23/30 (76.67)	9/23 (39.13)	10.4 ± 1.7	0/0/0	14/23 (60.87)	10.6 ± 1.9
CKDT-3	20/30 (66.67)	7/20 (35.00)	8.9 ± 3.0	0/0/0	13/20 (65.00)	9.7 ± 2.8
CTDK-4	21/30 (70.00)	7/21 (33.33)	9.3 ± 1.8	0/0/0	14/21 (66.67)	9.5 ± 2.3
CTDT-5	21/30 (70.00)	8/21 (38.10)	9.6 ± 1.7	0/0/0	13/21 (61.9)	9.7 ± 2.5
CKDT-6	21/30 (70.00)	8/21 (38.10)	9.3 ± 1.8	0/0/0	13/21 (61.9)	10.1 ± 2.1
CKDK-7	19/30 (63.33)	8/19 (42.11)	10.1 ± 4.8	0/0/0	11/19 (57.89)	9.8 ± 2.9
p(i-n)	> 0.05	> 0.05	> 0.05		> 0.05	> 0.05

The differences in conception rates of F1 generation mice in all test and control groups were not statistically significant (p>0.05). Also, no early or late fetal death or malformations were detected in all batches. The average numbers of offspring per litter did not differ remarkably (p>0.05). Besides, each mouse normally gave birth to 7 - 14 babies/litter while 1 mouse (code 209) in the control group only carries 2 fetuses.

Table 3. Conception and reproduction processes of F2 mice

Group (number of females = 30)	Evaluation indices					
	Conception rates (%)	Number of pregnant mice were operated on (%)	Number of fetuses/ 1 mother mouse ($\bar{X} \pm SD$)	Number of early died / late died / defect fetuses	Pregnant mice were raised until giving birth (%)	Number of pups/litter ($\bar{X} \pm SD$)
CTDK-1	21/30 (70.00)	10/21 (47.62)	10.7 ± 1.7	0/0/0	11/21 (52.38)	10.6 ± 2.0
CTDT-2	20/30 (66.67)	8/20 (40.00)	10.6 ± 1.8	0/0/0	12/20 (60.00)	10.8 ± 2.4
CKDT-3	22/30 (73.33)	9/22 (40.91)	9.3 ± 1.5	0/0/0	13/22 (59.09)	10.9 ± 2.7
CTDK-4	21/30 (70.00)	8/21 (38.10)	9.5 ± 1.8	0/0/0	13/21 (61.90)	9.6 ± 2.0
CTDT-5	22/30 (73.33)	9/22 (40.91)	9.3 ± 1.8	0/0/0	13/22 (59.09)	9.5 ± 1.9
CKDT-6	22/30 (73.33)	10/22 (45.45)	9.6 ± 1.9	0/0/0	12/22 (54.55)	9.6 ± 2.3
CKDK-7	23/30 (76.67)	10/23 (43.48)	8.8 ± 4.9	0/0/0	13/23 (56.52)	11.2 ± 2.6
p (i-n)	> 0.05	> 0.05	> 0.05		> 0.05	> 0.05

The conception rates of F2 generation mice in all test and control groups did not differ notably (p>0.05). Additionally, mice in all groups did not have early/late fetal death or fetal malformations. Moreover, the average numbers of offspring/litter were not statistically significant (p>0.05). Parameters of mice's growth and development in each generation are shown in Tables 4 - 6.

