

# Agglutination Reactions of Artemisinin-Type Drugs and Other Substances

Bin Yan<sup>1, a</sup>, Lihong Han<sup>1, b</sup>, Lijun Gao<sup>2, c</sup>, Wenbin Qin<sup>2, \*</sup>

<sup>1</sup>Putian University Department of Basic medical Science Putian, China

<sup>2</sup>Baotou Medical College Department of Hemoglobin Research Baotou, China

**Abstract:** Artemisinin is a good antimalarial drug independently developed in China. It is highly effective and low toxic. In the process of studying the antimalarial mechanism of artemisinin drugs, we found that there was agglutination when the drugs came into contact with blood. Methods: ABO positive typing test card was used to detect the agglutination reaction of artemisinin and dihydroartemisinin with whole blood, red blood cells, hemolytic solution, hemin chloride, ferrous sulfate, ferric chloride, sodium chloride, DMSO and artemether. Results: artemisinin can agglutinate with many substances, such as red blood cells, red blood cell hemolytic solution, hemin chloride, ferrous sulfate, ferric chloride, sodium chloride and so on. The agglutination reaction in this paper is not related to antigen and antibody, but the result of the interaction between artemisinin drugs and various substances. Whole blood, red blood cells and hemolytic fluid contain biological macromolecular components. Hemin belongs to low molecular organic compounds, and the rest are simple inorganic compounds. Artemisinin drugs can interact with such a wide range of substances and agglutinate, indicating their strong effect. The mechanism is not clear. It is speculated that it is related to the "oxygen bridge" in artemisinin molecule, but the details of the action and how to agglutinate need to be studied. Interestingly, when artemether interacts with artemisinin and dihydroartemisinin, there is no agglutination, but there is a tendency of agglutination in the control, which is contrary to other results. This is a phenomenon, indicating that there is interaction, and its mechanism and significance need to be further studied. Artemisinin can interact with many substances.

## 1 INTRODUCTION

For its significant efficacy in the prevention and treatment of malaria, artemisinin-type drugs are widely recognized by the world. People have been making efforts to understand the mechanisms of such drugs. Currently, the underlying mechanisms of artemisinin-type drugs are not yet fully understood, however, some excellent results have been accumulated in recent years (Meshnick, 1993; Eckstein-Ludwig, 2003; Haynes, 1996; Golenser, 2003; Efferth, 2007; Meshnick, 1991; Li, 2005; Haynes, 2004; Robert, 2002). At present, the hypothesis of artemisinin-type drugs mechanism has been proposed to act via two major aspects, including the activation of artemisinin and the target of artemisinin. Various opinions have been put forward on the activation of artemisinin. Regarding the activation of artemisinin, it has been suggested that iron (Meshnick, 1993; Eckstein-Ludwig, 2003; Haynes, 1996) and heme (Eckstein-Ludwig, 2003; Efferth, 2007; Meshnick, 1991) may all be involved in the activation of artemisinin. However, several controversial issues remain to be addressed. Meanwhile, it also has been advocated that the heme participates in the activation of artemisinin. Furthermore, there are also different opinions on whether iron is involved in the activation process of artemisinin

(Haynes, 1996; Haynes, 2004). In addition, it also has been advocated that the antimalarial activity of artemisinin is related to hemoglobin (Robert, 2002). Regarding the target of artemisinin, it is considered that the alkylation of heme is an important target (Robert, 2005; Kannan, 2005; Meunier, 2010; Loup, 2007), but there are also opposite opinions (Haynes, 2007; Coghi, 2009; Meshnick, 2003). Likewise, mitochondrial models (Srivastava, 1997; Wang, 2010) and other different views (del Pilar Crespo M, 2008; Afonso, 2006) are proposed as well.

