Lapin Mucosal Versus Systemic Humoral and Cellular Immune Responses to Oral *Helicobacter Pylori* Bacterin Administration

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Abstract

The gut compartment, the common mucosal immune system was attempted as a model for providing the immunological opinion that mucosal immunization induces mucosal as well as systemic immune responses specific to the stimulating antigen.

الحث المناعي الموضعي ومقارنته بالاستجابة المناعية الجهازية الخلطية والخلوية بعد التمنيع عبر الفم لبكترين *H.pylori* المقتول بالحرارة

الخلاصة

جرى استعمال حيز القناة الهضمية من الجهاز المناعي المخاطي لتحضير موديل تجريبي للتحقق من صحة الفرضية المناعية القائلة بان تمنيع السطوح المخاطية يحث استجابة مخاطية و جهازية مناعية متخصصة للمستضد الحاث . إذ تهدف هذه الدراسة إلى إجراء مقارنة بين طبيعة الاستجابة المناعية الموضعية و الجهازية الخلطية والخلوية في الأرانب الممنعة فمويا" ببكترين (HpHk) في فترات زمنية مختلفة.

Introduction

Nowadays, *Helicobacter pylori* is considered to be the most common human bacteria in the pathogenesis of chronic gastritis and peptic ulcer diseases [1-2]. It cause mucosal as well as systemic humoral and cellular immune responses , till now little references regarding the immune status of this bacteria and the time of development of these responses [2-3].

Objectives

The comparison study between mucosal and systemic humoral and cellular immune responses in rabbits after oral administration of heat killed *H.pylori* (HpHK) bacterin at different days.

Material and Methods

Antigens:

Heat killed *H.pylori* (HPHK) bacterin was prepared from 24 hour brain heart infusion agar plate culture then we added 6 ml of normal saline to the plate, collected the solution and put it in centrifuge at 4000 rm\ mint for 5 mints, double washed were done with normal saline then compared it with standard opacimeter (WHO) to obtain the concentration equals to 10 IU\ml . The solution put in water path at 60 degrees for 30 mints to killed the bacteria and obtained the antigen that was used in immunization the rabbits after done the sterility test . A cell free culture filtrate antigen was also prepared from microaerophilic 72 hour

brain heart infusion broth culture of *H.pylori* [4-5].

Animals:

Two groups, each of two rabbits *O. canniculus* were elected , adapted to laboratory conditions and housed under Ad libitum standardized conditions , one served as test and other as control group [6].

Immunization protocol :

Four successive doses of (HpHK) bacterin were administered via oral route into tested rabbits through four weeks , each dose about 2 ml of bacterin that had 10 IU\ml concentration . Control animals received sterile normal saline in same protocol and this protocol was specific for this research..

Mucosal samples and immunoglobulines separation :

Gut mucosal samples were obtained from four parts of gut mucosa included esophageous , stomach , duodenum and spleen which is used as lymphoid organ . Then the immunoglobulines were separated from these parts according to [7].

Blood Samples Blood with anticoagulant was processed for e-rosette test and for migration inhibition test. Coagulated blood were collected for the others immunological tests that include: tube agglutination test, measure the concentrations of IgG and IgM ; C3 and C4 and IL-4 and IL-8 [8].

Immunology:

Agglutination in microtiteration method, anti *H.pylori* IgG, IgM antibodies were be detected by using specialized kits were provided from the (DRG, USA) company , Measured the concentration of C_3 and C_4 the complement compartments , separation of lymphocytes by using e-rosette test to collected the number of T-cells that formed the rosette form, capillary migration inhibition test, IL-4 and IL-8 cytokines were be detected by using specialized kits were provided from (RayBio, USA) company as well as skin delayed type hypersensitivity were done respectively. In addition to the histopathological study which down in each of the gut mucosal parts [2,7,8].

Results and Discussion

We studied the development of immune responses at five days (8 , 12 , 16 , 20 and 27) after immunization with HPHK bacterin via oral route that stimulates *H.pylori* specific humoral systemic and mucosal immunoglobulins titers (Table 1) , also it played an important role in the increased the concentrations of IgG and IgM antibodies in serum and mucosal secretions of immunized rabbits(Table 2). The concentration of C_3 and C_4 which are the complement compartments were significantly increased in the rabbits sera (Table 3) [9-10].

The cellular systemic and mucosal immune responses were represented by significant increased in the lymphoid cells that formed the rosette form, significant migration inhibition index with more than 30% inhibition as well (Table 4), furthermore, the HPHK bacterin able to inducing tuberculin type delayed hypersensitivity, Hence, their epitopes can be of T dependent type through the activation of Th1 and Th2 (Table 5) [11-13].

IL-4 is one of anti-inflammatory cytokines and IL-8 is a proinflammatory cytokine were be detected in serum and mucosal secretions of rabbits with significant increased in there concentrations (Table 6) . Thus *H.pylori* antigens were B and T cells dependent types [14].

