International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: 10.7439/ijbar
CODEN: IJBABN

Original Research Article

Evaluation of BCG Vaccine in Excessive Dermal Reactivity Test

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Abstract

BCG vaccine has been in use globally to control the tuberculosis since the year 1921 and there has been significant achievement to curtail the disease. The important tests as per pharmacopoeial requirements including count of viable units (CVU) and excessive dermal reactivity (EDR) are required to be performed for ensuring the quality of BCG vaccine. In order to see the trend analysis of EDR of BCG vaccine used in India, a study has been carried out using 35 batches of BCG vaccine manufactured by two different manufacturers. The results of EDR of 16 batches of manufacturer-1 and 19 batches from manufacturer-2 ranged from 2.7mm to 3.2mm and1.9mm to 3.9mm respectively and the results are not markedly different from that of reference vaccine. The preliminary observation based on 16 batches suggests that there is some correlation between CVU and EDR of BCG vaccine, however more batches of BCG vaccine need to be tested to draw a clear correlation between these two tests. The critical EDR test is required to be performed regularly till the suitable alternate *in vitro* method is established. Overall results of the study suggest that BCG vaccine used in India is safe and potent.

Keywords: BCG vaccine, Excessive Dermal Reactivity (EDR), Pharmacopoeia, Guinea pig, Count of Viable Units (CVU).

1.Introduction

BCG vaccine is a live attenuated strain of *Mycobacterium bovis* that was originally isolated from cattle with tuberculosis and cultured for a period of 13years with a total of 231 passages [1]. The BCG vaccine was first used to immunize humans in 1921. Over the years, different BCG vaccine seed strains have evolved from the original vaccine strain. Worldwide, the most commonly used vaccine strains are: Danish 1331, Tokyo 172-1 and Russian BCG-I.BCG vaccine has a known reactogenecity profile after intradermal inoculation in children. Local reaction at the vaccination site is normal after BCG vaccination. It may take the form of a nodule that, in many cases, will break down and suppurate. The reaction developing at the vaccination site usually subsides within 2-5 months and in practically all children leaves a superficial scar of 2-10mm in diameter. The nodule may persist and ulcerate. Swelling of regional lymph nodes may also be seen, and this may be regarded as a normal reaction, but the size should be limited [2-4].

The dermal reactivity of each batch of BCG vaccine needs to show a comparable size of lesions/scars with reference vaccine. The dermal reactivity test, as stipulated by the Pharmacopoeia, can at present only demonstrate the consistency of the dermal reaction elicited by different batches of the BCG vaccines and should be comparable with reference vaccine. In order to see the trends of excessive dermal reactivity the present study has been carried out to evaluate the local tolerability of BCG vaccine in excessive dermal reactivity in guinea pigs.

2. Material and Method

2.1 BCG Vaccine Batches

A total of 35 batches of BCG vaccine from two different manufacturers were included in the present study. It includes 16 batches from manufacturer-1and 19 batches from manufacturer-2. Reference vaccine used wasalso obtained from the respective BCG manufacturers.

2.2 Animals

Guinea pigs used for testing the BCG vaccine were maintained in the Animal Facility of the Institute. All the experiments involving animals are approved by the Institutional Animal Ethics Committee (IAEC).

2.3 Method

Six healthy guinea pigs, each weighing not less than 250 gm and having received no treatment likely to interfere

with the test, were taken for testing each batch of BCG vaccine. Experiments were carried out to measure excessive dermal reactivity as per the Indian pharmacopoeia requirements. Briefly, 0.1ml each of neat vaccine, 1:10 and 1:100 dilutions were injected intradermally into six guinea pigs along with identical doses of reference vaccine at different sites of abdominal regions in same groups of guinea pigs. The guinea pigs were observed for lesion formation at the site of injection for four weeks following the injection. The diameters of lesion were measured and recorded using a digital Vernier Calliper at the site of injection of each guinea pig.

3. Results

The mean size of lesion formation in neat, 1:10 and 1:100 dilutions of 16 batches of BCG vaccine from manufacturer-1 was found to be 2.87mm, 1.54mm and 0.21mm respectively. Similarly, the mean size of lesion formation of reference vaccine in neat, 1:10 and 1:100 was 3.04mm, 1.54mm and 0.15mm respectively (Table1). The mean size of lesion formation in neat, 1:10 and 1:100 dilution of 19 batches of BCG vaccine from manufacturer-2 was found to be 2.80mm, 1.67mm and 0.0mm respectively. Similarly, the mean size of lesion formation of reference vaccine in neat, 1:10 and 1:100 was 2.83mm, 1.67mm and 0.0mm respectively (Table2).Overall trend analysis of size of lesions of 16 and 19 batches of test vaccine of manufacturer 1 and 2 are in concurrence with results of reference vaccine (Fig1, 2).Our preliminary observation suggest that there is some correlation between CVU of BCG vaccine and lesion size in millimetre (Mean CVU $5.96x10^6$ ±0.78 versus Mean EDR 2.79±0.55) however more batches of vaccine need to be tested to draw a clear correlation.

