AN EXPERIMENTAL STUDY OF THE EFFECT OF SIMULTANEOUS VACCINATION WITH BCG AND SMALLPOX VACCINE

Part 1. Experiments in Guinea Pigs and Rabbits

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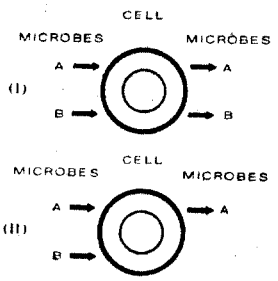
Introduction

Mass B.C.G. vaccination has been carried out in many countries for many years one of the effective measures for control of tuberculosis.[4, 5] Difficulties have often expressed by the field operators in reaching a satisfactory coverage of vaccination of eligible sector of population, especially the preschool children[1]. Therefore, if simultaneous vaccination with BCG vaccine and other vaccines, for instance, smallpox vaccine could be made, to newborn infants it would be saving staff-time and money to the heavily loaded health units, thus facilitating a more complete coverage. Lin[1] tried this experiment in humans in Taiwan, and this approach has been generally applied with a success in Taiwan since 1965.

However, even with BCG vaccination alone some untoward side-effects may be observed. Therefore, more side-effects would be expected at the vaccination region as well as systemically, if smallpox vaccination is given simul-taneously with BCG vaccination.

B.C.G. and vacciniia virus are all live microbes; therefore, if they interfere with each other inside the human bodies, the following impediments may occur in the way of immunizing process:

According to the "Interferon"[7] hypothesis, inteferon is conjugating with ribosomes the messangers of R.N.A. in the cell body to check the formation of synthesis of messangers of R.N.A. and stop the development of immunity; in other words, the interferon is considered to be a kind of polyribosomes, (glycoprotein) against the component of BCG as an antigen, i.e., polysaccharide. When BCG becomes an immuno-antigen, a complicated combination of enzyme and protein should be accomplished, but the above mentioned glyco-protein, "Interferon", would impede this combination and thus hinder the production of antibodies against tuberculoantigens. The definition of interference can be schematically illustrated as follows:



In case that a certain bacterium or virus coexists commensally with the other or without being inhibited or interfered by the other, in a cell body.

When one of the two microbes living together in a cell body passes out of it or there is the phenomenon of *rescue* or phagocytosis, it is call *interference*

In the field of simultaneous vaccination, so far no basic study has yet been attempted, although there are reports on the application of simultaneous vaccination with B.C.G. and smallpox vaccine. Further experiment will be carried out in animals with the methods such as isotope tracing and cellullar immunity.

This study was designed to answer, on basis of the findings in the animal experiments, some of the following questions:

(1) Will there be seen any adverse effects following such a procedure?

(2) How will the local reaction by one vaccination be changed by the other?

(3) Will there be any change in the multiplication of B.C.G. in the animals?

(4) Will there be any change in the acquisition of

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tuberculin sensitivity and immunity?

(5) Will there be any change in the effect of smallpox vaccination?

Materitl and Methods

Material:

Thirty tuberculin negative guinea-pigs, weighing approximately 400 gm each, were divided into five groups, each consisting of six animals. Another thirty tuberculin negative rabbits, weighing approximately 2 kg each, were also divided into five groups, each consisting of six rabbits.

Method:

Experimental desigfn

Each group of the study animals, both guinea-pigs and rabbits, were given either one of the following five vaccination regiments:

(A) B.C.G. vaccination with intradermal method, using 0.1 mg of 1 mg/ml B.C.G. vaccine.

(B) B.C.G. vaccination with multiple puncture method, using 80 mg/ml B.C.G. vaccine.

(C) Smallpox vaccination with the scratch method.

(D) Simultaneous vaccination with B.C.G. (0.1 mg, intradermal) and smallpox vaccine.

Table 1. Group of Vaccination Experiments on GuineaPigsRabbits

Group	Guinea Pigs	Rabbits	Doses	Vaccinated Site of Abdomen
A	BCG only	BCG only	0.1 mg. i.d.	Right lower area
B	BCG only	BCG only	80 mg/0.02 ml. Scarification	Richt lower area
C	Smallpox Vaccine only	Smallpox Vaccine only	10-5/0.02 ml. Scarification	Righ lower area
D	Simultaneous Vaccination	Simultaneous Vaccination	BCG 0.1 mg. i.d.	Right lower area
			Smallpox Vaccine 10-5/0.02 ml. Scarif.	Left lower area
E	Simultaneous Vaccination	Simultaneous Vaccination	BCG 80 mg/0.02 ml. Scarification	Right lower area
			Smallpox Vaccine 10-5/0.02 ml. Scarif.	Left lower area

(E) Simultaneous vaccination with BCG (80 mg, multiple puncture) and smallpox vaccine.