The histopathological study was showed the spleenic reactive hyperplasia in red pulp in the days 8 and 12 respectively (Picture 1),

dearrangement of epithelial cells that lining the mucosal layer of esophageous (Picture 2), increased the surface area of the pits of mucosal layer of rabbit 's stomach, also we seen the infiltration of neutophiles to the lamina propria of the stomach (Picture 3), while the duodenum had been normal in all treated rabbits.

Table 1 Titers of anti *H.pylori* antibodies

Titer						
Types of immune response	Days					Control
	8	12	16	20	27	
Humeral systemic(serum)	40	320	640	1280	2560	10
Cellular mucosal						
Esophageous	16	32	64	128	256	1
Stomach	32	32	128	256	512	
Duodenum	32	32	128	128	256	

Table 2 Concentrations of IgG and IgM antibodies

Types of immune response	IgG concentration(Iu\ml) M±S.D.					IgM concentration(Iu\ml) M±S.D.				
	Days					Days				
	8	12	16	20	27	8	12	16	20	27
Humeral systemic(serum)	4.066 ± 0.055	4.346 ± 0.002	4.859 ± 0.090	4.957 ± 0.142	4.964 ± 0.053	3.573 ± 0.006	3.612 ± 0.018	4.131 ± 0.093	4.432 ± 0.048	4.131 ± 0.001
Cellular mucosal										
Esophageous	3.931 ± 0.012	4.136 ± 0.094	4.140 ± 0.169	4.340 ± 0.004	4.341 ± 0.004	3.177 ± 0.000	3.496 ± 0.008	3.817 ± 0.134	3.817 ± 0.008	3.655 ± 0.074
Stomach	4.041 ± 0.178	4.156 ± 0.080	5.874 ± 0.007	6.037 ± 0.008	9.850 ± 0.001	3.491 ± 0.000	3.974 ± 0.007	4.551 ± 0.014	4.755 ± 0.003	4.550 ± 0.002
Duodenum	3.178 ± 0.014	4.134 ± 0.173	4.334 ± 0.154	4.341 ± 0.315	4.437 ± 0.271	3.178 ± 0.009	3.338 ± 0.007	3.813 ± 0.060	3.817 ± 0.029	3.973 ± 0.069
Control	4.076 ± 0.036					3.638 ± 0.001				

Table 3 Concentrations of C_3 and C_4 in the rabbits sera

Days	C_3 concentration (mg\dc) M±S.D.	C_4 concentration (mg\dc) M±S.D.
8	104.450±2.757	47.950±1.060
12	227.300±3.818	65.700±3.676
16	232.800±3.959	84.850±2.474
20	249.650±4.030	89.700±1.414
27	291.050±4.313	94.700±2.828
Control	110.500±0.000	37.200±0.000

Table 4 Percentage of E-rosette and LIF

Types of immune response	Percentage of E-rosette(%) M±S.D.					Percentage of LIF(%) M±S.D.				
	Days					Days				
	8	12	16	20	27	8	12	16	20	27
Humeral systemic(serum)	28.400 ± 0.707	30.550 ± 0.494	32.450 ± 0.636	33.600 ± 0.141	35.700 ± 0.141	90.850 ± 0.494	89.000 ± 1.626	82.950 ± 0.989	73.300 ± 1.484	61.500 ± 0.919
Cellular mucosal										
Esophageous	29.350 ± 1.060	33.200 ± 0.424	37.300 ± 0.282	38.150 ± 0.494	38.150 ± 0.070	78.250 ± 0.212	72.250 ± 0.636	69.500 ± 0.565	63.050 ± 0.494	60.700 ± 0.565
Stomach	30.550 ± 1.272	37.450 ± 0.494	40.000 ± 0.141	42.250 ± 0.353	45.550 ± 0.494	75.000 ± 0.141	63.200 ± 2.616	62.400 ± 0.141	55.750 ± 0.494	46.400 ± 1.555
Duodenum	30.850 ± 0.212	35.100 ± 0.282	39.750 ± 0.070	41.500 ± 0.282	42.600 ± 0.282	76.900 ± 1.131	66.500 ± 0.707	63.100 ± 1.414	59.300 ± 1.414	55.800 ± 0.989
Control	27.500 ± 0.427					93.500 ± 0.427				