Table1: The mean and standard deviation of size of lesion formation of16 batches of BCGvaccine and reference vaccine (manufacturer-1)

Type of vaccine	Test vaccines			Reference vaccine		
Dilutions	Neat	1:10	1:100	Neat	1:10	1:100
Mean (size in mm)	2.87	1.54	0.21	3.04	1.54	0.15
SD	0.50	0.36	0.23	0.45	0.37	0.25

Figure 1: Trend analysis of size of lesion formation of individual neat, 1:10 and 1:100dilutionsof testvaccine versus reference (manufacturer-1) in excessive dermal reactivity test

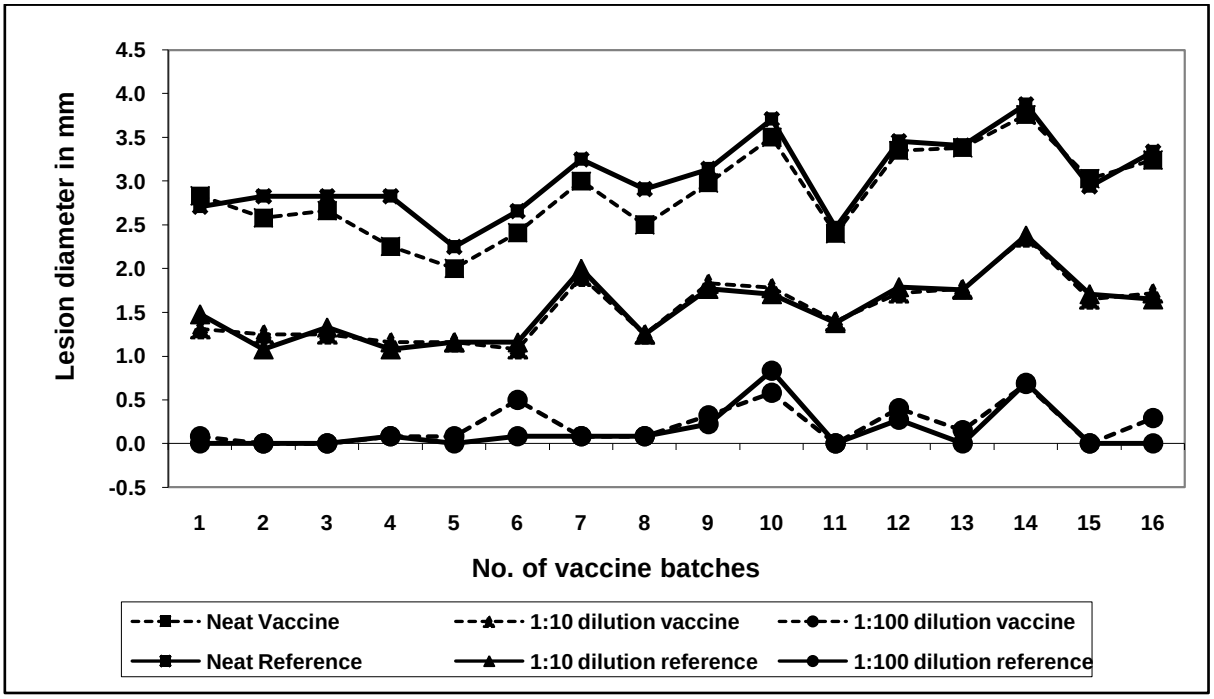
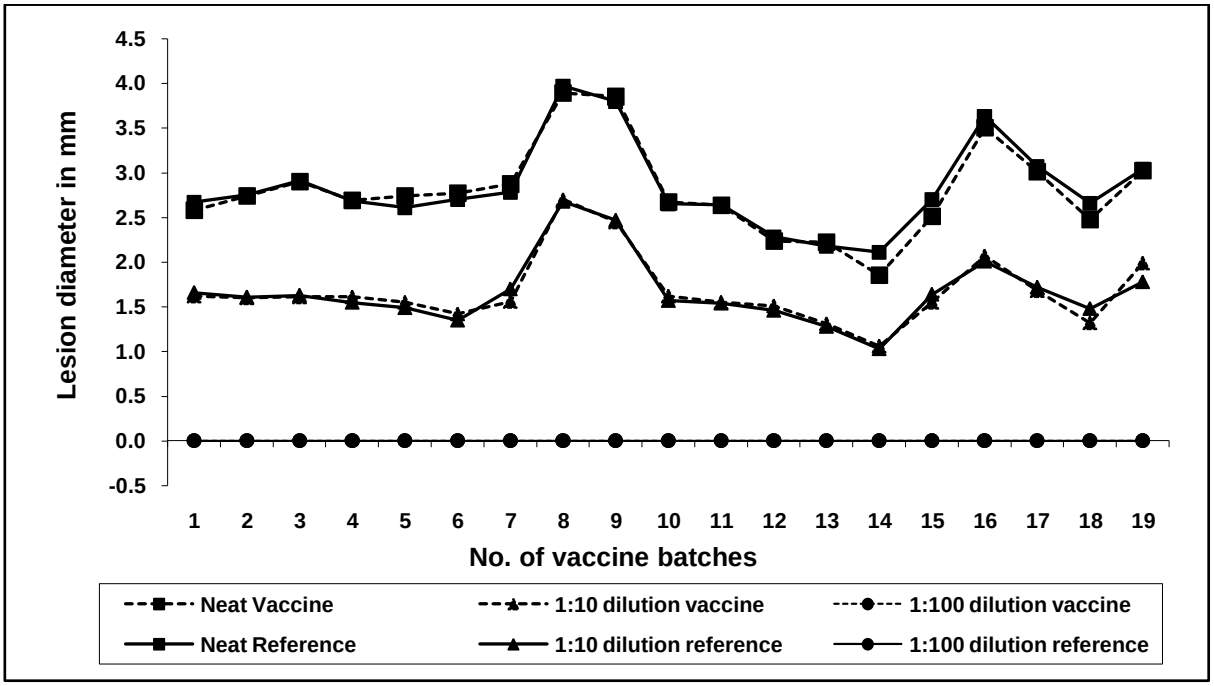


