Egyptian Journal of Surgery Vol. (19), No. (4), Oct., 2000

EFFECT OF TOTAL AND PARTIAL SPLENECTOMY ON BACTERIAL CLEARANCE IN PRAZIQUANTEL TREATED AND UNTREATED SCHISTOSOMAL MICE

By

N.E. Mikilail[1], E. El Sherbini[2], H. El Mohammadi[3], S. Botros[4] And I. El Attar[5]

Departments of Surgery[1], Bacteriology[2], Biochemistry[3] and Pharmacology[4] Theodor Bilharz Research Institute. Division of Statistics and Epidemiology National Cancer Institute[5]

Splenectomy for trauma or haematological diseases, affects the bacterial clearance as well as the immunological status of the patient. Over-whelming postsplenectomy sepsis syndrome carries 50-80% mortality. The ability of the spleen, liver and kidney to filter inoculated Staphylococcs aureus and the serum staphylococcal anti-DNase, was investigated in totally and partially splenectomized Schistosoma mansoni infected mices (n=120). Group Gl (n=60) was treated with the antischistosomal praziquantel six weeks post infection, while G2 (n=60) was left untreated control. Two weeks later, total (G1T and G2T), 75% partial (G1P and G2P) or sham splenectomy (G1C and G2C) were respectively performed. After four weeks, all animals were inoculated with ATCC 25923 Staphylococc aureus (3 x 107 C.F.U/ml saline/mouse). Twenty-four hours later, the liver, spleen, kidney, and serum were collected for bacterial counting and serum staphylococcal anti-Dnase testing. The Student's t, Wilcoxon two-sample and ANOVA tests were used for the statistical analysis. A significantly higher bacterial counts in the splenic remnant of G1P vs. G1C, and in the liver and kidney of G1T vs. G1C was found. Serum Staphylococcal anti-DNase level was more evident in G1 at almost the same mean log value of 0.6 and 0.5 but at different percentages of positivety (G1T=63 .6%, GlP=42.8%, G1C=29.4%). In conclusion, in the treated Schistosoma infected mice, the spleen and even a part of it, is an efficient bacterial filter and partial splenectomy may be preferred than total splenectomy. Praziquantel treatment favored the host's power to filter bacteria and to produce antibody.

Key Words: splenectomy, post splenectomy syndrome, bacterial inoculation, schistosomiasis.

INTRODUCTION

Schistosomal hepatic fibrosis with portal hypertension and splenomegaly is a major health problem in the third world. Splenectomy is often indicated in these patients and usually combined with porto-systemic disconnection. Splenectomy for trauma or haematological diseases, affects the bacterial clearance as well as the immunological status of the patient [1,2,3].

Postsplenectomy sepsis syndrome occurs in children and adults [4,5,6] and Overwhelming Postsplectomy Infection (OPSI) carries a 50-80% mortality usually occurring within the first two years [7,8].

AIM OF THE WORK

To test the effect of total and partial splenectomy on bacterial filtration and the humoral immune response in Schistosoma mansoni infected mice.

MATERIALS AND METHODS

Animals and groups: 120 Swiss albino mice (CD 1) 18-20g were infected with Schistosoma mansoni cercariae (80 cercariae /mouse) by subcutaneous injection. They were maintained under normal laboratory conditions and fed standard food and water. Six weeks post infection, the first group of mices (G1, n=70) received praziquantel orally in 2 % Cremophor EL in a dose of 500 mg/Kg body weight for two consecutive days. The second group (G2, n=50) was untreated control.

Surgical procedure: Two weeks later, under ether anaesthesia, a small left subcostal incision was done aseptically. The liver showed schistosomal fibrosis and, with the spleen, was enlarged in all animals. The spleen was delivered and the splenic vessels were isolated into upper and lower polar pedicles. The animals in G1T and G2T, G1P and G2P, and G1C and G2C underwent total, 75 %, and sham splenectomy respectively. Exploration for any accessory spleen was done and the abdominal wall closed with 3/0 prolene suture. The animals were allowed to recover in their cages.

