Disponible en ligne sur
ScienceDirect
www.sciencedirect.com
Elsevier Masson France
EM|consulte
www.em-consulte.com
Médecine et maladies infectieuses 45 (2015) 29–33

Original article

Low specificity of 2 tetanus rapid tests in Cambodia

Faible spécificité de 2 tests de diagnostic rapides tétanos au Cambodge

M. Schlumberger[a,*,1], B. Yvonnet[b,2], G. Lesage[b,2], B. Tep[a,3]

[a] *Association « SOS-Cambodge », 12, avenue du Général-Leclerc, 75014 Paris, France*
[b] *UFR de pharmacie « Philippe Maupas », 31, avenue Monge, 37200 Tours, France*

Received 21 October 2014; received in revised form 6 November 2014; accepted 29 December 2014
Available online 20 January 2015

Abstract

Objectives. – Rapid testing for tetanus on serum or blood allows for an immediate evaluation of individual protection against tetanus in developed countries, using a "single step" immunochromatographic technique using tetanus toxoid. The specificity of these tests, compared to the reference method for tetanus, mouse serum neutralization testing, has however never been assessed in these countries, due to the difficulty to perform serum neutralization titration in mice, because of animal testing bioethical regulations.

Population and methods. – A collection of sera from adult volunteers in Cambodia, living in rural environment, was tested for tetanus antibodies by ELISA in France, and by mouse serum neutralization in Vietnam. This allowed estimating the sensitivity and specificity of 2 rapid tetanus tests, available on the market: TQS™ and Tetanotop™.

Results. – The sensitivity of these tests was adequate, compared to mice serum neutralization test, for a test threshold of 0.01 IU/mL, (100% for TQS™, 91% for Tetanotop™), but their specificity was very low (1% for TQS™ and 13% for Tetanotop™).

Conclusion. – The results prove that these rapid tests for the assessment of individual protection against tetanus should not be used in the adult rural Cambodian population.

Keywords: Quick test; Tetanus; Mouse serum neutralization test; ELISA; Cambodia

Résumé

Objectifs. – Les tests de diagnostic rapides tétanos (TDRT), sur sang ou sur sérum, permettent de tester par immunochromatographie « en un temps » et en utilisant de l'anatoxine tétanique, le degré de protection de sujets contre le tétanos en pays développés. La spécificité de ces tests, vis-à-vis du test de référence pour le tétanos, la séroneutralisation sur souris, n'a cependant jamais été estimée dans ces pays, vu la difficulté de mettre en œuvre la technique de dosage par séroneutralisation sur souris, avec les lois de bioéthique animale.

Population et méthodes. – Une collection de sérums provenant de volontaires adultes, vivant en milieu rural au Cambodge, avait été titrée pour les anticorps antitétaniques en Elisa en France, et en séroneutralisation sur souris au Vietnam. Ceci a permis de tester la sensibilité et la spécificité de deux tests de diagnostic rapides tétanos, disponibles sur le marché: TQS™ et Tetanotop™.

Résultats. – Si la sensibilité de ces tests, vis-à-vis du dosage par séroneutralisation sur souris, pour une limite de protection à 0,01 UI/mL, est satisfaisante (100 % pour TQS™ et 91 % pour Tétanotop™), leur spécificité est très basse (1 % pour TQS™ et 13 % pour Tetanotop™).

* Corresponding author.
E-mail addresses: mschlumberger@wanadoo.fr (M. Schlumberger), bernard.yvonnet@univ-tours.fr (B. Yvonnet), gerard.lesage@univ-tours.fr (G. Lesage), thaknatep@yahoo.fr (B. Tep).
[1] Designed the protocol, selected the sera, analyzed the results, and wrote the article.
[2] Managed storage of sera and rapid test blind assessments at the Tours School of Pharmacy.
[3] Organized the technical and financial support with the community of donors of the SOS-Cambodge association for the study.

http://dx.doi.org/10.1016/j.medmal.2014.12.002
0399-077X/

Conclusion. – Ceci conduit à ne pas recommander l'utilisation de ces tests rapides en milieu rural au Cambodge pour estimer la protection individuelle des adultes contre le tétanos.