Table2: The mean and standard deviation of size of lesion formation of 19 batches of BCG vaccine and reference vaccine (manufacturer-2)

Type of vaccine	Test vaccines			Reference vaccine		
Dilutions	Neat	1:10	1:100	Neat	1:10	1:100
Mean (size in mm)	2.80	1.67	0.00	2.83	1.67	0.00
SD	0.51	0.39	0.00	0.50	0.38	0.00

Figure 2: Trend analysis of size of lesion formation of individual neat, 1:10 and 1:100 dilutions of vaccine versus reference (manufacturer-2) in excessive dermal reactivity test



4. Discussion

The residual virulence of BCG vaccine in excessive dermal reactivity test determines both its reactogenicity and immunogenicity, therefore, a good BCG vaccine entails a compromise between these two properties [5, 6]. Thus, dermal reactivity of a vaccine is a good measure not only of its reactogenicity, but also of its immunogenicity [6]. The use of the term "excessive" in the pharmacopoeia implies that the BCG monograph is more concerned with reactogenicity. However, in order to ensure satisfactory immunogenicity, the dermal reactivity of a BCG vaccine should not be too low. Therefore, it is recommended that both upper and lower limits be defined for dermal reactivity, and that the potency of the test vaccine shall be required to correspond to that of the reference vaccine. According to Lugosi [5], five factors determine the reactogenicity of a BCG vaccine, and only one of these, the so called residual virulence, is assessed in the dermal reactivity test. The other factors are production technology, the injection technique of the vaccinator, the susceptibility of the vaccine, and the viability of the vaccine.

As other reliable *in vivo* or *in vitro* tests are currently not available for studying reactogenicity[7, 8]the excessive dermal reactivity test is the only means in use ensuring consistency in immune reaction elicited by different batches of BCG vaccine.Since it is difficult to narrow down clearly the vaccine properties which result in excessive local reactions, therefore, it is difficult to define and correlate these properties which could be measured *in vitro*. In addition to the dermal reactivity test, the RPC-test (relative persistence capacity test) issued as a test for residual virulence in mice, which determines the growth of mycobacteria within the organs [5, 6]. However, it does not seem to be a suitable alternative, because it is yet another animal model with no clear relationship to BCG vaccine reactogenicity in children.

In present study, BCG vaccine batches from both the manufacturers were tested for excessive dermal reactivity and mean lesion diameters produced by these vaccines were not markedly different from that produced by reference vaccine. Thus, *in vivo* method is verified and found as per the pharmacopoeial requirements for excessive dermal reactivity test of BCG vaccine. Our preliminary observation suggest that there is some correlation between CVU of BCG vaccine and lesions size in millimeter (Mean CVU $5.96x10^6$ ±0.78 versus Mean EDR 2.79±0.55) however more batches of vaccine need to be tested to draw a clear correlation. Some variations in excessive dermal reactivity among different batches of BCG vaccine may be due to some differences in CVU of vaccine and guinea pigs used in the experiment but all the vaccine batches showed good correlation in excessive dermal reactivity test with the in-house working reference standard provided by different manufacturers. It is felt that regular efforts in forthcoming time may result in suitable/alternate *in vitro* test to the existing excessive dermal reactivity test performed in guinea pigs. Overall study suggests that BCG vaccine used in India is safe and potent.

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