Artemisinin, characterized by highly efficacious and relatively safe, is a new antimalarial compound discovered by Chinese scientists. In view of its good antimalarial effect, different artemisinin derivatives have become the first-line antimalarial drugs in recent years, which have been fully affirmed by the medical and health community around the world. The 2015 Nobel Prize in Physiology was awarded to a Chinese scientist, Tu Youyou, for the discovery of artemisinin as a tribute and acknowledgement to China's contribution in this field (Tu, 2016). Known for many years, artemisinin and its derivatives have been identified to play the role of immunomodulation, anti-tumor, antibacterial besides their antimalarial effects, which have broad prospects for development. Meanwhile, reports have credited the

<sup>a</sup>972938864@qq.com, <sup>b</sup>vhanlihong@163.com

<sup>c</sup>756287086@qq.com, \*362446866@qq.com

malaria-inhibiting activity of artemisinin requiring the digestion and absorption of hemoglobin. Suppressing the hemoglobin enzyme activity, interfering of hemoglobin degradation pathway or direct removing the red blood cell lysate from culture medium may significantly attenuate the antimalarial activity of artemisinin (Klonis, 2011). Plasmodium completes the invasion of hepatocytes and red blood cells through the blood route, and may result in batch rupture of red blood cells in the process of parasitic reproduction. Of these, red blood cells can carry out functions such as transporting oxygen and carbon dioxide, as well as buffering the acidic and alkaline substances produced by the body. The above functions were accomplished primarily by hemoglobin. However, hemoglobin can only fulfil its intended role when it exists within red blood cells. Upon rupture and lyse of the red blood cells, escape of hemoglobin may occurs, leading to a marked loss of its functions (Zhang, 2021; Mancuso, 2021; Huai, 2021; Hu, 2021; Dolivo, 2021; Lu, 2021).

Artemisinin compounds have unique oxygen bridge structure and have significant therapeutic effect on malaria and are not easy to produce drug resistance. Artemisinin and its derivatives also have a variety of pharmacological activities, such as antischistosomiasis, anti-tumor, anti-microbial, anti-inflammatory, anti-virus, anti-fibrosis and so on (Cazelles, 2002; Asawamahsakda, 1994; Haynes, 2007).

The aforementioned viewpoints and hypotheses indicate that the underlying mechanisms of artemisinin-type drugs are fairly complex, involving several multifactorial interaction simultaneously. During the experiment, it was found that artemisinin drugs can agglutinate with some substances, which is also a kind of interaction. Nevertheless, its relationship with the above-mentioned studies remains unclear.

## 2 MATERIALS AND METHODS

Artemisinin powder and dihydroartemisinin powder were provided by Manyuan Wang, School of Traditional Chinese Medicine, Capital Medical University, and kept away from light. Artemether injection was purchased from Kunming Pharmaceutical Group Co., Ltd., China (lot#: 14HM20 1-11). Hemin was purchased from Hefei Bomei Biotechnology Co., Ltd (imported products, highly pure, lot#: 180137). Ferrous acid was purchased from Xinxiang Chemical Reagent Factory (analytically pure, lot#: 840918). DMSO was kindly provided by Prof. Ruili Shi.

Blood samples were obtained from the Laboratory Department of Baotou Steel Third Hospital, provided by Baowu Renbiliger.

The ABO blood type positive test cards were purchased from Changchun Bode Biotechnology Co., Ltd..

Agglutination reaction of whole blood, red blood cells, hemolytic blood and artemisinin drugs. The ABO blood type positive test card was prepared. Preparation of required reagents: ① DMSO stock solution was used as the first solution; ② 0.055 g artemisinin was dissolved in 1 mL DMSO solution as the second solution; ③ 0.035 g

dihydroartemisinin was dissolved in 1 mL DMSO solution as the third solution.

Sampling: ① The three holes on the left side of the test card were 50  $\mu$ L of whole blood, 50  $\mu$ L of red blood cells with the same amount of saline and 50  $\mu$ L of hemolytic blood; ② The three holes on the right side of the verification card were the upper hole with 50  $\mu$ L of the first liquid, the middle hole with 50  $\mu$ L of the second liquid and the lower hole with 50  $\mu$ L of the third liquid. Pulled and mixed the liquid in the left and right holes with bamboo sticks: ① Pulled one time from left to right; ② Pulled 3 times from left to right.

