Sensitivity of Oncomelania hupensis to Niclosamide: A Nation-Wide Survey in China

Jianrong Dai 1,2,3, Youzi Li 1,2,3, Wei Wang 1,2,3,*, Yuntian Xing 1,2,3, Guoli Qu 1,2,3 and Yousheng Liang 1,2,3,*

1 Jiangsu Institute of Parasitic Diseases, 117 Yangxiang, Meiyuan, Wuxi 214064, Jiangsu, China; E-Mails: djr0008@163.com (J.D.); muziyouzi@sina.cn (Y.L.); xingyuntianok@163.com (Y.X.); quguoli83@163.com (G.Q.)
2 Jiangsu Provincial Key Laboratory of Molecular Biology of Parasites, 117 Yangxiang, Meiyuan, Wuxi 214064, Jiangsu, China
3 Key Laboratory on Technology for Parasitic Disease Prevention and Control, Ministry of Health, 117 Yangxiang, Meiyuan, Wuxi 214064, Jiangsu, China

* Authors to whom correspondence should be addressed; E-Mails: wangweijipd@163.com (W.W.); wxliangyousheng@163.com (Y.L.); Tel.: +86-510-6878-1022 (W.W.); +86-510-6878-1003 (Y.L.); Fax: +86-510-6878-1022 (W.W.); +86-510-8551-0263 (Y.L.).

Received: 24 January 2014; in revised form: 27 February 2014 / Accepted: 4 March 2014 / Published: 12 March 2014

Abstract: Schistosomiasis japonica, transmitted by the intermediate host snail Oncomelania hupensis of the causative agent Schistosoma japonicum, remains a major public-health concern in China, and control of this snail is one of the major approaches used in attempts to interrupt the transmission of this neglected tropical disease. Niclosamide is currently the only commercial molluscicide available for the control of O. hupensis snails in China. The purpose of this study was to evaluate the current sensitivity of O. hupensis to niclosamide in China. O. hupensis snails derived from 17 sampling sites from eight schistosomiasis-endemic provinces of China were used for the molluscidal tests. Active adult snails (10 for each drug concentration), were immersed in solutions of 1, 0.5, 0.25, 0.125, 0.063, 0.032, 0.016 and 0.008 mg/L of 50% wettable powder of niclosamide ethanolamine salt (WPN) for 24 and 48 h at 25°C, and then the snail mortality was estimated and LC50 values were calculated. All field-derived O. hupensis snails were dead following immersion in 0.5 and 1 mg/L WPN for 24 h, whereas no death was observed after immersion in 0.008 mg/L WPN for 24 h. Immersion in 0.5, 0.25,
0.125, 0.063, 0.032 and 0.016 mg/L WPN for 24 h resulted in 80%–100%, 63.33%–100%, 0%–85%, 0%–50%, 0%–15%, and 0%–5% snail mortalities, respectively. The 24 h WPN LC₅₀ values for the *O. hupensis* snails derived from the 17 sampling sites in China ranged from 0.0743 to 0.2285 mg/L, and no significant difference was detected by the Kolmogorov-Smirnov test (*p* = 0.2). The results indicate that there is no regional variation in the current susceptibility to niclosamide in *O. hupensis* populations in China. It is suggested that the current sensitivity of niclosamide against *O. hupensis* remains high and has not changed after more than two decades of repeated, extensive application for snail control in the main endemic areas of China.

**Keywords:** *Oncomelania hupensis*; *Schistosoma japonicum*; niclosamide; molluscicidal activity; China

1. Introduction

Schistosomiasis, a snail-transmitted parasitic disease, is a major neglected tropical disease affecting more than 207 million people in the tropical and subtropical regions around the world [1]. Although great success has been achieved in its control in China, schistosomiasis japonica is still one of the four communicable diseases that have been given high priority by the central government [2–4]. It is estimated that over 0.7 million people are infected with the parasite in China, and the snail host *Oncomelania hupensis* is detected in habitats with an area of 3.73 billion m² [5–7]. It has been proved that the transmission of *S. japonicum* is governed by the geographical distribution of its snail host; therefore, control of the *O. hupensis* snails, as a major part of the National Schistosomiasis Control Program, is currently one of the major approaches used for schistosomiasis control and elimination in China [8–10].

