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Establishing the 1st Chinese National Standard for inactivated hepatitis A vaccine

Fan Gao, Qun-Ying Mao, Yi-Ping Wang, Pan Chen, Zheng-Lun Liang*

National Institutes for Food and Drug Control, Beijing, PR China

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ABSTRACT

A reference standard calibrated in the International Units is needed for the quality control of hepatitis A vaccine. Thus, National Institutes for Food and Drug Control launched a project to establish a non-adsorbed inactivated hepatitis A vaccine reference as the working standard calibrated against the 1st International Standard (IS). Two national standard candidates (NSCs) were obtained from two manufacturers, and designated as NSC A (lyophilized form) and NSC B (liquid form). Six laboratories participated in the collaborative study and were asked to use their in-house validated enzyme-linked immunosorbent assay methods to detect hepatitis A vaccine antigen content. Although both exhibited good parallelism and linear relationship with IS, NSC B showed a better agreement among laboratories than NSC A. And based on suitability of the candidates, NSC B was selected. The accelerated degradation study showed that NSC B was stable at the storage temperature (≤−70 °C). Therefore NSC B was approved as the first Chinese national antigen standard for inactivated hepatitis A vaccine, with an assigned antigen content of 70 IU/ml.

1. Introduction

Hepatitis A is an acute infectious disease of liver caused by hepatitis A virus (HAV) and is mostly transmitted through the fecal-oral route. It is associated with poor sanitation and particularly correlated with poverty. HAV, one of the major global public health threats, is responsible for 1.4 million cases of hepatitis A worldwide annually [1]. Vaccination is the most successful and cost-effective measure to prevent and control HAV. Inactivated hepatitis A vaccine Havrix® was first developed by Glaxosmithkline in 1992 [2], followed by Merck Sharp & Dohme Corp (Vaqta®) [3], Sanofi Pasteur S.A. (Avaxim®) [4] and Crucell Switzerland AG (Epaxal®) [5,6]. In 2001, Healive®, a preservative-free inactivated HAV vaccine invented by Sinovac Biotech Co. Ltd., was licensed in China [7]. In 2006, another inactivated vaccine produced by the Chinese Academy of Medical Sciences is also available in the Chinese market [8]. HAV vaccination has been recommended by the Expanded Programme on Immunization and has been administrated to 20 million children annually in mainland China.

The 1st World Health Organization (WHO) International Standards (IS) for inactivated hepatitis A vaccine (code number: 95/500) was established in 1999 [9]. However, so far except a non-adsorbed inactivated HAV as the European working standard calibrated against the 1st WHO IS in 2010 [10], national standards and other secondary standards for hepatitis A vaccine were neglected. Currently, in the Chinese market the dosage units of inactivated hepatitis A vaccine from most manufacturers were still determined using the manufacturer's own internal references expressed as either u, U, El.U. or EU (Supplemental Table 1), making it difficult to standardize vaccine quality control (QC). Therefore a national standard for hepatitis A vaccine is urgently needed in China.

Based on the requirements of the WHO manual for the establishment of national and other secondary standards for vaccines [11] and the Chinese Pharmacopoeia (CP, 2010 version) regarding the preparation of national standards and the calibration of biological products, a national collaborative study was organized by the National Institutes for Food and Drug Control, China (NIFDC).

Abbreviations: CP, Chinese pharmacopoeia; CV, coefficients of variations; ELISA, enzyme-linked immunosorbent assay; HAV, hepatitis A virus; IS, international standard; IU, international units; NIBSC, National Institute of Biological Standards and Control; NIFDC, National Institutes for Food and Drug Control; NSCs, national standard candidates; OD, optical density; QC, quality control; R&D, research and development; WHO, World Health Organization.

* Corresponding author. National Institutes for Food and Drug Control, No. 2, Tiantan Xili, Beijing 100050, PR China. Tel.: +86 10 67095357; fax: +86 10 67022977.

E-mail address: lzhenglun@126.com (Z.-L. Liang).

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Two non-adsorbed hepatitis A antigen preparations were calibrated against the current WHO IS in antigen content assays using in-house ELISA methods. Based on the study results, the first Chinese national antigen standard for inactivated hepatitis A vaccine was established, which would play an important role in vaccine manufacturing quality control and dosage comparability.

2. Materials and methods

2.1. Participants

A total of six laboratories participated in the study, including two vaccine manufacturers, three research and development (R&D) laboratories and one national control laboratory (see Appendix A). Participants were randomly assigned with aliases from Lab 1 to Lab 6, and not necessarily related to the order of listing.

2.2. International standard (IS)

1st WHO IS for hepatitis A vaccine (inactivated), provided by the National Institute of Biological Standards and Control (NIBSC, code number 95/500), with an assigned antigen content of 100 IU/ml, stored at −20 °C.