Table 4. Growth and development processes of F1 mice

Group (number of females = 30)	Evaluation indices			
	Total number of offspring in the whole group/number of mice giving birth	Average weights of pups (g, $\bar{X}$ ± SD)	Number of Pups born with birth defects	Numbers of days needed to raise pups to 20g ($\bar{X}$ ± SD)
CTDK-1	130/14	1.79 ± 0.176	0	29.0 ± 0.96
CTDT-2	145/14	1.79 ± 0.118	0	28.4 ± 1.08
CKDT-3	118/13	1.83 ± 0.179	0	28.5 ± 1.45
CTDK-4	137/14	1.86 ± 0.130	0	29.2 ± 1.67
CTDT-5	134/14	1.84 ± 0.178	0	28.7 ± 1.64
CKDT-6	120/13	1.83 ± 0.143	0	28.5 ± 1.27
CKDK-7	136/14	1.83 ± 0.383	0	28.7 ± 2.16
p (i-n)	> 0.05	> 0.05		> 0.05

F1 mice in all groups had relatively similar average weights with no significant differences (p>0.05) and did not have birth defects. Furthermore, the average numbers of days needed to raise pups from birth until reaching weights of approximately 20g were 28.4 - 29.2 days with no remarkable differences among groups (p>0.05).

Table 5. Growth and development processes of F2 mice

Group (number of females = 30)	Evaluation indices			
	Total number of offspring in the whole group/number of mice giving birth	Average weights of pups (g, $\bar{X}$ ± SD)	Number of Pups born with birth defects	Numbers of days needed to raise pups to 20g ($\bar{X}$ ± SD)
CTDK-1	116/12	1.89 ± 0.298	0	27.3 ± 2.57
CTDT-2	149/14	1.72 ± 0.187	0	27.8 ± 2.08
CKDT-3	126/13	1.84 ± 0.284	0	28.7 ± 2.84
CTDK-4	133/14	1.83 ± 0.185	0	28.9 ± 1.75
CTDT-5	126/13	1.83 ± 0.205	0	28.8 ± 1.59
CKDT-6	131/13	1.81 ± 0.145	0	28.5 ± 1.51
CKDK-7	108/11	1.72 ± 0.270	0	28.6 ± 2.84
p (i-n)	> 0.05	> 0.05		> 0.05

Average weights of F2 mice born in the experimental and control groups were not statistically different (i > 0.05). No mice had birth defects. Besides, the number of days needed to raise pups from birth until reaching average weights of about 20g did not differ significantly among groups (p > 0.05).

Table 6. Growth and development processes of F3 mice

Group (number of females = 30)	Evaluation indices			
	Total number of off-spring in the whole group/number of mice giving birth	Average weights of pups (g, $\bar{X} \pm SD$)	Number of Pups born with birth defects	Numbers of days nee-ded to raise pups to 20g ($\bar{X} \pm SD$)
CTDK-1	117/11	1.79 ± 0.253	0	28.6 ± 1.36
CTDT-2	130/12	1.69 ± 0.219	0	28.9 ± 1.51
CKDT-3	142/13	1.72 ± 0.076	0	28.5 ± 1.51
CTDK-4	125/13	1.73 ± 0.135	0	28.5 ± 1.33
CTDT-5	124/13	1.71 ± 0.099	0	28.9 ± 1.26
CKDT-6	115/12	1.71 ± 0.105	0	28.3 ± 1.23
CKDK-7	145/13	1.62 ± 0.127	0	28.6 ± 1.45
p (i-n)	> 0.05	> 0.05		> 0.05

Average weights of F3 mice born in different batches were not statistically significant (p>0.05). No mice had birth malformations. Numbers of days needed to raise pups from birth until reaching a weight of approximately 20g ranged from 28.3 to 28.9 days on average, with no notable differences among groups (p>0.05).

DISCUSSION

The effects on chromosome mutations and reproductive processes are often chosen to evaluate the long-term toxicity of drugs. There are two methods of researching mutations comprising chromosomal and gene mutations. In particular, the genetic mutation method is very complex and currently mainly focuses on studying indirect traits in the offspring. The Dominant lethal test is often applied to evaluate the genetic toxicity of drugs in 1 to 4 generations. Therefore, in this study, we evaluated the effects of compound (32) with two oral doses of 288 and 576 mg/kg/day × 7 consecutive days on the parent generations (P) of white mice and their F1, F2 and F3 progeny.