In China, many approaches have been used for the control of *O. hupensis*; however, snail control with chemicals remains the most widely used method to kill the snail intermediate host till now [11–13]. Niclosamide is recommended by the WHO as the only molluscicide for snail control in the endemic foci [14]; however, the agent is difficult to dissolve in both water and organic solvents. Therefore, many novel niclosamide formulations have been developed attempting to improve its water solubility. Of these a 50% wettable powder of niclosamide ethanolamine salt (WPN) has been recommended as the only market-available molluscicide since the initiation of the World Bank Loan Project for Schistosomiasis Control in China, due to its high efficacy and easy use [15]. Currently, WPN is recommended by the Ministry of Health, P. R. China at a dose of 1 mg/L for snail control in the field [15]. Following extensive, long-term, repeated use for more than two decades, the possible emergence of resistance to niclosamide in the intermediate host snails has received much attention [16]. Therefore, a systematic survey of the molluscicidal activity of niclosamide against *O. hupensis* would be of great significance for understanding the current sensitivity of niclosamide in China, and the prevention and rapid management of niclosamide resistance in snail populations. Here, a nationwide determination was carried out in regions where *O. hupensis* snails are present in China to assess the current efficacy of *O. hupensis* to niclosamide.
2. Materials and Methods

2.1. Snails

Adult *O. hupensis* snails were collected by individual picking with forceps [17], from 17 sampling sites from eight schistosomiasis-endemic provinces of China (Table 1, Figure 1). After feeding in the laboratory for 24 h, active adult snails with 7–8 spirals were randomly divided into groups for the molluscicidal test.

**Table 1.** Location and environmental types of the snail sampling sites in China

| Snail Sampling Site                | Environmental Type       | Code | East Longitude | North Latitude |
|------------------------------------|--------------------------|------|----------------|----------------|
| Weishan County, Yunnan Province    | Hill                     | A    | 100.33°        | 25.23°         |
| Shimen County, Hunan Province      | Hill                     | B    | 110.47°        | 29.51°         |
| Linli County, Hunan Province       | Hill                     | C    | 111.64°        | 29.44°         |
| Yuanjiang County, Hunan Province   | Lake and marshland       | D    | 112.20°        | 28.52°         |
| Nanxian County, Hunan Province     | Lake and marshland       | E    | 112.39°        | 29.37°         |
| Gong’an County, Hubei Province     | Lake and marshland       | F    | 112.00°        | 30.05°         |
| Jingshan County, Hubei Province    | Hill                     | G    | 113.11°        | 31.03°         |
| Wucheng County, Jiangxi Province   | Lake and marshland       | H    | 115.54°        | 28.05°         |
| Pengze County, Jiangxi Province    | Lake and marshland       | I    | 116.32°        | 29.58°         |
| Duchang County, Jiangxi Province   | Lake and marshland       | J    | 116.24°        | 29.25°         |
| Yongxiu County, Jiangxi Province   | Lake and marshland       | K    | 115.82°        | 29.04°         |
| Nanchang County, Jiangxi Province  | Lake and marshland       | L    | 115.89°        | 28.68°         |
| Xuancheng County, Anhui Province   | Hill                     | M    | 118.77°        | 30.74°         |
| Dongtai County, Jiangsu Province   | Plain with waterway networks | N  | 120.31°        | 32.84°         |
| Zhenjiang County, Jiangsu Province | Plain with waterway networks | O  | 119.44°        | 32.20°         |
| Pinghu County, Zhejiang Province   | Plain with waterway networks | P  | 121.02°        | 30.70°         |
| Yinxin County, Fujian Province     | Hill                     | Q    | 119.35°        | 25.72°         |

**Figure 1.** The sampling sites of *Oncomelania hupensis* snails in China.
2.2. Niclosamide Formulation

WPN was provided by the Nanjing Essence Fine Chemical Co., Ltd. (Nanjing, China; lot: 1012031). WPN was weighed and solutions of 1, 0.5, 0.25, 0.125, 0.063, 0.032, 0.016 and 0.008 mg/L of niclosamide were prepared in dechlorinated tap water for the subsequent experiments.

2.3. Molluscicidal Test

One hundred milliliter flasks were filled with the solutions, then 10 active adult snails were added and the flasks covered with gauze to prevent their escape. Snails in flasks of dechlorinated water served as controls. After being immersed for 24 and 48 h at 25°C, the snails were washed with dechlorinated water and fed for a further 48 h. Those suspected of being dead were tested by the knocking method [18,19], and the snail mortality was estimated. All tests were performed in triplicate, and LC50 values were calculated.