These animals were vaccinated according to the manner described in Table 1.

Technique of vaccination and vaccine products

BCG vaccination: 0.1 ml of 1 mg/ml and 80 mg/ml liquid BCG vaccine prepared freshly(not older than two weeks) were given to the animals at the flank region by intradermal or multiple puncture method. Two batches of BCG vaccine were used. The viability counts of these batches of vaccine were 23×10^6 and 25×10^6, respectively.

Smallpox vaccination: Liquid smallpox vaccine, Lot No. 6810, which was producted on March 1968 and kept below 5°C in the refrigerator, was used throughout the study period. The vaccination was given by the scratch method at the right of left deltoid region. After 0.02 ml of vaccine were dropped on to the skin, two linear scratches, 3 mm in length and 3 mm apart, were made with a sewing needle.

Tuberculin test: One and two TU of PPD RT 23 with Tween 80 were applied by intradermal injection. Reading was made after 72 hours and the size of reactions measured.

Necropsy of the animals: All the animals were sacrificed after 3 months. Observation was made of the internal organs of the animals in relation to the vaccinations given.

Quantitative tests: A portion of the tissues was excised at the vaccination site and ground thoroughly in a porcelain mortar. In case of BCG vaccination, the ground material was diluted with 1% sodium hydroxide and inoculated on to the slants of five tubes of Lowenstein media. At the end of 4 week incubation, the number of colonies was counted. In case of smallpox vaccination, the ground material was diluted with antibiotics and then inoculated on to the 11-day-old egg C.A.M. Counts of the number of vaccine pocks were made at the end of 5 day incubation.

Blood was taken from each animal, centrifuged and

diluted with antibiotics. 0.2 ml of this diluent each was inoculated on 5 tubes of Lowenstein media and 5 egg C.A.M., respectively. Observation was made at the end of 4 week incubation for viability counts of BCG and at the end of 5 day incubation for count of the number of vaccina pocks.

Results

Change in reactions at the vaccination sites of guinea-pigs

Table 2 (A) shows the size of the local lesions of guinea-pigs caused by each kind of vaccination. In case of BCG vaccination. the local lesion reached the maximum size after two weeks when the reaction to smallpox vaccination began to wane. In the simultaneous group in which BCG vaccination was given by intracutaneous method with 0.1 ml of 1 mg/ml BCG vaccine, i.e., Group D as shown in Table 1, each of the two types of vaccination took its own course without much difference from those of the prespective single vaccination groups. However, the reactions to both vaccination in the animals of the other simultaneous group, i.e., the Group E, in which BCG vaccination was given by multiple puncture method with 80 mg of BCG vaccine,

Table 2. Change in Reaction at Vaccination Sites on Guinea Pigs.

Groups \ Days	Period After Vaccination										
	1	2	3	4	5	6	7	8	9	10	14
B.C.G. 0.1 mg	1.9	2.2	2.2	2.4	2.8	2.8	3.0	2.0	1.5	0.4	0.2
B.C.G. 80 mg Scarify	3.8	4.5	4.5	5.3	5.4	6.2	6.5	6.5	7.0	7.0	5.0
Smallpox Vaccination	11.3	11.8	11.0	10.5	10.2	6.5	5.6	4.8	3.8	3.5	2.0
Simultaneous B.C.G. 0.1 mg	3.0	3.5	3.6	3.8	3.5	3.4	3.0	2.4	2.2	1.4	1.0
Simultaneous Smallpox Vaccination	8.4	8.5	8.6	8.0	6.5	6.0	6.0	5.5	5.2	3.8	3.0
Simultaneous B.C.G. 80 mg. Scarify	11.6	11.5	12.0	11.0	9.6	10.5	9.3	9.0	6.8	5.3	4.5
Simultaneous Smallpox Vaccination	8.3	8.4	8.8	8.6	8.5	8.0	8.2	7.2	6.0	5.8	5.0

Indicated by Average size (mm) of Vaccination Lesions.

Table 3. Change in Reaction at Vaccination Sites on Rabbits.