Bacterial strain: Varian S 634 of coagulase positive Staphylococcus aureus ATCC 25923 which gave a reading of 1.4 at 600 n.w spectrophotometricaly was used. An inoculum of 3 x 10^7 C.F.U/ml saline was given orally to all animals two weeks postoperatively.

The number of animals studied ranged from 17-22 in the treated and 7-12 in the untreated subgroups according to the number of survivors. They were sacrificed 24 hours after bacterial inoculation by decapitation. The liver, spleen, left kidney, and blood were collected and the serum was kept at -70°C until anti-DNase enzyme was assayed.

Nutrient count agar for deep agar colony count, blood agar for isolation of different types of organisms, and toluidine blue agar (TDA) [9] for determination of anti--DNase level were used. Each harvested organ was weighed separately and the volume completed with sterile saline up to 10 ml. The organ was homogenized with an electric homogenizer. Two samples of 0.l ml each of the mixture were cultured on blood and deep agar count respectively. Serum anti-DNase enzyme level was estimated as described by Hoie and Gudding [10]. The titres of the anti-DNase in the sera was defined as log of dilution of serum that prevented any change of the TDA DNase agar colour from blue to pink.

Statistical Methods: Student's t-test, Wilcoxon two-sample test (for small sample size) and ANOVA (F-test or Kruskal-Wallis test) [12] were used. *p* value of< 0.05 was considered significant.

RESULTS

In total absence of the spleen (GIT), the liver (Table 1) and the kidney (Table 2) were the main filtering organs. The bacterial count in the liver and kidney in GlT were significantly higher than in GlC (P 0.026) and G1P and GlC (P =0.01) respectively. Furthermore, both G1T and G1P had significantly higher count in the kidney than G2T and G2P (P=0.039 and 0.062 respectively).

After partial splenectomy (G1P), the bacterial count per gram of splenic tissue was significantly higher than in GIC (P= 0.026) (Table 3). The liver tissue showed a higher bacterial count, not statistically significant compared to G1C, but significantly higher compared to G2P (P= 0.016) (Table 1). The same was observed in the kidney tissue (Table 2). Similarly, the count in G1P was higher than in GIC and significantly higher than G2P (P = 0.062).

The untreated schistosomal mice had a poor survival and a very low bacterial count in the spleen, liver, and kidney. No significant difference was found when these counts were compared in all subgroups (p >0.05).

The mean log value and the percentage of positivity of the anti DNase enzyme in both groups are shown in (Table 4) and (Fig 1). G2 did not show any response, except for G2T at a low titre.

Table (1): ***Mean (± S.D) of bacterial counts in the liver tissue of all groups.***

Groups	*Total Splenectomy*	*Parital Splenectomy*	*Control*	*P value*
G1	**G1T**	**G1P**	**G1C**	
Number of mice	22	21	17	
Mean	61663	12533	3418	
S.D.	168310	15790	3880	
ANOVA	A	AB	B	0.026
G2	**G2T**	**G2P**	**G2C**	
Number of mice	7	7	12	
Mean	7629	1629	2808	
S.D.	11699	3695	5715	
ANOVA	A	A	A	>0.05

Note: G1T and G1C were significantly different (P = 0.026) G1P had significantly higher reading thean G2P (p = 0.016).

Table (2): ***Mean (±S.D) of bacterial counts in the kidney tissue of all groups.***

Groups	*Total Splenectomy*	*Partial Splenectomy*	*Control*	*P value*
G1	**G1T**	**G1P**	**G1C**	
Number of mice	20	21	17	
Mean	40800	1326	4970	
S.D.	54566	26798	16339	
ANOVA	A	B	B	0.01
G2	**G2T**	**G2P**	**G2C**	
Number of mice	7	7	12	
Mean	7543	164	5000	
S.D.	14740	200	6077	
ANOVA	A	A	A	>0.05

Note: G1T was significantly different than G1P and GlC (P **=**0.01), GlT and GIP had significantly higher reading than G2T and G2P (P = 0.039 and 0.062, respectively).

