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Visible Malformations in Mice, *Mus musculus* Experimentally Exposed in Utero to Antimalarials (Manalaria®, Syrup Kilma® versus Quinine)

Joseph Kangudia Mbaya, Damien Mbanzulu Pita Nsonizau, Nene Mbanzulu Nsolani, Gauthier Mesia

Department of Basic Sciences, Faculty of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo
Email: drguymonzango@gmail.com

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Abstract

The antimalarial phytomedicines, Manalaria® and Sirop Kilma®, also frequently used in Kinshasa at the start of pregnancy, would not have previously undergone embryotoxicity tests. This study aims to determine the embryotoxicity of Manalaria® and Sirop Kilma® on mice, *Mus musculus*. An experimental study in which 54 prepubescent mice were exposed during embryogenesis, including 16 mice to Manalaria®, 20 to Syrup Kilma® and 18 to Quinine. Careful observation by visual inspection and enlarged photography enabled the search for malformations in the exposed mice. As a result, all the mice exposed to the 3 antimalarials did not reveal visible malformations on visual inspection or on enlarged photography. In conclusion, under the experimental conditions of this study, no visible malformation was observed in mice exposed to the two phytomedicines in utero.

Subject Areas

Gynecology & Obstetrics

Keywords

Embryotoxicity, *Mus musculus*, Manalaria, Syrup Kilma, Quinine

1. Introduction

Malaria in endemic areas is a major cause of maternal and fetal morbidity and mortality [1] [2]. Indeed, in Sub-Saharan Africa, nearly 25 to 30 million women become pregnant each year. They are at risk of contracting Plasmodium falcipa-

rum malaria. Among these women, 10,000 die from malaria and its complications and 15% of these deaths occur in the first trimester of pregnancy.

The fall in immunity induced by pregnancy increases the risk of maternal and fetal mortality due to malaria [3]. Early treatment of uncomplicated malaria in pregnant women with effective antimalarials is one of the strategies that the WHO proposes to combat this pathology and its complications during pregnancy [4]. In accordance with WHO guidelines, the National Malaria Control Program (PNLP) of the Democratic Republic of the Congo recommends, taken orally, 4 antimalarial molecules including 3 therapeutic combinations based on artemisinin (artesunate amodiaquine, artemether-lumefantrine and artesunate-pyronaridine) as well as quinine in combination with Clindamycin [5]. Alongside the antimalarials recommended by the PNLP, there are also other antimalarials on the Congolese pharmaceutical market, in particular Manalaria® and Sirop Kilma®, the subject of this research. According to the indications provided by the manufacturers, Manalaria® is an extract of Sarcocephalus, Latifolia and Senna occidentalis, while Sirop Kilma® is a hydroalcoholic extract of *Gardenia ternifolia*, *Crossopterix febrifuga* and *Lantana camara*. These two antimalarial phytomedicines would have obtained marketing authorization without having been subjected to embryotoxicity tests, considering that the total plant extracts are not toxic in general [6] [7] [8]. This argument would probably have weighed on the decision to grant this approval. Interestingly, for more than two decades, these two antimalarials have been prescribed even to pregnant women in early pregnancy. To our knowledge, they have never been incriminated as responsible for malformations in newborns exposed in utero. The surveys carried out in Kinshasa among doctors on the prescription of antimalarials at the start of pregnancy confirm the frequent use of these antimalarials [9].

This experimental study aims to:

- List the visible malformations attributable to antimalarials in mice experimentally exposed in utero during embryogenesis to Manalaria® and Syrup Kilma®, in comparison with quinine, a non-teratogenic agent at therapeutic doses;
- Describe any visible malformations encountered during this research.

2. Methods

This experimental study was conducted at the Human Embryology Laboratory of the Department of Basic Sciences of the Faculty of Medicine of the University of Kinshasa (DR Congo), from May to October 2021. It consisted of experimentally exposing during embryogenesis mice, *Mus musculus* in utero, to the antimalarials Manalaria®, Sirop Kilma® and Quinine; then to look for visible malformations in the same mice.

2.1. Exposure of Mice to Antimalarials

Eighteen (18) adult mice, *Mus musculus* (9 females and 9 males) weighing an

average of 25 grams, without visible malformations and free from carbohydrate metabolism disorder, were kept in a well-ventilated room whose ambient temperature did not exceed 23˚C. They were fed cakes made by the Minoterie de Matadi and Alpina brand water. The female mice were isolated and each kept in its own cage. For mating, we introduced a male into each cage already inhabited by a female. Each pair of mice cohabited for 7 days, a period deemed sufficient for habituation, mating and successful fertilization. After this time, the female mice were separated from the males. And all the male mice were reared in the animal facility. Females were therefore eligible for experimental treatment with antimalarials.