Groups \ Days	Period after Vaccination										
	1	2	3	4	5	6	7	8	9	10	14
B.C.G. 0.1 mg.	1.5	1.6	1.8	2.0	2.0	1.4	1.2	0.8	0.5	0	0
B.C.G. 80 mg. Scarify	3.5	4.0	4.0	4.5	5.0	4.4	4.2	4.0	3.5	2.0	0
Smallpox Vaccination	11.8	12.0	12.2	12.5	11.0	10.5	9.5	8.5	8.5	8.0	6.0
Simultaneous B.C.G. 0.1 mg.	2.4	2.8	2.8	3.2	3.2	3.5	3.0	2.0	1.8	1.6	0
Simultaneous Smallpox Vaccination	9.0	9.4	10.5	11.5	12.0	11.0	10.0	9.2	8.0	6.0	4.0
Simultaneous B.C.G. 80 mg. Scarify	3.0	3.2	3.5	3.6	3.6	3.8	4.2	3.0	3.0	2.5	2.0
Simultaneous Smallpox Vaccination	9.2	10.5	11.0	12.0	12.5	12.0	11.5	9.2	7.4	5.0	4.5

Indicated by Average size (mm) of Vaccination Lesions.

were slightly larger than the single vaccination groups although the same trend was still observable as in the Group D.

Change in reactions at the vaccination site of rabbits

The per-cutaneous vaccination of smallpox vaccine with the scratch method in rabbits gave rise to an induration surrounded by red areola after 48 hours. If the reaction was strong, it often became necrotic after 4 to 5 days. The erythema was measured every day for 14 days.

In the two simultaneous groups, i.e., Groups D and E, local reactions to smallpox vaccination were found to be of almost the same size as in the single vaccination group, i.e., Group C. However, the smallpox lesions in the rabbits were definitely larger than those in the guinea pigs, whereas the opposite was true for the BCG lesions. Table 3.

Vaccinial immunity test

All the animals (only rabbits) vaccinated with smallpox vaccine showed vaccinal lesions, no matter what group they belonged to. On revaccination with the same vaccine one month after the initial vaccination showed no reaction at all in all animals which indicates appearance of immunity (immune reaction). In the two groups of animals given only BCG vaccination originally, i.e., Groups A and B, a typical reaction was observed at the site of smallpox vaccination, which means that the smallpox vaccine used for this test was still sufficiently potent. Thus, no significant difference in the local reaction to the vaccinial test was found between the group given smallpox vaccination alone (Group C) and the simultaneous groups(Groups D and E).

Viability counts of BCG colonies and vaccinia pocks from the site of vaccination and blood

Results of quantitative culture tests carried out with the tissues from the sites of the vaccination as well as the blood are shown in Figure 2. No significant change was noted between the viability counts of BCG from the tissues between the BCG alone groups and the simultaneous Groups. No BCG colonies were found in blood culture in all groups of animals. Pocks appeared in the tissue cultures in the three groups of animals (guinea-pigs and rabbits) given smallpox vaccination, i.e., Groups C.D and E. The quantitative level of the pocks from culture of tissues was higher in the rabbits than in the guinea-pigs. Only a few Pocks were recovered from blood of animals(rebbits) in Groups C and E.

Change in body weight of guinea-pigs

The changes in the average body weight of the guinea pigs are shown in Table 4. The body weight did not increase during the first weeks of experiment, but one week after the vaccination the weight increased steadily during the period of experiment in all groups of gurinea-pigs.

Changes in body weight of rebbits

The changes in the average body weight of the rabbits of the five groups are shown in Table 5. The weight did not increase up to

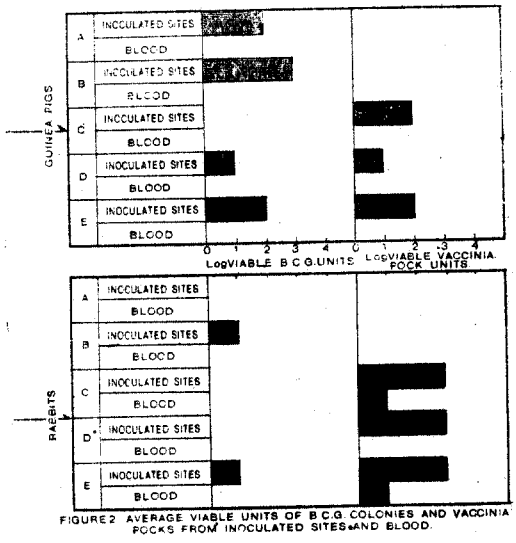

FIGURE 2 AVERAGE VIABLE UNITS OF B.C.G. COLONIES AND VACCINIA POCKS FROM INOCULATED SITES AND BLOOD.