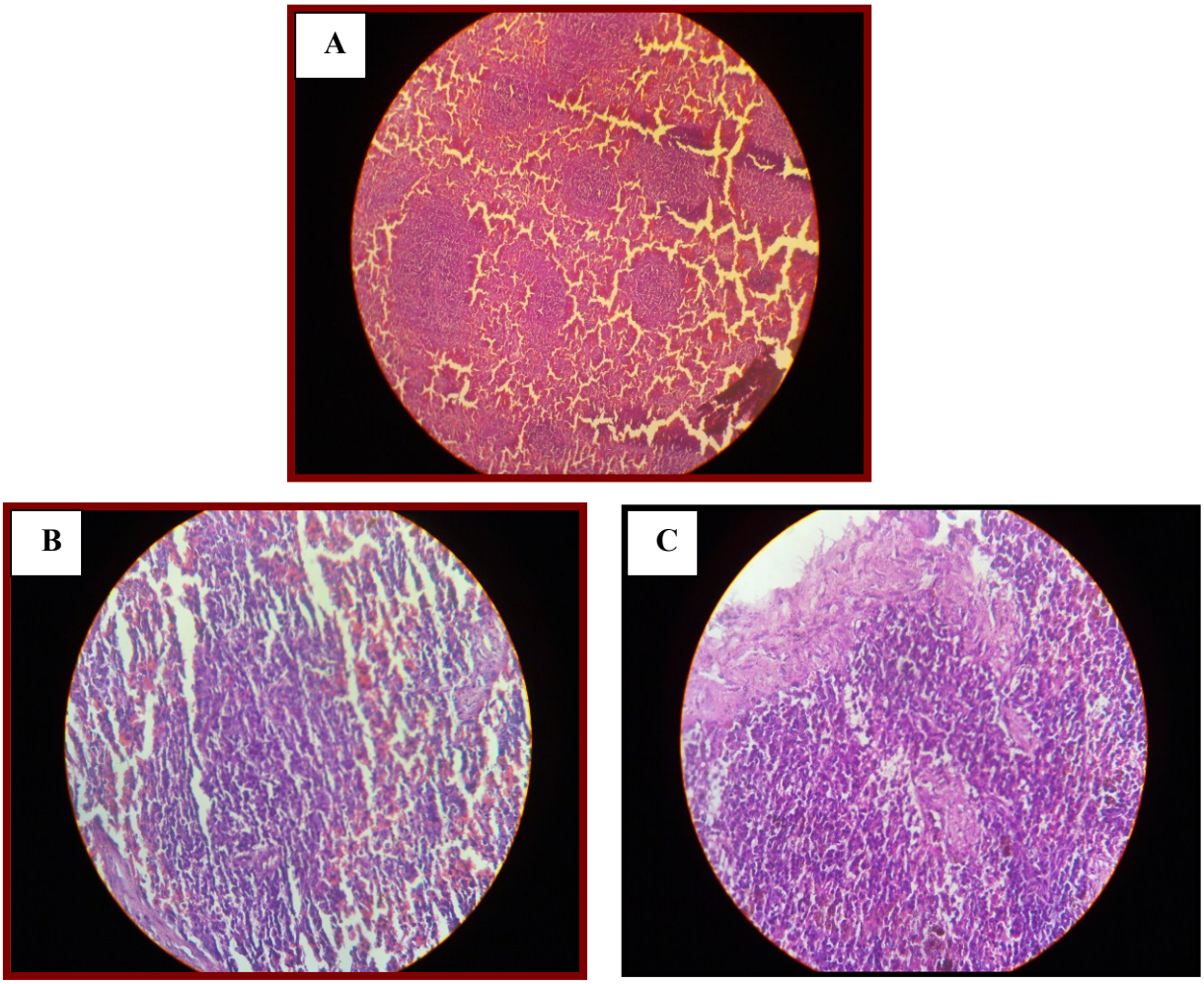
Table 5 Skin test

Date of inoculation of CFC \ Days	Skin test \ Hures					Immune reaction
	6	18	٢٤	48	72	
6	-	E	EI	EIN	EIN	Notes
	-	-	15	15	15	Reaction area (mm)
10	-	E	E	EIN	EIN	Notes
	-	-	-	16.5	16.5	Reaction area (mm)
14	-	E	E	EI	EI	Notes
	-	-	-	12	12	Reaction area (mm)
18	-	E	E	E	EI	Notes
	-	-	-	-	10	Reaction area (mm)
25	-	E	E	-	-	Notes
	-	-	-	-	-	Reaction area (mm)
Control	-	-	-	-	-	Notes
	-	-	-	-	-	Reaction area (mm)