2.2. Treatment of Mice

After the cohabitation stay with the males, the pregnant female mice were ready for treatment. We divided the 9 female mice into 3 groups of 3, while keeping them each in its own cage. We identified the cages belonging to each group by the letters A, B and C; thus the mice of group A were intended for the treatment of Manalaria®, of group B for Syrup kilma® and those of group C for Quinine. The antimalarials tested are: Manalaria® supplied by the Luozi Pharmaceutical Research Center in Kongo Central; Syrup Kilma® by the Harmonie Pharmaceutical Laboratory in the Municipality of Kisenso in Kinshasa; Quinine by Pharmakina in Bukavu in South Kivu, all in DR Congo. Drugs were administered to mice by gavage using Gilson (20 - 100 µL), Ppetman (1 - 10 µL) micropipettes. For practical reasons, we administered the antimalarial treatment for 3 days for each group at the rate of 2 doses per day. The daily dose of each drug was calculated from the therapeutic daily dose recommended by the manufacturers.

Thus for Manalaria®, the proposed dose between 0 - 1 year: 2 × 1 cc per day, for 3 days, *i.e.* a total of 2 cc per day, which corresponds to 10 ml of Manalaria®. For a newborn who weighs around 3000 g, the daily dose would be 10 ml. And for the mouse that weighs about 25 gm, its calculated dose amounts to 0.083 ml, or 83 µL. This dose is divided into two intakes of approximately 42 µL. For Syrup Kilma®, the suggested dose for a newborn of about 3000 g is 4x1tsp per day for 3 days and the total daily dose is equivalent to 20 ml. If the newborn of 3000 grams must receive 20 ml of Sirop kilma® per day, the dose for the mouse of 25 grams amounts to 0.167 ml, that is to say 167 µL; with for each take about 84 µL. For Quinine, the proposed dose is 10 mg/Kg in 3 daily intakes, *i.e.* a total of 30 mg/Kg per day. The galenic form used was syrup, 5 ml of which contains 100 mg of Quinine. The daily dose of 30 mg/Kg converted into syrup amounts to 1.5 ml/Kg, *i.e.* 1.5 ml per 1000 grams of body weight. This brings for a mouse of 25 grams a daily dose of 0.038 ml, or 38 µL. This dose divided into 2 amounts to more or less 20 µL per dose.

2.3. Embryonic Period for in Utero Exposure of Mice

Susceptibility to teratogens varies with the stage of development at the time of

exposure. If the period of great vulnerability corresponds during embryogenesis to gastrulation (when the fundamental embryonic tissues are formed), no stage of development is completely free from risk.

In this study, in order to increase the chances of obtaining the malformations induced by the antimalarials, we targeted the period of gastrulation to expose the mice to the tested agents. Gastrulation of the mouse, *Mus musculus* during its development begins at 6.5 days after fertilization. It begins with the establishment of the primitive streak, the total duration of gestation in mice, *Mus musculus* being 21 days. In practice for this experimental study, we started the treatment of the mice on the 7th day after the separation of the females from the males. After 3 days of treatment, the 9 pregnant mice were kept under observation until the birth of a total of 54 pups. The latter were left with their mothers until the search for visible malformations 30 days later.

2.4. Methods for Detecting Visible Malformations in Mice

The search for visible malformations in the mice consisted of a meticulous observation of their general morphology. After this observation, each mouse was photographed with the IPAD brand tablet with image enlargement. The different sections of the mice (head, trunk, limbs, legs, tail and genitals) were examined on enlarged photos. All ethical rules related to the handling of laboratory animals were strictly respected during this research.

3. Results

The number of mice exposed to antimalarials in utero and their sex. Table 1 summarizes the number and sex of mice exposed in utero to antimalarials.

A total of 54 mice were experimentally exposed to antimalarials: Manalaria®

Table 1. Number and sex of mice exposed in utero.

Medecines	Mice pregnant number	Mice exposed		Total	%
		Males	Females		
Group A Manalaria®	Mouse n˚ 1	3	2	5	
	Mouse n˚ 2	3	3	6	
Subtotal 1	Mouse n˚ 3	1	4	5	
	3	7	9	16	29.7
Group B Syrup Kilma®	Mouse n˚ 1	2	4	6	
	Mouse n˚ 2	1	4	5	
Subtotal 2	Mouse n˚ 3	4	5	9	
	3	7	13	20	37
Group C Quinine	Mouse n˚ 1	1	4	5	
	Mouse n˚ 2	3	3	6	
Subtotal 3	Mouse n˚ 3	4	3	7	
	3	8	10	18	33.3
Total A, B et C	9	22	32	54	100

16 (29.7%), Syrup kilma® 20 (37%) and Quinine 18 (33.3%).