Agglutination reaction of hemin, ferrous sulfate, ferric chloride, sodium chloride, DMSO, liquid artemether and artemisinin drugs. The ABO blood type positive test card was prepared. The same as the whole blood test above. Hemin solution: 0.01 g hemin was dissolved in 1 mL of 1% sodium hydroxide. Ferrous acid dyeing solution: 0.025 g ferrous sulfate was dissolved in 1 mL of double distilled water. Ferric chloride dyeing solution: 0.1 g ferric chloride was dissolved in 1 mL of double distilled water, completely dissolved in 10 min, showing yellow color, then diluted three times, showing light yellow color for later use. 0.9% sodium chloride solution, DMSO solution and artemether solution were prepared. Sampling: ① The three holes on the left side of the verification card are all 50  $\mu$ L of hemin, 50  $\mu$ L of ferrous sulfate solution, 50  $\mu$ L of ferric chloride solution, 50  $\mu$ L of 0.9% sodium chloride solution, 50  $\mu$ L of DMSO and 50  $\mu$ L of liquid artemether; ② Three holes on the right side of the verification card: the same as the whole blood experiment mentioned above. Pulled and mixed two holes of liquid with bamboo sticks: ① Pulled one time from left to right; ② Pulled three times from left to right; ③ Pulled furiously from left to right; ④ After 10 minutes, lifted the right side of the paper to make the liquid flow from the right to the left.

## 3 RESULTS

Agglutination reaction of whole blood and artemisinin-type drugs. The results were shown in Figure A. It could be seen from Figure A that there are obvious agglutinating particles in the right middle pore and lower pore. The whole blood has no agglutination reaction with DMSO. The whole blood has agglutination reaction with artemisinin and dihydroartemisinin. The effect of dihydroartemisinin is stronger than that of artemisinin.

Agglutination reaction of red blood cells and artemisinin-type drugs. The results were shown in Figure B. It could be seen from Figure B that there are obvious agglutination particles in the right middle hole and lower hole, especially in the lower hole. Erythrocytes have no agglutination reaction with DMSO. Erythrocytes have agglutination reaction with artemisinin and dihydroartemisinin. The effect of dihydroartemisinin is stronger than that of artemisinin.

Agglutination reaction of hemolysis and artemisinin-type drugs. The results were shown in Figure C. It could be seen from Figure C that there are obvious

agglutination particles in the right middle hole and lower hole, and the middle hole is not as obvious as the lower hole. The hemolytic solution has no agglutination reaction with DMSO, but has agglutination reaction with artemisinin and dihydroartemisinin. The effect of dihydroartemisinin is stronger than that of artemisinin.

Agglutination reaction of heme chloride and artemisinin-type drugs. The results were shown in Figure D. It could be seen from Fig. D that agglutination occurs in the right mesopore and lower mesopore, especially in the mesopore. "Fingers" appear in the right middle hole and lower hole, indicating the solidification trend. Hemin does not agglutinate with DMSO, while hemin agglutinates with artemisinin and dihydroartemisinin.

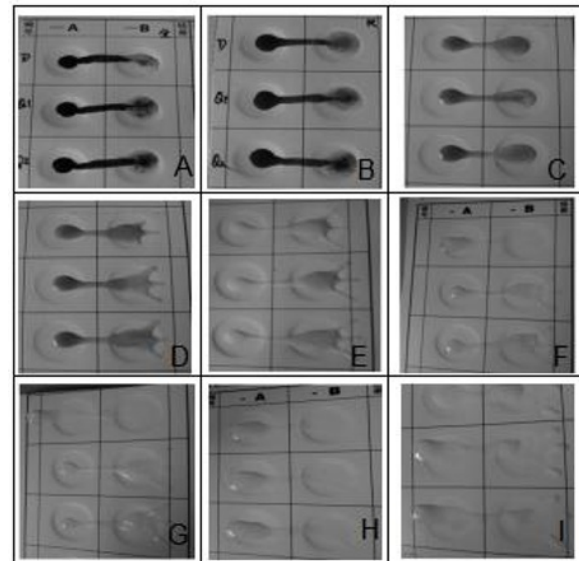
Agglutination reaction of ferrous sulfate and artemisinin-type drugs. The results were shown in Figure E. It could be seen from Figure E that "fingers" appear in the right middle hole and lower hole, indicating that there has the solidification trend, and the upper hole is not solidified and is in the flow state. Ferrous sulfate has no agglutination reaction with DMSO, while ferrous sulfate has agglutination reaction with artemisinin and dihydroartemisinin.

Agglutination reaction of ferric chloride and artemisinin-type drugs. The results were shown in Fig. F. It could be seen from Fig. F that the right middle hole and lower hole appear "fingers" without deformation, indicating that the solidified upper hole is in a flow state without solidification. Ferric chloride has no agglutination reaction with DMSO, while ferric chloride has agglutination reaction with artemisinin and dihydroartemisinin.