2.3. National standard candidates (NSCs)

Two HAV bulk materials were included in this study. Candidate A (NSC A) is a bulk material (batch No. 01-E1312-032) provided by Sinovac Biotech Ltd (Beijing, China). The HAV strain was TZ84, and was cultivated in 2BS cell line (human fetal lung diploid fibroblast) by cell factory technology. It was produced through virus harvest, trichloromethane extraction, chromatographic purification, ultrafiltration, concentration, and formalin inactivation. This bulk material was aliquot into 3000 vials. The mean volume was 0.59 ml per vial (CV 1.4%). After lyophilization, the vials were closed with rubber-stoppers, sealed with tear-off aluminum caps, and stored at −20 °C. Moisture content was less than 1%. NSC A should be reconstituted in 0.8 ml purified water before use. The HAV antigen content was 57 IU/ml detected against WHO IS by the manufacturer's in-house method, and the CV value of antigen content homogeneity was 8.9%.

Candidate B (NSC B) is a bulk material (batch No. L201208-01) provided by the Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming, China). The HAV strain was L-8, and was cultivated in KMB_{17} cell line (human fetal lung diploid fibroblast) by cell factory technology. It was produced through virus harvest, ultrafiltration, concentration, trichloromethane extraction, chromatographic purification, and formalin inactivation. This bulk material was aliquot into 3000 vials. They were closed with rubber-stoppers and sealed with tear-off aluminum caps. NSC B was in liquid form supplemented with 2.5% bovine serum albumin. The mean volume was 0.53 ml per vial (CV 2.5%). The vials were stored at −70 °C. The HAV antigen content was 69 IU/ml detected against WHO IS by the manufacturer's in-house method, and the CV value of antigen content homogeneity was 6.6%.

2.4. Reagents and methods

ELISA was a well accepted method to determine antigen content of inactivated HAV, and was used for the establishment of WHO IS (95/500) [9]. All participants in this study were asked to use their own in-house ELISA methods/reagents to detect HAV antigen in the collaborative study. All in-house antigenicity assays were performed using sandwich ELISA. In brief, 96-well microtiter plates were coated with monoclonal antibody against HAV. According to the linearity range of each ELISA method, plates were incubated with HAV antigen at appropriate serial dilutions. Detection was performed using horseradish peroxidase-conjugated polyclonal or monoclonal secondary antibody, followed by the addition of the tetramethylbenzidine substrate. The absorbance at 450/630 nm was measured by an ELISA plate reader.

2.5. Calibration of candidate materials against WHO IS

For the calibration of the candidate materials, each participant performed at least 6 independent assays. Each assay included the WHO IS and the two NSCs, freshly reconstituted or thawed. At least four dilutions were required per sample and each dilution was performed in duplicates for each independent assay. Participants were requested to report both raw data (Optical Density, 450/630 nm) and calculated results for HAV antigen content.

2.6. Suitability study

To assess the suitability of the candidates as standards for antigen bulks, there were nine samples included in this study. Samples 1–4 were formalin inactivated HAV antigen (non-adsorbed) bulks from different manufacturers or institutes developing HAV vaccine and Samples 5–9 were final HAV (aluminum adjuvanted) inactivated vaccines in China. In order to simulate the real situation, each laboratory (Lab 2,3,4,6) tested HAV bulks produced by themselves (samples 1–4) using their in-house ELISA methods. Antigen contents of Samples 1–4 were expressed against WHO IS, NSC A and NSC B, respectively. Each participant performed six independent assays, and was requested to report both raw data (Optical Density, 450/630 nm) and calculated results. To evaluate hepatitis A vaccine final products (Samples 5–9), desorption step was needed before antigenicity assays. However the desorption protocols were manufacturer-specific and manufacturers do not wish their desorption methods disclosed to others. Therefore only Lab 1 was able to perform the tests (three independent assays per sample) on the final products (Samples 5–9). The antigen contents of these vaccines were individually expressed against WHO IS and NSC B after appropriated desorption step. The details of these samples are shown in Table 1.

2.7. Stability studies

Accelerated degradation studies were carried out to analyze the stability of NSC B. Sample vials were stored at −150 °C, −70 °C, −20 °C, +4 °C and +25 °C for a period of 0.5–12 months. The antigen contents of vials were measured against WHO IS by Lab 1 using its in-house ELISA method. Vials at all temperature and storage time points were evaluated in quadruplicates in two independent assays. The vials stored at −70 °C (incubated for 0 day) were used as baseline and the degradation rate of antigen content was calculated for each temperature. The baseline (69.4 IU/ml) was obtained from the result of antigen content homogeneity detected by the same in-house ELISA method as above.

For the freeze–thaw stability, the antigen content of NSC B with 3, 6, 9, 12 and 14 freeze–thaw cycles was expressed against that from vials without freeze–thaw cycle, which was assumed as 70 IU/ml. Vials with all freeze–thaw cycles were evaluated in duplicate in two independent assays.

2.8. Statistical analysis

The raw data was submitted to NIFDC and analyzed using the statistical software package “statistical analysis”. The parallel line model was applied to analyze the log-transformation

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