2.4. Statistical Analysis

All data were double entered into Microsoft Excel 2003 (Microsoft Corporation; Redmond, WA, USA) and all statistical analyses were performed using the statistical software SPSS version 16.0 (SPSS Inc.; Chicago, IL, USA). Differences of LC50 values were tested for statistical significance with Kolmogorov-Smirnov test. A \( p \) value < 0.05 was considered statistically significant.

3. Results

All field-derived \( O. \ hupensis \) snails were dead following immersion in 1 mg/L WPN for 24 h, whereas no death was observed after immersion in 0.008 mg/L WPN for 24 h. Immersion with WPN at concentrations of 0.5, 0.25, 0.125, 0.063, 0.032 and 0.016 mg/L for 24 h resulted in 80%–100%, 63.33%–100%, 0%–85%, 0%–50%, 0%–15%, and 0%–5% mortalities of snails, respectively (Table 2). The 24 h WPN LC50 values for the \( O. \ hupensis \) snails derived from the 17 sampling sites in China ranged from 0.0743 to 0.2285 mg/L, and no significant difference was detected by the Kolmogorov-Smirnov test (\( p = 0.2 \)).

Table 2. Comparison of mortality rates and LC50 values of \( Oncomelania \ hupensis \) snails sampled from different regions of China following immersion in various concentrations of WPN for 24 h.

| Snail Population | Mortality of Snails in Different Concentrations of WPN | LC50 (mg/L) |
|------------------|------------------------------------------------------|-------------|
|                  | 1 mg/L | 0.5 mg/L | 0.25 mg/L | 0.125 mg/L | 0.063 mg/L | 0.032 mg/L | 0.016 mg/L | 0.008 mg/L |
| Weishan           | 100    | 100      | 90        | 20         | 15         | 5          | 0          | 0         | 0.1436     |
| Shimen            | 100    | 100      | 80        | 80         | 50         | 10         | 0          | 0         | 0.0770     |
| Linli             | 100    | 95       | 75        | 15         | 10         | 5          | 5          | 0         | 0.1708     |
| Ruanjiang         | 100    | 95       | 95        | 70         | 25         | 0          | 0          | 0         | 0.0981     |
The *O. hupensis* snails derived from 16 sampling sites (the number of snails sampled from Shimen county of Hunan province was not enough, due to death, to be used for assessing the mortality following immersion in WPN for 48 h) were employed to test the efficacy of WPN following immersion for 48 h. No snails survived the treatment with WPN at concentrations of 0.5 and 1 mg/L for 48 h, whereas all snails were alive by immersion in WPN at a concentration of 0.008 mg/L for 48 h. Immersion in WPN at concentrations of 0.25, 0.125, 0.063, 0.032 and 0.016 mg/L for 48 h resulted in 95%–100%, 10%–100%, 0%–55%, 0%–10%, and 0%–5% mortalities of snails, respectively (Table 3).

### Table 3. Comparison of mortality rates and LC50 values of *Oncomelania hupensis* snails sampled from different regions of China following immersion in various concentrations of WPN for 48 h.

| Snail Population | Mortality of Snails in Different Concentrations of WPN | LC50 (mg/L) |
|------------------|-------------------------------------------------------|--------------|
|                  | 1 mg/L | 0.5 mg/L | 0.25 mg/L | 0.125 mg/L | 0.063 mg/L | 0.032 mg/L | 0.016 mg/L | 0.008 mg/L |
| Weishan          | 100    | 70       | 10        | 5          | 0          | 0          | 0          | 0.1015     |
| Shimen*          |        |          |          |            |            |            |            |            |
| Linli            | 100    | 25       | 20        | 5          | 5          | 0          | 0          | 0.1208     |
| Ruanjiang        | 100    | 75       | 35        | 0          | 0          | 0          | 0          | 0.0825     |
| Nanxian          | 100    | 95       | 75        | 15         | 0          | 0          | 0          | 0.0981     |
| Gong’an          | 100    | 60       | 10        | 0          | 0          | 0          | 0          | 0.1088     |
| Jingshan         | 100    | 40       | 3.33      | 3.33       | 0          | 0          | 0          | 0.1309     |
| Wucheng          | 100    | 90       | 13.33     | 3.33       | 0          | 0          | 0          | 0.0864     |
| Pengze           | 100    | 63.33    | 10        | 0          | 0          | 0          | 0          | 0.1063     |
| Duchang          | 100    | 80       | 33.33     | 0          | 0          | 0          | 0          | 0.0806     |
| Yongxiu          | 100    | 100      | 55        | 30         | 0          | 0          | 0          | 0.0490     |
| Nanchang         | 100    | 100      | 100       | 0          | 0          | 0          | 0          | 0.0718     |
### Table 3. Cont.