Table (3*): Mean (±S.D) of bacterial counts in splenic tissue in partially and unsplenectomized mices.*

Groups	*Partial Splenectomy*	*Control*	*P value*
G1	**G1P**	**G1C**	
Number o mice	21	17	
Mean	15540	4912	
S.D	19935	9908	
ANOVA	A	B	0.026
G2	**G2P**	**G2C**	
Number of mice	7	12	
Mean	10343	1633	
S.D.	12013	2059	
ANOVA	A	A	>0.05

Note: G1P and G1C were significantly different (P=0.026). No significant difference was found between treated and untreated mice.

Table (4): ***The mean log value and percentage of positivity of serum anti-DNase ezyme in all groups.***

Groups	*Number of mice*	*+ve anti-Dnase*	*Mean log value*	*%*
G1T	22	14	0.5	63.6
G1P	21	9	0.5	42.8
G1C	17	5	0.6	29.4
G2T	7	3	0.3	42.9
G2P	7	-	-	-
G2C	12	-	-	-

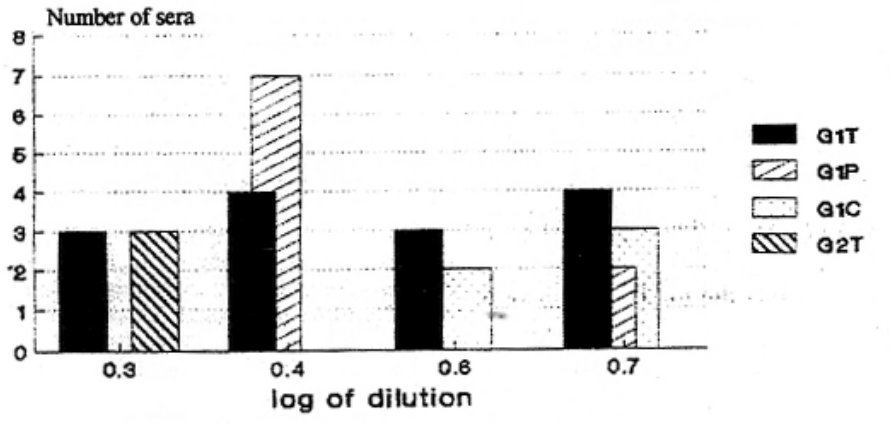

Fig (1): ***Log Value of anti Dnase Enzyme in G1 and G2T***

DISCUSSION

Postsplenectomy sepsis has been observed in children and adults [4,13]. Pneumococcus and Streptococcus pneummoniae, gram-negative bacteria, Staphylococcus aureus, some viruses, and fungi are among the agents involved in such infection [6,14,15]. Previous experimental studies have used an inoculus of Streptococcus pneummoniae [16] or E.coli [1].

In this work, we used a strain of Staphylococcus aureus capable of causing infection, susceptible to filtration by the host tissues and could induce an immunological response. The acquired immunoglobulins could be traced by a specific anti-DNase enzyme [17]. The oral route was chosen because it is a natural way of infection while the intravenous or intraperitoneal routes have a quicker onset of bacteraemia, which is atypical to occur in nature, and gives no time for an immunological reaction's. [18,19]. The spleen, liver, and kidney are normal bacterial filters [20]. But the spleen has a greater capacity per unit weight for the uptake of organisms and filters a relatively greater amount of bacteria than the liver and kidney[21]. It was also found that the absence of the spleen impedes bacterial filtration, phagocytosis as well as antibody synthesis [22,23]. The splenic bacterial filtration capacity resides mainly on its peculiar anatomical site, blood flow and parenchymal architecture rather than its reticulo-endothelial cells function [23,24,25]. The spleen as a filter has a greater importance in rapidly evolving sepsis while its immunological function is inefficient in this situation [18].