General morphology and hair color: Indeed, all the mice of 3 groups exposed in utero to the 3 antimalarials each had a normal morphology (54/54), *i.e.* 100%.

Examination of mouse body sections on magnified images:

- Head: No abnormality was detected in the heads of 54 young mice exposed in utero to Manalaria®, Syrup Kilma® and Quinine (54/54), *i.e.* 100%.
- Trunk: Examination of enlarged images of the trunks of 54 young mice exposed in utero to the antimalarials tested revealed no malformation (54/54), *i.e.* 100%.
- Forelimbs: The examination focused on 108 enlarged images of the forelimbs of all 54 young mice exposed in utero to antimalarials. They were without visible abnormalities (108/108), *i.e.* 100%.
- Forelimb paws: Careful examination of 108 mouse forelimbs noted each well-formed paw with 5 distinct toes bearing claws. No abnormality was observed (108/108), *i.e.* 100%.
- Hind limbs: the 108 hind limbs of the mice examined were normally formed without visible abnormalities (108/108), *i.e.* 100%.
- Legs of hind limbs; as shown in the enlarged image, all 108 legs of the hind limbs of the mice had no visible malformations (108/108), *i.e.* 100%.
- Tails: The tails of 30-day-old mice examined after in utero exposure to antimalarials were normal (54/54), or 100%.
- Genital organs: 22 male mice examined had normal external genitalia (22/22), *i.e.* 100% and 32 female mice examined had normal external genitalia (32/32), *i.e.* 100%.

4. Discussion

To date, a large proportion of the population in Africa south of the Sahara still uses traditional medicine to treat malaria [1]-[7].

The antimalarials in question in this research are total plant extracts, presented in an improved galenic formulation [10]-[19]. Surveys of prescribers of these phytomedicines have not reported the presence of malformations in children born to mothers treated with these phytomedicines during pregnancy [10]. Since the tragedy of thalidomide®, a drug that gave birth to thousands of children suffering from phocomelia, the marketing of a new drug is subject to very restrictive requirements, in particular those making embryotoxicity. Most legislation currently imposes the study on 2 unrelated animal species [14] [15]. However, there are significant legislative variations between different countries [20]-[26]. It is therefore important that the embryotoxicity tests that would have failed for Manalaria® and Sirop Kilma® be carried out a posteriori, given the large number of users of these drugs [9]. The choice of the mouse, *Mus musculus* is judicious for this study. Indeed the model of the development of the mouse, *Mus musculus* is very close, at least in its earliest stages to that of man; also, this species is genetically well known. This model is well suited for observing the dysfunctions of ontogenetic processes [27].

From an embryotoxicity point of view, the 2 drugs tested were compared in this study with Quinine, which is a drug certified as non-teratogenic at therapeutic doses [13]. It was therefore important to give the mice the therapeutic doses recommended by the manufacturers. This led us to calculate, for the 3 drugs tested, the dose according to the body weight of the mice.

Under the experimental conditions of this study, no visible congenital malformation was detected in young prepubescent mice in the 3 groups tested, exposed in utero to Manalaria®, Syrup Kilma® and Quinine (reference drug). It is therefore important to point out that this result only concerns visible, *i.e.* external, malformations. Malformations involving the internal organs of mice as well as tissue and molecular malformations were not examined. Therefore, it is risky to claim that Manalaria® and Sirop Kilma® are not teratogenic. Other more in-depth studies, in particular those of tissue and molecular embryotoxicity, are necessary. Moreover, in relation to the visible external malformations explored, only one animal species was used. It would be desirable to test again with another unrelated species, as required by certain legislations, to better establish the results obtained in this study [28]-[32]. Given that these drugs are frequently prescribed by health care providers in Kinshasa, there is a need to set up sustained pharmacovigilance to monitor women who use these drugs during pregnancy as well as their children at birth.

5. Conclusion

We carried out an experimental study by exposing *Mus musculus* mice in utero to Manalaria®, Sirop Kilma® and Quinine, which are herbal medicines widely used in sub-Saharan Africa in pregnant women suffering from malaria. Under our experimental conditions on *Mus musculus* mice, no visible malformation was observed in mice exposed in utero to Manalaria®, Sirop Kilma® and Quinine.

Contribution of the Authors

- Kangudia Mbaya J. contributed to all stages of project execution.
- Mbanzulu Pita Nsonizau D. is the designer of the project and took part in all stages of its execution.
- Mbanzulu Nsolani N. took an active part in the discussion of the results, in typing and text processing.
- Mesia G: correction of the manuscript.

Conflicts of Interest

This article does not contain any conflict of interest.

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