| Snail Population | Mortality of Snails in Different Concentrations of WPN | LC$_{50}$ (mg/L) |
|------------------|--------------------------------------------------------|------------------|
|                  | 1 mg/L | 0.5 mg/L | 0.25 mg/L | 0.125 mg/L | 0.063 mg/L | 0.032 mg/L | 0.016 mg/L | 0.008 mg/L |
| Xuancheng        | 100    | 100      | 96.67     | 16.67      | 3.33       | 0         | 0         | 0         | 0.1575     |
| Dongtai          | 100    | 100      | 100       | 25         | 0          | 0         | 0         | 0         | 0.1487     |
| Zhenjiang        | 100    | 100      | 97        | 57         | 13         | 10        | 0         | 0         | 0.1037     |
| Pinghu           | 100    | 100      | 96.67     | 23.33      | 0          | 0         | 0         | 0         | 0.1539     |
| Yinxi            | 100    | 100      | 100       | 10         | 5          | 0         | 0         | 0         | 0.1593     |

Note: *The number of *O. hupensis* snails sampled from Shimen county of Hunan province is not enough to be used for assessing the mortality following immersion in WPN for 48 h.

The 48 h WPN LC$_{50}$ values for the *O. hupensis* snails derived from 16 sampling sites in China ranged from 0.049 to 0.1593 mg/L, and no significant difference was detected by the Kolmogorov-Smirnov test ($p = 0.197$).

### 4. Discussion

In China, the description of schistosomiasis japonica dates back more than two millennia [2]. Since the control efforts initiated in the 1950s, snail control has been a key part of the National Schistosomiasis Control Program of China [8,12], and many approaches have been used for the control of the intermediate host snails, including environmental improvement, physical methods, building trees, molluscicide treatment and biological control [11]. Among all these strategies, molluscicide treatment is the most widely used method for snail control due to the wide application coverage, easy procedure and fast action [20]. A recent meta-analysis revealed that it is necessary to continuously apply molluscicidal treatments more than twice a year in the field to consolidate the schistosomiasis control achievements gained [21], and different molluscicidal treatment strategies should be utilized at different stages of the control programme to maximize cost-effectiveness [20].

Since 1950s, many chemicals have been tested for molluscicidal activity and several agents have been used for snail control in the schistosomiasis-endemic fields of China, including sodium pentachlorophenate, bromoacetamide, nicotinanilide, calcium cyanamide, niclosamide, META-Li, and so on [22–25]. In addition, some plants have been screened and tested for molluscicidal activity against *O. hupensis* in laboratories [26–30]. Since 1992 when the World Bank Loan Project for Schistosomiasis Control was initiated in China, WPN was introduced and has replaced other molluscicides to become the only molluscide now used for snail control in the endemic areas of China [15]. Following the repeated, long-term, extensive use for more than two decades, there is a concern about the potential development of resistance to niclosamide in *O. hupensis* snails [16]. It is therefore of great importance to monitor the molluscicidal activity of niclosamide against the *O. hupensis* snails in regions where this is currently the only available chemical that is widely used in China.

WPN at a dose of 1 mg/L is recommended for snail control in the field [15]. Therefore, drug solutions at concentrations of 1, 0.5, 0.25, 0.125, 0.063, 0.032, 0.016 and 0.008 mg/L were designed for the molluscicidal tests in the laboratory. Our findings showed that all *O. hupensis* snails were dead following immersion in 1 mg/L WPN for 24 and 48 h or in 0.5 mg/L WPN for 48 h, and
only 0%–20% survived the treatment with WP N at a concentration of 0.5 mg/L for 24 h. In addition, the 24 h WPN LC50 values for the *O. hupensis* snails derived from 17 sampling sites in China ranged from 0.0743 to 0.2285 mg/L, with no significant differences detected (*p* = 0.2), and the 48-h WPN LC50 values for the *O. hupensis* snails derived from 16 sampling sites in China ranged from 0.049 to 0.1593 mg/L, with no significant differences detected (*p* = 0.197). The results from this study demonstrate that the molluscicidal activity of niclosamide against *O. hupensis* is still high in the main endemic areas of China after more than two decades of repeated, extensive use. This is important information for both the public health workers and health policy makers in the field of schistosomiasis control, considering that niclosamide, the currently only chemical of choice for snail control, plays an essential role in the current Chinese National Schistosomiasis Control Program of China.