Table 4. Change In Body Weight of Guinea Pigs

Group No. \ Weeks after Vaccination	0	1	2	3	4	6	8	10	12
A	345	342	386	388	383	392	405	415	434
B	352	360	366	382	388	395	407	418	425
C	361	364	376	392	405	418	421	442	461
D	406	415	427	435	448	454	465	472	506
E	384	382	386	406	421	448	464	483	525

Indicated by Average body Welght (gm.) of Respective Groups.

Tabal 5. Change in Body Weight of Rabbits.

Group No. \ Weeks After Vaccination	0	1	2	3	4	6	8	10	12
A	1.66	1.65	1.68	1.72	1.74	1.82	1.86	1.89	1.92
B	1.85	1.72	1.82	1.93	2.04	2.11	2.18	2.25	2.42
C	1.79	1.78	1.81	1.95	2.10	2.19	2.25	2.40	2.55
D	1.84	1.82	1.84	1.96	2.03	2.07	2.09	2.26	2.32
E	1.82	1.80	1.83	1.92	2.04	2.06	2.10	2.28	2.35

Indicated by Average body Weight (Kgm) of Respecctive Groups.

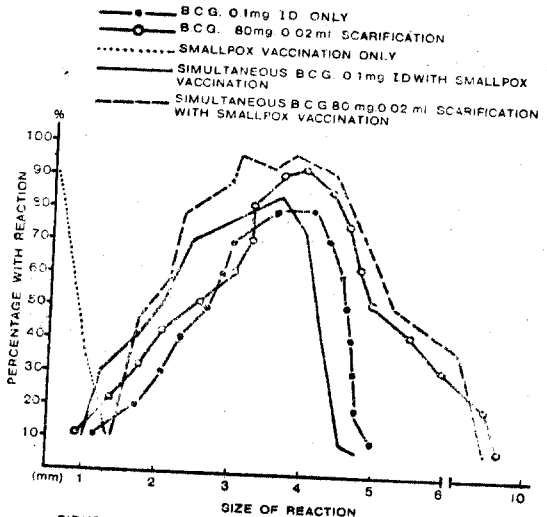

FIGURE3 COMPARISON OF TUBERCULIN REACTIONS BY SIZE AT 12 WEEKS ON GUINEA PIGS.

the end of the second week, but after three weeks the animals in all groups gained weight throughout the rest of the experiment period.

Results of comparison of the size of tuberculin reactions in guineapigs at 12 *weeks*

In the groups vaccinated with BCG alone or with BCG and smallpox vaccine simultaneously, the tuberculin reaction increased in size rapidly from the third week to the fifth week after BCG vaccination. Then the size of reaction decereased slowly up to the 12th week. There was no significanti dfference in the size of tuberculin reactions at 12 weeks between the four groups vaccinated eith with BCG alone or simultaoeously with smallpox vaccine (Groups A,B,D and E), as shown in Figure 3.

Discussion

There is no basic information available as to the simultaneous vaccination with BCG and smallpox vaccine except a few reports concerning vaccination on humans in Korea.

In order to carry out animal experiments on this subject, the authors used guinea-pigs for the immunity test of BCG vaccination and smallpox vaccination, and rabbits for the test of acquisition of BCG and vaccinia.

Consequently, between the four groups vaccinated with BCG, i.e., the two BCG alone groups and the two simultaneous vaccination groups, no signifcant difference was observed either in the strength of the local reaction, the multipication of BCG and vaccinia in the animal bodies, the intensity and duration of sensitivity to tuberculin allergy.

The local reactions in the simultaneous vaccination groups appeared in the same manner as reactions to each kind of vaccination, and no particular intensification of the reactions in the simultaneous groups was observed. Since the rabbits are less susceptible to BCG vaccination than the guinea-pigs, the results of BCG Vaccinotion tuberculin allergy with rabbits were rather unstable as compared with those in guinea-pigs.

In order to observe to observe the vaccinal immunity, rabbits were vaccinated with the same smallpox vaccine one month after the initial vaccination. No significant difference in the immune reactions was found between the group given smallpox vaccine alone and the gronps given smallpox and BCG vaccination simultareously.

Results of the count of the viable units units of BCG and pock units of vaccinia showed normal quantitative trend in this experiment.

The above findings suggest that the simultaneous vaccinations with BCG vaccine and smallpox vacine exert no unfavourable effect on the occurrence of complication and the acquisition of immunity. Since the present experiments used animals which do not necessarily have the same susceptivility to these vaccines as the humans, further studies on human bodies, probably by use isotope tracing and cellular immunity tests, are required.