Mots clés : Tests de diagnostic rapides ; Tétanos ; Test de séroneutralisation sur souris ; Elisa ; Cambodge

1. Introduction

The rapid tetanus diagnostic tests (RTDT) allow quickly assessing the state of immunization against tetanus for patients hospitalized in developed countries especially in the emergency unit (EU), or before gastrointestinal tract surgery [1–5]. They also allow you to check the state of immunization against tetanus for individual going to countries with poorly equipped medical facilities [6,7]. The test sensitivity and specificity were assessed in developed countries, taking as reference an ELISA; but they were not assessed with the reference test for immunization against tetanus, seroneutralization (SN) in mice. This test gives a better assessment of the sensitivity and specificity of these tests, if a patient is tested positive by RTDT and thought to be protected against tetanus [8–13]. RTDT are still little used in Developing Tropical Countries (DTC), given their costs [14]. Yet it is in these countries that greater number of individuals are tested negative, and so where these tests would be the most useful.

The sera collected in Cambodia (Southeast Asia) from 1992 to 1994 were tested for tetanus antibodies, by ELISA and SN in mice [15,16], then stored at −80 °C at the "Philippe Maupas" School of Pharmacy in Tours; they allowed testing the sensitivity and especially the specificity of 2 RTDT available on the market: Tetanos Quick Stick test (TQS™) [17] and Tetanotop™ [18].

2. Material, population, and method

2.1. Material

2.1.1. Reagents used

These 2 tests are semi-quantitative; they do not use the level considered as protective by the WHO (0.01 IU/mL [19]), but give a positive response if the ELISA tetanus antibody level was > 0.1 IU/mL, a level considered as to better guarantee of protection against the disease [20].

2.1.1.1. TQS™. This test uses a "single step" immunochromatographic technique using a tetanus toxoid attached to a membrane, and toxoid marked with colloidal gold. A reaction between a positive serum or blood sample (with tetanus antibodies) and toxoid marked with colloidal gold forms a complex that migrates along the membrane. A "capture" antigen (a purified toxoid immobilized at a test area) will fix the complex migration and form a colored deposit (gray). So, this colored complex reveals the presence of tetanus antibodies. A purple internal control strip allows checking the proper functioning of the test [17].

2.1.1.2. Tetanotop™. This test also uses a "single step" immunochromatographic technique for the detection of tetanus antibodies in the blood or serum. It also uses a combination of tetanus toxoid attached on a membrane, and a latex labeled tetanus toxoid that can form a complex with tetanus antibodies in the blood or serum. A reaction between a positive sample of blood or serum (with tetanus antibodies) and latex labeled toxoid forms a complex that migrates along the membrane. The purified tetanus toxoid (capture antigen) attached to the membrane at a test area retains the complex migration, making the test strip gray, proving the presence of tetanus antibodies. A pink internal control strip allows checking the performance of the test (positive control). The absence of a pink background noise, which prevents reading the test, serves as a negative control [18].

One hundred and sixty individual RTDT of each type were purchased by SOS-Cambodge (August 2013), routed and stored as recommended between 2 °C and 8 °C, at the Tours School of Pharmacy. These tests were used within a year, maximum shelf life recommended by the manufacturers.

2.2. Study population

The study population lived in the rural district of Angkor-Thom, Cambodia (DTC), near the town of Siem-Reap. Three samples were performed between 1992 and 1994, just after the Paris Peace Treaty, when the Ministry of Health initiated a catch up tetanus vaccination (CTV). This serum bank was created to check the quality of tetanus seroconversion in Cambodian adults, which had never been done up to then. The adult population, at the time, had just come out of Khmer Rouge occupation, during which vaccinations were not performed by health services. The Siem-Reap area, occupied by the Khmer Rouge since 1970, had not benefited from CTV during the previous regimes (Monarchy and Khmer Republic) for safety reasons. Ninety percent of the male volunteer population, unlike women who were beginning to be vaccinated by the expanded program on immunization (EPI) in the global plan for maternal and neonatal tetanus elimination (MNT), was therefore seronegative for tetanus before CTV. These volunteers had signed an informed consent in the Khmer language [15,16].

2.3. Sera used

Blood samples were centrifuged and attributed a number in the Siem-Reap HIV screening center, carried in cold boxes by airplane, and stored frozen (−65 °C) at the Cambodia Pasteur Institute (CPI) in Phnom-Penh. They were then renumbered for dosage blind, i.e. blinding sex and vaccine history of volunteers.