It has been found that the environmental factors including temperature, vegetation, sunlight, soil and rainfall, the quality and concentration of the chemicals, as well as the technical skills may affect the molluscicidal actions in the endemic foci [11], whereas in the laboratory, the volume of drug solution [31], and the quantity, laboratorial breeding duration and the sampling time of snails used for molluscicidal test [32–34] are reported to affect the evaluation of the molluscicidal actions in the laboratory. Currently, a single WPN dose (1 mg/L) is employed for the field snail control in China [15], however, the variations in snail habitats, climate, temperature and rainfall may affect the concentration and duration of snail contact with niclosamide, thereby resulting in various molluscicidal efficacies. It is suggested that the dose of molluscicides should be appropriately adjusted based on the actual field settings, and temperature, climate and rainfall should be taken into account during the snail control in the field, so as to achieve the optimal molluscicidal efficacy.

5. Conclusions

The results indicate that there is no regional variation in the current susceptibility to niclosamide in *O. hupensis* populations, and the current activity of niclosamide against *O. hupensis* snails in China appears satisfactory; however, it does not mean that resistance cannot occur nor that in different geographical regions the response of *O. hupensis* snails will be the same. We, therefore, should not reduce our vigilance to the possible development of resistance to niclosamide in the intermediate host snails. Further periodical studies monitoring both the sensitivity of *O. hupensis* to the chemical and the development and epidemiology of niclosamide resistance of different geographical isolates of *O. hupensis* in China are still required, which would be of great significance for the elimination of schistosomiasis japonica in China.

Acknowledgements

Many thanks are due to the staff from Yunnan Provincial Institute of Endemic Diseases Control, Sichuan Provincial Center for Disease Control and Prevention, Hunan Institute of Parasitic Diseases, Hubei Provincial Center for Disease Control and Prevention, Jiangxi Institute of Parasitic Diseases, Anhui Institute of Schistosomiasis Control, Zhejiang Academy of Medical Sciences, Shanghai Center for Disease Control and Prevention and Fujian Provincial Center for Disease Control and Prevention, for their kind help to collect the snails. Thanks are also given to Jianying Wei and Na Guo for
their work in breeding snails in laboratory. This work was supported by National Science and Technology Pillar Program of China (2009BAI78B06), the National Important Sci-Tech Special Projects (2012ZX10004-220), the Natural Science Foundation of China (81071379), the Jiangsu Province’s Outstanding Medical Academic Leader Programme (LJ201132), and Jiangsu Department of Health (X201103). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Author Contributions**

Jianrong Dai, Wei Wang and Yousheng Liang conceived and designed the study; Jianrong Dai, Youzi Li, Wei Wang, Yuntian Xing and Guoli Qu conducted the study. Jianrong Dai and Youzi Li collected the data and performed analysis of data. Jianrong Dai and Wei Wang prepared the first draft of the manuscript; Wei Wang and Yousheng Liang provided strategic advice and assisted with editing of the manuscript. All authors read and approved the final version of the manuscript.

**Conflicts of Interests**

The authors declare no conflicts of interest.

**References**

1. Steinmann, P.; Keiser, J.; Bos, R.; Tanner, M.; Utzinger, J. Schistosomiasis and water resources development: Systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis.* 2006, 6, 411–425.

2. Wang, L.D.; Utzinger, J.; Zhou, X.N. Schistosomiasis control: Experiences and lessons from China. *Lancet* 2008, 372, 1793–1795.

3. Manus, D.P.; Li Y.; Gray, D.J.; Ross, A.G. Conquering “snail fever”: Schistosomiasis and its control in China. *Expert Rev. Anti Infect. Ther.* 2009, 7, 473–485.

4. Utzinger, J.; Zhou, X.N.; Chen, M.G.; Bergquist, R. Conquering Schistosomiasis in China: The long march. *Acta Trop.* 2005, 96, 69–96.

5. Zhou, X.N.; Guo, J.G.; Wu, X.H.; Jiang, Q.W.; Zheng, J.; Dang, H.; Wang, X.H.; Xu, J.; Zhu, H.Q.; Wu, G.L.; et al. Epidemiology of Schistosomiasis in the People’s Republic of China, 2004. *Emerg. Infect. Dis.* 2007, 13, 1470–1476.

