Homeopathy (2016) 105, 225–232

http://dx.doi.org/10.1016/j.homp.2016.02.006, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Preparation, standardization and *in vitro* safety testing of *Mycobacterium* nosodes (Emtact- polyvalent nosode)

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Background: **Most of the nosodes in the homeopathic pharmacopeia have been sourced from obscure pathological material over a century ago; of which no scientific documentation is available.**
Method: **A method for preparation and standardization of univalent and polyvalent *Mycobacterium* nosodes (labeled as Emtact), using different strains of *Mycobacterium tuberculosis* was developed. The committee comprising microbiologists, scientist, pharmacist, homeopaths and clinicians had reviewed and approved the method of preparation of nosode. Preparation of the nosode was based on the reference in the Homeopathy Pharmacopoeia of India (HPI), group N-IV. Strains of *M. tuberculosis* viz. Standard strain H37Rv, multi-drug resistant (MDR) *M. tuberculosis*, *Mycobacterium bovis* (BCG vaccine) and *Mycobacterium avium* were identified, procured and documented. Twenty billion viable cells for each strain were taken for Original Stock Nosode (OSN). The original stock was prepared by suspending the microbial cells into water for injection (WFI) (1 ml). As per the Indian Pharmacopoeia (IP) monograph, sterility testing was done for different potencies. Polymerase Chain Reaction (PCR) was performed for 30c potency for detection of any DNA material of the source organisms.**
Result: **A polyvalent (multi-strain) and univalent *M. tuberculosis* nosodes were prepared for research and clinical use. No growth of *Mycobacterium* was observed in any of the samples above 5c potency. The *in-vitro* testing for nosode (30c) was found to be free from any organism and DNA material.**
Conclusion: ***Mycobacterium* nosodes sourced from individual strain and polyvalent Emtact nosode *in vitro* testing results found to be satisfactory for its handling and utilization. The nosode seems to be safe and may be tested further *in vivo* to explore its therapeutic application.** *Homeopathy* (2016) **105**, 225–232.

Keywords: Nosode; *Mycobacterium tuberculosis*; Emtact; Standardization; Polyvalent; Potentization; Safety; PCR

Introduction

Homeopathy is an alternative system of medicine introduced by Samuel Hahnemann, MD, in 1794, in Germany. The homeopathic medicines prepared by using the process of serial dilution and rigorous shaking, called potentization, have been found to contain nano-particles[1] in a recent study. Nosodes are broad-spectrum, widely used homeopathic preparations sourced from biological materials such as cultures or clinical samples of microorganisms (e.g. bacteria, fungi and viruses) or from parasites, diseased tissues (cancerous tissues), or decomposition products from humans or animals.[2] This category of drugs has been used in homeopathic profession since 1830 for the treatment of acute as well as chronic diseases.[3] Results from Homeopathy Pathogenetic Trials,

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Received 13 July 2015; revised 21 January 2016; accepted 29 February 2016

clinical trials and data collection in homeopathic practice show a long and safe track record with the use of nosodes. The manufacturing methods essentially meet pharmacopoeia requirements and must guarantee biological safety. Therefore, patients, practitioners and professional organizations insist on the preservation of nosodes for homeopathic treatment for acute as well as chronic diseases.

As per the European Central Council of Homeopaths (ECCH) survey, the five most frequently prescribed nosodes[4] were found to be Tuberculinum (22.6%), Carcinosinum (20.5%), Medorrhinum (15.0%), Psorinum (14.9%) and Syphilinum (10.9%). The survey was based on rating the importance of nosodes in homeopathy practice by various practitioners. It was found that for 38.2% of patients in the age group of 0−4 years and 5−11 years, nosodes had been indispensable to the improvement of chronic complaints while an average of 41.4% of cases were recorded for the age group of 12 years and above. In the treatment of acute problems, the average percentage of cases where nosodes were considered indispensable was 17%.[5]

Practically all of the nosodes have been sourced from obscure pathological material over a century ago; of which very few literature references[6,7] are available. The nosodes available presently are largely sourced from the previously potentized (called as back-potencies) preparations which have been passed on from very old pharmacies; having no documented information about the original source materials, thus, presenting many uncertainties. The ill-defined nature of the source, non-reproducibility and limited antigenicity are some of the major drawbacks providing limited immune protection. Also, the organisms have evolved over the time, making it imperative for development of fresher preparations from the recent strains.

With the advancement in microbiology, histopathology, immunology and medical science; and with the newer strain of organisms available, it necessitates re-visiting and re-developing of nosodes with scientific validation and standardization. Newer preparation will also allow further research using the current *in-vitro* and *in-vivo* studies involving safety, infectivity and possibly mechanism of action.

There are approximately forty five major nosodes[8] quoted in the literature and about twenty have been found clinically effective in the treatment of infectious and non-infectious diseases. Limited research work on nosodes such as *in-vitro* testing, animal studies, cell line studies, clinical research, veterinary research have been done.[9]

Nosodes sourced from Leptospirosis and dengue have been used in past for prophylactic treatment which have shown noteworthy efficacy.[10−12]

Hepatitis C nosode[13] and HIV[14] nosode have been introduced using a standardized method. Many such newer preparations will also encourage further research using the latest methods including but not limited to clinical trials, *in-vitro*, *in-vivo* studies and protein profiling.

Aims and objectives

Preparation and standardization of polyvalent *Mycobacterium* nosode (Emtact) using different *Mycobacterium* strains. *In-vitro* evaluation of safety of Emtact nosode for further research and therapeutic application.

Material and method

Nosode preparation has been based on the guidance in the Homeopathy Pharmacopoeia of India (HPI), Volume IV[15] group N-IV (using microorganism as source material). The standard operating procedure (SOP) was developed for preparation, standardization and evaluation of safety of the nosode. The project review committee comprising microbiologists, scientists, pharmacist, homeopaths and clinicians had reviewed and approved the method of preparation of the nosode. The procedure was executed and implemented under the supervision of investigator and authorized personnel at Haffkine Institute[16] and Life Force.

Steps

Source material: Different *Mycobacterium* strains as mentioned below were employed for the study (Refer Table 1):

a. *Mycobacterium tuberculosis* Standard strain H37Rv (culture)
b. Multi-drug resistant (MDR) *M. tuberculosis* (culture)
c. *Mycobacterium bovis* (BCG vaccine)
d. *Mycobacterium avium*

Preparation of stock, dilution and potentization

As per the general method of preparation of nosode stocks, 20 billion viable cells[14] for each strain were selected. In Emtact (*M. tuberculosis* polyvalent nosode), total 20 billion cells (5 billion for each strain) were taken for Original Stock Nosode (OSN). The original stock was prepared by suspending the microbial cells into water for injection (WFI) (1 ml). As per the HPI requirement, 20 billion counts of *Mycobacterium* strains were achieved and used for the preparation of the nosode. Potencies 1c, 2c and further up to 30c were prepared by using individual strains (univalent) as well as combined strains.

Potentization

For preparation of 1c potency, one part (0.03 ml) of OSN was mixed with 99 (2.97 ml) parts of WFI (vehicle), and ten strokes given with potentization machine. The impact parameters of the potentization machine were documented (Torque = 404.3 Nm).[2] Similarly, potencies up to 6c were prepared using WFI; and stored. For preparation of 6c and above potencies, dispensing alcohol was used in place of WFI. Conventionally, the homeopathic potencies are prepared and stored in dispensing alcohol for long term use.

Potencies up to 6c were stored at Haffkine Institute, at temperature between 4°C and 6°C and not allowing freezing. The bio-safety measures were followed during