Agglutination reaction of sodium chloride and artemisinin-type drugs. The results were shown in Fig. G. It could be seen from Fig. G that the right middle hole and the lower hole appear "fingers" without deformation, indicating solidification, and the upper hole is in flow state without solidification.

Agglutination reaction of DMSO and artemisinin-type drugs. The results were shown in Figure H. It could be seen from Figure H that there is no difference between the results of the right middle hole, the lower hole and the upper hole.

Agglutination reaction of artemether and artemisinin-type drugs. The results were shown in Figure I. It could be seen from Figure I that the results of the right middle hole and the lower hole are opposite to those of the upper hole, that is, the upper hole is solidified, the middle hole and the lower hole are not solidified.

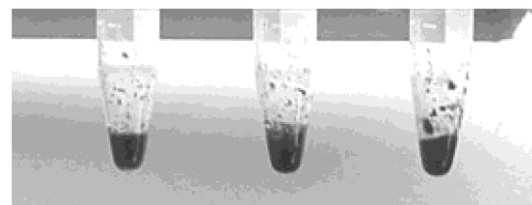


**Fig. 1** Agglutination reaction of different substances and artemisinin-type drugs

Note: From Figure A to Figure I, the agglutination reaction of whole blood, red blood cells, hemolytic blood, hemin, ferrous sulfate, ferric chloride, sodium chloride, DMSO and liquid artemether with artemisinin-type drugs was shown in sequence. The three holes on the right side of each figure were DMSO, DMSO-dissolved artemisinin and DMSO-dissolved dihydroartemisinin in top to bottom sequence.

## 4 DISCUSSION

Agglutination reaction is a manifestation occurred as a result of two interacting influences. In the present study, agglutination reaction was found when artemisinin-type drugs were mixed with hemolytic blood, as shown in Figure 2. The results showed that the mixture of dihydroartemisinin and hemolytic blood produced the most agglutinative particles, followed by artemisinin, and the control tube was the least. In view of the unclear boundary of agglutination reaction observed in the test tube, we used ABO blood type positive test card and got the above-mentioned results.



**Fig. 2** Agglutination reaction of red blood cell hemolysis and artemisinin-type drugs

Note: The left tube was hemolytic solution+DMSO; The middle tube was hemolytic solution+DMSO (dissolved with artemisinin); The right tube was hemolytic solution+DMSO (dissolved with dihydroartemisinin)

Agglutination refers to the visible agglutination of granular antigens, including bacteria or red blood cells, with corresponding antibodies in the presence of

appropriate electrolytes. The agglutination reaction is mainly used for the detection of antigen and antibody in clinic. For the detection of antigen, reverse indirect agglutination test is commonly used in clinic, for example, the latex agglutination inhibition test is used to detect chorionic gonadotropin. For antibody detection, blood type identification and cross matching are commonly used clinical agglutination reactions.

In this study, we found for the first time that artemisinin-type drugs can interact with a variety of substances to cause agglutination, which has not been recorded in the past literature. It was previously believed that agglutination reaction could be found in antigen antibody reaction, but this discovery does not seem to belong to this type of reaction. Early research by our team had found the phenomenon of hemoglobin A2, namely, the interaction between HbA2 and HbA1 in red blood cells, and the interaction among HbA, HbB, HbC and HbD in red blood cells of rats. None of the above phenomena belong to antigen-antibody reaction, and thus whether agglutination can occur remains to be tested.

## 5 CONCLUSIONS

The agglutination reaction discussed in this paper is not related to antigen and antibody, but closely related to the interaction between artemisinin drugs and various substances. Therein, the agglutination reactions of artemisinin drugs with whole blood, red blood cells, hemolytic solution and sodium chloride are included. Whole blood, red blood cells and hemolytic solution contain biological macromolecular components, while hemin belongs to low molecular organic compounds, and the rest are simple inorganic compounds. Artemisinin-type drugs have the ability to interact and agglutinate with substances of such a large spectrum of substances, which proves its excellent effect. Unfortunately, the potential mechanism of artemisinin-type drugs remains unclear so far. Although it is speculated that the "peroxide bridge" in artemisinin molecule is involved, the specific details of the action and the process of agglutination have not been fully clarified. Interestingly, agglutination did not occur when artemether interacted with artemisinin and dihydroartemisinin, but the control showed an agglutination trend, which was contrary to other results and the reason remains to be further studied.

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