6. Li, S.Z.; Luz, A.; Wang, X.H.; Xu, L.L.; Wang, Q.; Qian, Y.J.; Wu, X.H.; Guo, J.G.; Xia, G.; Wang, L.Y.; Zhou, X.N. Schistosomiasis in China: Acute infections during 2005–2008. *Chin. Med. J.* 2009, 122, 1009–1014.

7. Zheng, H.; Zhang, L.J.; Zhu, R.; Xu, J.; Li, S.Z.; Guo, J.G.; Xiao, N.; Zhou, X.N. Schistosomiasis situation in People’s Republic of China in 2011 (in Chinese). *Chin. J. Schisto. Control* 2012, 24, 621–626.

8. Zhou, X.N.; Wang, L.Y.; Chen, M.G.; Wu, X.H.; Jiang, Q.W.; Chen, X.Y.; Zheng, J.; Utzinger, J. The public health significance and control of Schistosomiasis in China—Then and now. *Acta Trop.* 2005, 96, 97–105.
9. Zhou, X.N.; Bergquist, R.; Leonardo, L.; Yang, G.J.; Yang, K.; Sudomo, M.; Olveda, R. *Schistosomiasis japonica* control and research needs. *Adv. Parasitol.* 2010, 72, 145–178.

10. Wang, L.D.; Guo, J.G.; Wu, X.H.; Chen, H.G.; Wang, T.P.; Zhu, S.P.; Zhang, Z.H.; Steinmann, P.; Yang, G.J.; Wang, S.P.; Wu, Z.D.; Wang, L.Y.; Hao, Y.; Bergquist, R.; Utzinger, J.; Zhou, X.N. China’s new strategy to block *Schistosoma japonicum* transmission: Experiences and impact beyond schistosomiasis. *Trop. Med. Int. Health* 2009, 14, 1475–1483.

11. Zhou, X.N. *Science of Oncomelania Snail* (in Chinese); Science Press: Beijing, China, 2005; pp. 97–108, 317–318.

12. Yuan, Y.; Xu, X.J.; Dong, H.F.; Jiang, M.S.; Zhu, H.G. Transmission control of *Schistosomiasis japonica*: Implementation and evaluation of different snail control interventions. *Acta Trop.* 2005, 96, 191–197.

13. Lin, D.D.; Hu, G.H.; Zhang, S.J. Optimal combined approaches of field intervention for Schistosomiasis control in China. *Acta Trop.* 2005, 96, 242–247.

14. WHO. *The Role of Mollusciciding in Schistosomiasis Control*; Division of Control of Tropical Diseases: Geneva, Switzerland, 1992; pp. 1–10.

15. Xianyi, C.; Liying, W.; Jiming, C.; Xiaonong, Z.; Jiang, Z.; Jiagang, G.; Xiaohua, W.; Engels, D.; Minggang, C. Schistosomiasis control in China: The impact of a 10-year World Bank Loan Project (1992–2001). *Bull WHO* 2005, 83, 43–48.

16. Cao, Z.G.; Wang, T.P.; Zhang, S.Q.; Tian, X.G.; Zhu, L.; Zhang, L.S.; Yao, G.X.; Jin, W.; Yang, W.P. Experimental study on the resistance of *Oncomelania* snails to niclosamide (in Chinese). *Chin. J. Pathogen Biol.* 2012, 7, 352–353, 376.

17. Mao, C.P. *Biology of Schistosome and Control of Schistosomiasis* (in Chinese); People’s Medical Publishing House: Beijing, China, 1990; 87–92.

18. Webbe, G. Laboratory and field trials of a new molluscicide, Bayer 73, in Tanganyika. *Bull WHO* 1961, 25, 525–531.

19. Gönnert, R. Results of laboratory and field trials with the molluscicide Bayer 73. *Bull WHO* 1961, 25, 483–501.

20. Yang, G.J.; Sun, L.P.; Hong, Q.B.; Zhu, H.R.; Yang, K.; Gao, Q.; Zhou, X.N. Optimizing molluscicide treatment strategies in different control stages of Schistosomiasis in the People’s Republic of China. *Parasit. Vector.* 2012, 5, doi:10.1186/1756-3305-5-260.