Toxicity of (32) on mices' reproduction

Reproductive toxicity represents adverse effects on sexual function and fertility in adult animals as well as developmental toxicity in offspring [5]. The study showed that in all three generations P, F1 and F2, the conception rate, number of fetuses per mother mouse, and number of pups born between the experimental and control groups were not statistically different (p > 0.05). In the P generation, mice in all (32)-treated groups did not have early or late fetal deaths or fetal malformations; nonetheless, the control group had a mouse (code 187) carrying 1 stillborn fetus in the 3rd week of pregnancy. This phenomenon of stillbirth in mice is very low, but this is completely natural and consistent with the reproductive reality of mice. For the F1 and F2 generations, no early or late fetal deaths, fetal or congenital malformations were detected in the pups born in all 7 batches. These proves that, with oral doses of 288 mg/kg/day (equivalent to the human therapeutic dose) and 576 mg/kg/day × 7 consecutive days, the compound (32) did not cause mutations in both female mice (egg line mutant) and male mice (sperm line mutant) in three generations P, F1 and F2. Therefore, (32) did not affect gene mutations in mice.

These results are also similar to that of Truong Van Nhu et al. (2005) when evaluating the effects of 10α-trifluoro methyl dihydroartemisinin (BB101) at a dose of 50 mg/kg/day × 5 consecutive days on P generation white mice. Conception rate, embryonic development status (damage in the embryo, number of implanted eggs, rates of early or late fetal death), the number of offspring per litter between the experimental and control groups were not statistically different (p > 0.05) [7].

However, T. E. White et al. (2006) when studying the effects of artesunate at a single oral dose of 17 mg/kg/day on pregnant rats at the 10th - 11th day found cardiac

abnormalities in approximately 25 - 60% and 100% of their embryos within 24 and 48 hours, respectively. Additionally, embryonic delay in limb and tail development occurred at the 13th day of gestation. These embryos survived until the 13th gestational day but 77% of them died before the 14th gestational day probably due to lack of oxygen or cardiac abnormalities [8].

Toxicity of (32) on mice's development

Developmental toxicity is understood in the broadest sense to include any effect that interferes with the normal development of an animal before or after its birth due to exposure of its parents to a chemical before conception, or exposure of the offspring itself during prenatal or postnatal development until its reproductive maturity [5].

Research results show that in all three generations P, F1 and F2, the number of pups born, the pubs' average weights, and the number of days needed to raise the pups to reach average weights of 20g between the test and control groups did not differ significantly (p>0.05). No fetal malformations or birth defects were detected in the pups. Also, when observing baby mice from their birth until adulthood, we found that white mice have very rapid growths and development cycles. Specifically, at 6 weeks of age, the pups have fully developed genitalia and can begin mating. Mice's gestation period is short with only 21 days. These are completely consistent with ordinary white mouse reproductive cycles. Moreover, the pups all grew and developed normally, with no abnormalities in their activities, eating, urination, respiration, central nervous systems and fertility. These affirm that the compound (32) did not affect the development of white mice over 3 generations at oral doses of 288 and 576 mg/kg/day × 7 consecutive days.

The above results are also consistent with those of Truong Van Nhu et al. (2005) when studying the effects of BB101 at a dose of 50 mg/kg/day × 5 consecutive days on P generation mice. However, in mice that were 6 - 10 days pregnant and were given BB101 at the same dose as above, the rates of early and late fetal deaths had a statistically significant difference compared to that of the control group (p<0.05) [7].

CONCLUSION

The compound (32) did not cause genetic mutations in mice at oral doses of 288 mg/kg/day (equivalent to expected human dose) and 576 mg/kg/day for 7 consecutive days.

The fertilization rates, numbers of nested eggs, numbers of pups born, average weights of pups, and numbers of days needed to raise pups until adulthood among all experimental and the control groups were not statistically different (p>0.05).

Pups born from the P, F1 and F2 generations all grew and developed ordinarily.

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