In G1, the quarter of a spleen (G1P) filtered more than the intact spleen per gram unit of tissue (Table 3). This means that the schistosomal spleen is an efficient bacterial filter and can increase its capacity for filtration. Nevertheless, the liver and kidney tissues of G1P had a more active role as bacterial filters. Malangoni and colleagues [26] reported that the hepatic reticuloendothelial system may show a compensatory change after splenectomy and the hepatic uptake of colloid sulphur was inversely proportional to the splenic weight. This seemed to be also true for the schistosomal spleen since in G1T, the liver showed a significantly higher filtering capacity. The kidney also augmented its bacterial filtering function in this sub-group. Although a different animal model has been used in this study, the results are different from those of Capitan Morales and colleagues [1] who found the liver; spleen, and kidney acting alike as bacterial filters.

In absence of the splenic filter, although compensated for by other tissue filters, still there was a sustained bacteraemia [14] that enhanced development of an immunological response [2] and possibly total leucocytic function [16]. The increased plasma fibronectin in presence of bacteraemia, after total or partial splenectomy, [26,27,28] can explain the rise in the percentage of positivity of anti-DNase observed in G1T, G1P, and G2T although the latter had a low titre.

Untreated Schistosomal mansoni mice (G2) in all subgroups had no special bacterial filtering capacity, and a poor survival and immune response (Table 1,2,3 and 4). This study acknowledges the importance of the schistosomal spleen as a bacterial filter. The liver and kidneys could compensate its absence with stimulation of specific antibody reaction in Staphylococcus aureus induced sepsis. To

conserve the schistosomal spleen in clinical practice by splenorrhaphy or segmental splenectomy [30] seems to be advantageous. Maintaining part of the spleen in its anatomical site, with its vascular system, is superior to autotranspiantation of splenic tissue, which can only preserve its immunological capacity [18,31].

Treatment of the Schistosoma mansoni infestation in mice improves the filtering function of the spleen, liver and kidney and the antibody production when challenged with Staphylococcus aureus.

REFERENCES

1. Capitan Morales, L.C., Garcia Iglesias, M.C., Ortega Bevia, J.M. et al. (1990) Liver, Spleen and Kidney cultures in different conditions of Splenic preservation. Int. Surg: 75: 215-219.

2. Sawmiller, C.J.; Dudrick, S.J.; Hamzi, M. (1998) Postsplenectomy Capnocytophaga canimorsus sepsis presenting as an acute abdomen. Arch. Surg; 133(12): 1362-1365.

3. Benoist; S. (2000) Median and long-term complications of splenectomy. Ann Chir; 125(4):317-324.

4. Idowu; O., Hayes; Jordan. A. (1998) Partial splenectomy in children under 4 years of age with hemoglobinopathy. J. Pediatr. Surg. 33(8): 1251-1253.

5. Remkova'; A. (1998) Perioperative care by internists in splenectomy. Bratisl Lek. Listy. 99(6):291-295.

6. Zarabi, M.H. and Rosner, T. (1984) Serious infections in adults following splenectomy for trauma. Arch. Intern. Med.; 144:1421-1424.

7. Green, GB., Shackford, S.R., Sise, U.J. et al. (1986) Late septic complications in adult following splenectomy for trauma: A prospective analysis in 144 patients. J.Trauma; 26: 999-1004.

8. Feliciano, D.V., Bitondo, C.G., Mattox, K.L., et al. (1985) A four year experience of splenectomy versus splenorrhaphy. Ann. Surg. ; 201(5): 568-573.

9. Lachica RVF., genigeorgis C. and Hoeprich PD. (1971) Metachromatic agar diffusion methods for detecting Staphylococcal nuclease activity Appl. Micrabiol.; 25:585-587.

10. Hoie, S. and Gudding, R. (1990) A method for monitoring antibodies against Staphylococcal DNases. Act. Vet. Scand.; 31: 223-226.