Summary

1. When BCG vaccine nod smallpox vaccine are va-

ccinated in guinea-pigs and rabbits no particular difference was observed in the body weight, the local reaction and the development of tuberculin allergy.

2. At necropsy of the vaccinated animals, no significant difference was observed in the degree of tuberculous involvement between the simultaneous groups and BCG alone groups.

3. As a result of viability counts of BCG and vaccinia pocks, no normal trend of quantitative culture on the Lowenstien's media for BCG and egg C.A.M. for vaccinia of both the tissue from the vaccination sites and the blood, However, did not shown a significant difference between the simultaneous and the single vaccination groups.

4. In conclusion, under the conditions of the present experiments in animals, the simultaneous vaccination with BCG and smallpox vaccine seems to be as safe and effective as the single vaccination as far as each vaccination is concerned.

Acknowledgements

This study was supported by the Korean National Tuberculosis Association. The authors are grateful to Dr. J.C. Tao, Regional Tuberculosis Adviser of the Western Pacific Regional Office of the World Health Organization, Manila, for his technical advice and constant encouragement. The authors wish to express their appreciation to Dr. H.T. Lin, WHO Tuberculosis Adviser in Korea, for his advice in the design of the study. The authors are also obliged to Dr. D.B. Kim, Mr. H. O. Shin and the other staff of the BCG Laboratory Seoul, Korea, for their untiring efforts in the conduct of this study.

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—國文抄錄—

B.C.G. 接種과 牛痘同時接種時에 일어나는 相關關係에 對하여

第1報 動物實驗에서 일어나는 相關關係

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B.C.G.와 牛痘(vaccinia)의 同時接種問題는 結核의 集團豫防接種事業을 普及하는데 接種要員의 人力과 時間節約에 큰 도움을 주고 있으나 免疫學的으로 問題되는 點은

첫째 B.C.G.만을 接種하여도 그 副作用이 些少는 있는데[3] 牛痘하고 같이 接種할 때 牛痘의 善感에 따르는 副作用과 겹쳐서 接種部位와 全身에 副作用이 相乘될 念慮가 없지 않다.

둘째 B.C.G.와 牛痘는 모두 살아있는 生菌 백신임으로 人體內에서 干涉作用(Interference)이 일어나서 奮還現象(Rescue)을 받게되면 免疫過程에서 防害를 받게 될 수도 있다. 即 干涉物質(Interferon)은 細胞를 자극하여 Ribosome을 產生한다. 이 Ribosomes은 宿主細胞의 Massanger R.N.A.의 感染 Virus의 變化들을 抑制하여 Polyribosomes을 形成하지 않게 되면 Virus의 Synthesis에서 生成하는 Protein이 없게됨으로 Enzymes과 Protein의 作用이 이루어지지 않음으로 Virus의 繁殖이 되지 않을 것이므로 免疫機轉에서 防害를 받을 수 있는 理論은 B.C.G. 菌이 Polysaccharide라고 볼 때 干涉作用 物質은 Polyribosomes(Glyco-protein)으로 보면 抗原이 免疫抗體가 되기까지는 Antigenicity의 Phagocytosis을 일으키는데 Enzyme과 Protein의 複雜한 結合이 必要하며 Polyribosomes이 이 結合을 防害함으로써 Virus나 菌의 增殖이 되지 않음으로 免疫抗體產生이 어려워질 수도 있을 것이다.

셋째 牛痘와 B.C.G.를 同時接種할 때 個個의 免疫效果를 얻을 수 있는지의 與否 等에 關한 實驗을 動物에서 實施하여 아래와 같은 結果를 얻었다.

1. B.C.G.와 牛痘(Vaccinia)를 Guina-pig과 家兎에 同時接種 할 때에 個個의 單獨接種群에 比하여 局所反應, 反應經過 Tuberculin Allergy反應 및 實驗動物의 體重變動이 없었다.
2. 實驗動物剖檢所見에서도 同時接種과 個個接種群의 差를 確認할 수 없었다.
3. 接種한 B.C.G.와 牛痘의 再分離試驗의 定量培養成績은 一定한 推移를 判定하기 어려웠다.
4. 動物實驗의 結果로서는 B.C.G.와 牛痘를 同時接種하였을 때 免疫에 影響을 미치지 않음은 勿論 여러 가지 反應成績이 滿足스러웠고 安全한 것을 確認할 수 있었음을 報告한다.

※ 本論文의 內容은 1969年 第28次 大韓結核學會에 報告되었음