21. Yang, G.J.; Li, W.; Sun, L.P.; Wu, F.; Yang, K.; Huang, Y.X.; Zhou, X.N. Molluscicidal efficacies of different formulations of niclosamide: Result of meta-analysis of Chinese literature. *Parasit. Vectors* 2010, 3, doi:10.1186/1756-3305-3-84.

22. Zhou, P.S.; Ma, L.; Huang, J.L. Research progress on molluscicides and their application (in Chinese). *Chin. J. Vector Biol. Control* 2002, 13, 231–233.

23. Wu, X.Y.; Yang, L.Q.; Zhang, L.H.; Ge, Q.J. Progress of research on molluscicides (in Chinese). *Chin. J. Schistosomiasis Control* 2006, 18, 474–476.

24. Zhu, D.; Yao, P.; Bao, Z. Mollusciciding action and toxicity of bromoacetamide (in Chinese). *Chin. J. Parasitol. Parasit. Dis.* 1999, 17, 244–246.

25. Zhu, D.; Zhou, X.N.; Zhang, S.Q.; Zhang, G.; Liu, H.X.; Lu, D.B.; Cai, G.Y.; Ni, Q.Z.; Cao, Z.G.; Wu, W.D. Study on the molluscicidal effect of META-Li against *Oncomelania hupensis* (in Chinese). *Chin. J. Parasitol. Parasit. Dis.* 2006, 17, 244–246.
26. Wei, F.H.; Xu, X.J.; Liu, J.B.; Dai, Y.H.; Dussart, G.; Trigwell, J. Toxicology of a potential molluscicide derived from the plant *Solanum xanthocarpum*: A preliminary study. *Ann. Trop. Med. Parasitol.* **2002**, *96*, 325–331.

27. Wang, H.; Cai, W.M.; Wang, W.X.; Yang, J.M. Molluscicidal activity of *Nerium indicum* Mill, *Pterocarya stenoptera* DC, and *Rumex japonicum* houtt on *Oncomelania hupensis*. *Biomed. Environ. Sci.* **2006**, *19*, 245–248.

28. Yang, X.M.; Chen, S.X.; Xia, L.; Chen, J. Molluscicidal activity against *Oncomelania hupensis* of *Ginkgo biloba*. *Fitoterapia* **2008**, *89*, 250–254.

29. Zou, F.C.; Duan, G.; Xie, Y.J.; Zhou, Y.; Dong, G.D.; Lin, R.Q.; Zhu, X.Q. Molluscicidal activity of the plant *Eupatorium adenophorum* against *Oncomelania hupensis*, the intermediate host snail of *Schistosoma japonicum*. *Ann. Trop. Med. Parasitol.* **2009**, *103*, 549–553.

30. Peng, F.; Liu, M.; Huang, Q.; Liu, N.; Yang, H.; Sun, H.; Hu, Q.; Feng, F.; Jiang, C. Molluscicidal effect of *Eomecon chionantha* alkaloids against *Oncomelania hupensis* snails. *Southeast Asian J. Trop. Med. Public Health* **2011**, *42*, 289–296.

31. Dai, J.R.; Zhang, Y.P.; Jiang, Y.J.; Xi, W.P.; Yang, G.J.; Liang, Y.S. Studies on standardization of methods for screening molluscicides in laboratory I Volume of molluscicidal solution to influence the efficacy (in Chinese). *Chin. J. Schisto. Control* **2002**, *14*, 122–124.

32. Dai, J.R.; Xi, W.P.; Zhang, Y.P.; Liang, Y.S. Studies on standardization of methods for screening molluscicides in laboratory II Quantity of snails used to influence the efficacy (in Chinese). *Chin. J. Schisto. Control*. **2002**, *14*, 263–265.

33. Dai, J.R.; Liang, Y.S.; Zhang, Y.P.; Xu, M.; Li, H.J.; Zhu, Y.C. Studies on standardization of methods for screening molluscicides in laboratory III Breeding time of snails to influence the efficacy (in Chinese). *Chin. J. Schisto. Control*. **2003**, *14*, 346–348.

34. Li, Y.Z.; Xing, Y.T.; Li, H.J.; Qu, G.L.; Wang, W.; Wei, J.Y.; Liang, Y.S.; Dai, J.R. Studies on standardization of methods for screening molluscicides in laboratory IV breeding time of snails to influence the efficacy (in Chinese). *Chin. J. Schisto. Control*. **2012**, *24*, 35–39.