11. Erickson, A. and Deibel, R.H. (1973) Turbidimetric assay of Staphylococcal nuclease. Appl. Microbial.; 25: 337-341.

12. Armitage, P. and Berry, G. (1987) Statistical Methods in Medical Research. Research. Boston: Blackwell Scientific Publications.

13. Thurman, W. (1963) Splenectomy and Immunity. A.J.D.C.; 105:138-140.

14. Leemans; R., Manson; W., Snijder; J.A. et al. (1999) Immune response capacity after human splenic autotransplantation: restoration of response to individual pneumococcal vaccine subtypes. Ann. Surg. 229(2):279-285.

15. Standage, B.A. and Goss, J.C. (1982) Outcome and sepsis after splenectomy in adults. Am. J. Surg.; 545-549:143.

16. Kharazmi, A., Shokouk-Hamiri, M.H., Rahimi-Saber et al. (1990) Phagocytic function during bacterial challenge following spleen autotransplantation. Res. Surg.; 2(2): 58-61.

17. Sandivik, 0. (1974) The occurrence of antibodies against Staphylococc deoxyribonucleases in blood sera from different species. Acta. vet. Scand.; 15: 631- 635

18. Garcia Caballero, M., Vara Thorbeck, C., Navarro, A., et al. (1990) Multiple auto transplants of the spleen. Res. Surg.; 2(3): 120-122.

19. Eber; S.W., Langendörfer C.M., Ditzig; M. et al. (1999) Frequency of very late fatal sepsis after splenectomy for hereditary spherocytosis: impact of insufficient antibody response to pneumococcal infection. Ann. Hematol. 78(11):524-528.

20. Cooney, D., Dearth, J., Swanson, S., et al. (1979) Relative merits of partial splenectomy. Splenic reimplantation and immunization in preventng postsplenectomy infection. Surg. ; 69: 311-313.

21. Cheslyn-Curtis, S., Aldridge, M.C., Begun, J.E.J., et al. (1988) Effect of splenectomy on Gram negative bacterial clearance in the presence, and absence of sepsis. Br. J. Surg.; 75:177-180.

22. Deitch, E. and O'Neal, B. (1982) Neutrophil function in adults after traumatic splenectomy. J. Surg.Res.; 33: 98-102.

23. Leonard, A., Giebink, G., Baesl, T., et al. (1980) Over-whelming post-splenectomy sepsis problems.; 4:423-426.

24. Sherman, R. (1981) Rationale for. and methods of splenic preservation following trauma. Surg.Clin. North. Am.; 61:127-134.

25. Manssur R., Margarido; N.F., Nahas; S.C. et al. (1998) Experimental injury to the spleen study of interaction: contaminationl/preservation. Rev. Hosp. Clin. Fac. Med. Sao. Paulo. 53(6):293-298.

26. Malangoni, M.A., Evers, B.M., James, C-.; et al. (1988) Reticuloendothelial clearance and splenic mononuclear cell populations after resection and autotransplantation. Am. J. Surg.; 155(2): 298-302.

27. Grossman, J., Pholman, T., Koerner, F. et al. (1983) Plasma fibronectin concentration in animal models of sepsis and endotoxaemia. Surg. Res.; 34: 145-150.

28. Mosher, D.F. and Proctor, R.A. (1980) Binding and factor XIII a-mediated cross linking of a -27kilodalton fragment of fibronectin to Staphylococcus aureus Science; 20: 927-929.

29. Foster, P.N., Bowen, M., Howdle, P.D. et al. (1983) Low fibronectin in portal hypertension. Digestion; 28:122-124.

30. Kamel, R. and Dunn, M.A. (1982) Segmental splenectomy in schistostosomiasis. Br. J. Surg. ;69: 311-313.

31. Rahimi- Saber, S., Bayat, M., Shokouh- Amiri, et al:(1990) 'Multiple "Omental Pouches versus a Single Pouch in Splenic Autotransplantation. Res. Surg. 2(3): 117- 119.