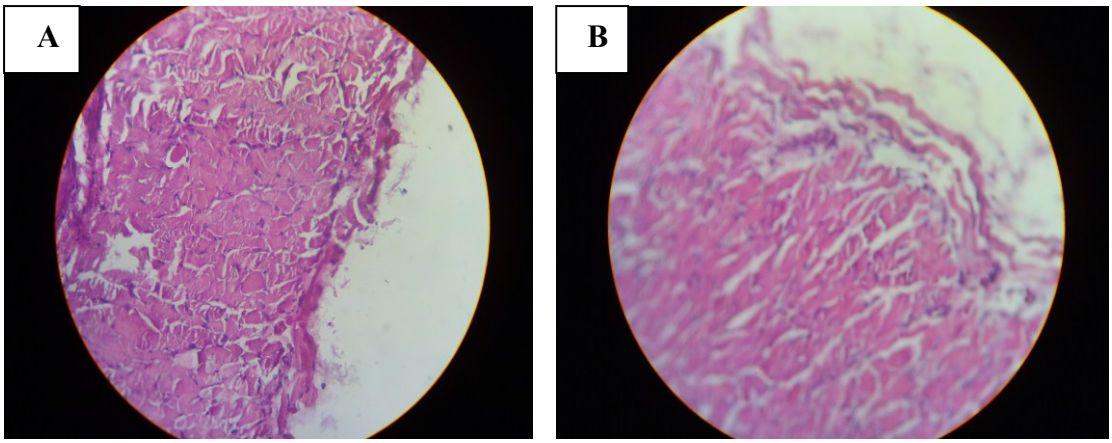
E- Erythema
I- Induration
N- Necrosis

Table 6 Concentrations of IL-4 and IL-8

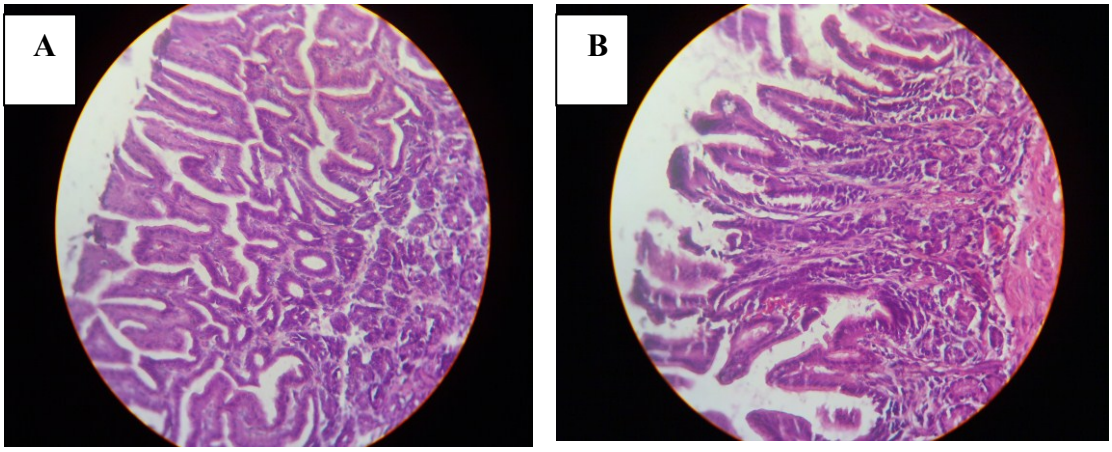
Types of immune response	IL-4 concentration(pg\ml) M±S.D.					IL-8 concentration(pg\ml) M±S.D.				
	Days					Days				
	8	12	16	20	27	8	12	16	20	27
Humeral systemic(serum)	10.144 ± 0.086	28.488 ± 0.270	155.918 ± 0.292	155.964 ± 1.231	167.544 ± 0.782	18.916 ± 0.975	19.767 ± 0.609	20.230 ± 0.148	21.376 ± 0.875	21.440 ± 0.579
Cellularmucosal										
Esophageous	6.772 ± 0.115	7.092 ± 0.015	8.200 ± 0.138	9.844 ± 0.634	6.772 ± 0.041	16.620 ± 0.330	19.896 ± 0.135	24.876 ± 0.452	26.568 ± 0.329	26.580 ± 0.350
Stomach	5.316 ± 0.169	8.700 ± 0.079	13.520 ± 0.100	15.936 ± 0.084	7.272 ± 0.212	19.920 ± 0.100	19.956 ± 0.059	29.628 ± 0.258	43.116 ± 0.161	49.752 ± 1.069
Duodenum	5.320 ± 0.029	8.696 ± 0.022	12.080 ± 0.100	12.084 ± 0.043	7.004 ± 0.137	18.264 ± 0.074	20.352 ± 0.178	23.232 ± 0.458	31.500 ± 0.701	49.752 ± 0.275
Control	7.004±0.012					19.814±0.050				



Picture 1 Section in spleen **A-** control group , **B-** and C- test group



Picture 2 Section in Esophageous **A-** Test group, **B-** Control group



Picture 3 Section in Stomach A- Test group, B- Control group

Conclusions

According to the results of this study we can conclude that serum and mucosal anti *H.pylori* antibodies, elevated serum C_3 and C_4 levels , lymphocytes and their secretion of cytokines were correlated to the status of *H.pylori* immunity and protection against recurrent infections.

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