The Role of Fibrin Glue for Splenic Salvage in Grade I and II Splenic Injuries in Albino Rats and Rabbits

Authors
Dr Shashikant Verma¹, Dr Ashutosh Silodia²*, Dr Mallika Sinha³
¹Senior Resident, Department of General Surgery, NSCBMCH Jabalpur
²Associate Professor, Department of General Surgery, NSCBMCH Jabalpur
³Assistant Professor, Department of Surgery, BMHRC Bhopal
*Corresponding Author
Dr Ashutosh Silodia
Associate Professor, Department of General Surgery, NSCBMCH Jabalpur, India

Abstract

Introduction: Spleen is one of the most commonly injured abdominal organ in blunt trauma. Iatrogenic splenic injury accounts for 20% of all splenectomy. A better understanding of importance of the spleen, its role in immune response and increased awareness of "post splenectomy sepsis" syndrome has led to the concept of splenic salvage surgery. Thus this experimental study was done to assess the role of fibrin glue for splenic salvage in grade I and II splenic injuries in albino rats and rabbits

Method: In this study, 60 healthy albino rats and 20 rabbits were used. All albino rats were randomly divided into three groups and rabbits into two groups. Laparotomy was done in all groups and superficial lacerations were made, then fibrin glue application or splenorrhaphy or no intervention was done according to different groups.

Observation: In rabbits all were show healing, there was no difference between the fibrin glue and splenorrhaphy group (P>0.05). There was higher grade of adhesion in higher grade of tear and the difference was statistically significant (P<0.05). In rats, 3 of the 20 rats (15%) in the control group without intervention showed no healing while all of the 20 rats (100%) in the fibrin glue & splenorrhaphy group respectively healed. Even in rats the difference between the 3 groups was not statistically significant (P>0.05).

Conclusion: Splenorrhaphy and fibrin glue application had no significant difference in healing. Splenorrhaphy had significantly more and higher grades of adhesions than fibrin glue application. Grade of adhesions increased with increasing grade of tear in both rats and rabbits.

Keywords: Fibrin glue, Splenorrhaphy, Adhesion, Shackford’s classification.

Introduction
Spleen is one of the most commonly injured abdominal organs in blunt abdominal trauma due to road traffic accidents. Iatrogenic splenic injury accounts for 20% of all splenectomy. A better understanding of importance of the spleen, its role in immune response and increased awareness of "post splenectomy sepsis" syndrome has led to the concept of splenic salvage surgery. With the advent of diagnostic peritoneal lavage (DPL), and more recently, CT and ultrasound, have made early diagnosis and conservative
management for less severe injuries standard care. Management of splenic trauma depends on - Extent of injury, associated injuries and Hemodynamic stability. Salvage of ruptured spleen has now become universally accepted policy. Conservative management comprises– Non-operative management and Operative management. Fibrin glue is a biological tissue adhesive based on the final stage of coagulation wherein thrombin acting on fibrinogen converts it into fibrin. It has two components, fibrinogen and thrombin. Fibrin glue augments the induction of entire process of wound healing as whole of the coagulation cascade is bypassed and readymade fibrin is immediately produced with its adhesive function due to fibrin polymers. Fibrin sealant acts naturally by fibrin polymerization and cross-linking; a possible disadvantage of using fibrinogen made from human plasma in fibrin sealant preparation is possibility of transfer of hepatitis or HTLV-III. By careful selection of plasma donors, heat-treating of fibrinogen in liquid solution and sterilization of sealant with gamma rays, this risk can be minimized. Based on this principal this experimental study was done to assess the role of fibrin glue for splenic salvage in grade I and II splenic injuries in albino rats and rabbits

Method
This experimental study “The role of fibrin glue for splenic salvage in grade I and II splenic injuries in albino rats and rabbits” was carried out from June 2005 to July 2007 in Experimental Surgery unit, Department of Surgery, NSCB Medical College & Hospital, and Jabalpur (M.P.) with approval from the Animal ethics committee. This was a study of effects of fibrin glue in splenic salvage surgery in comparison to splenorrhaphy and non-operative management in grades I and II splenic injuries. In this study 60 healthy albino rats and 20 rabbits were used, both male and female. All albino rats were randomly divided into three groups and rabbits into two groups, laparotomy was performed, splenic lacerations of grade I or II were made in all study group.

Study on rats
Group A (Control group) - managed conservatively without any intervention as control group.
Group B (spleenorrhaphy) - spleenorrhaphy was done using absorbable suture.
Group C (fibrin glue application) - fibrin glue was applied over the laceration.

Study in rabbits
Group D (rabbits with spleenorrhaphy) - spleenorrhaphy was done using absorbable suture.
Group E (rabbits with fibrin glue application) - fibrin glue was applied over the laceration.

Animals were sacrificed at 10th postoperative day and splenectomy was performed for examination of repair sites. Splenic specimens were taken in all cases for gross and histological examination.

Method of fibrin glue application - Fibrin sealant (FS) was applied to bleeding site by a specially made syringe and an 18-gauge blunt tip needle, splenic defects were filled from the base of the injury at first and then gradually towards the surface. Additional FS was applied to the surface defect as well. Fibrin sealant begins to solidify within seconds; thus, bleeding was usually slowed after the first application. Gentle blotting with a dry gauge sponge was performed, before applying additional FS.

Splenorrhaphy: Splenorrhaphy was done with vicryl 6-0 on atraumatic round body needle, hemostasis was checked before closure. There were no cases of active or recurrent bleeding.

Control group: Slight pressure was applied for 5 minutes and absorbable gelatin sponge was kept near laceration, thereafter closure was done.

Identification: Subject’s ear tip was cut for identification as follows —
Group A (Control group)- Both ears intact.
Group B (rats with spleenorrhaphy)- One ear tip cut (wedge shaped).
Group C (rats with fibrin glue application)-Both ears tip cut (wedge shaped).
Group D (rabbits with splenorrhaphy)- No identification mark was made.
Group E (rabbits with fibrin glue application)- Silk suture was applied in right ear.

Re exploration
Day 10 was chosen because it is sufficient time for healing to occur and after that there is progressive absorption of fibrin glue. Animals were sacrificed on 10th postoperative day and specimen of spleen was harvested. Each subject was assessed on the basis of: Gross findings (splenic disruption, hematomas), Healing at laceration site, Presence of intra-abdominal abscess.

Procedure
With all aseptic precaution, midline laparotomy was performed and spleen was delivered into the wound and two types of experimental splenic injuries were made as follows: (1) superficial lacerations involving capsule only, (2) lacerations to capsule along with parenchymal injury 0.5 to 1 cm in length in peripheries (not extending into splenic hilum). All injuries were made using a No. 11 scalpel blade according to Shackford’s grading system.

Modified grading system of splenic ruptures according to Shackford’s
Grade I- Localised capsular rupture without significant parenchymal injury.
Grade II- Localised capsular rupture with local parenchymal injury.
Grade III- Parenchymal injury not extending into the hilum or involving major vessels.
Grade IV- Severe parenchymal injury extending into the hilum or involving major vessels.
Grade V- Completely shattered or fragmented spleen or separation from the blood supply.

Observation and Result
This experimental study was carried out on 60 rats and 20 rabbits. Five groups were studied. In all groups first laparotomy was done on day 0 and superficial lacerations were made, then fibrin glue application or splenorrhaphy or no intervention was done according to different groups. Animals were sacrificed on 10th day and spleen was harvested.

| Adhesions Tear | Control | FIBRIN GLUE | SPLENORRHAPHY |
|----------------|---------|-------------|---------------|
| Grade-I        | Grade-II| Grade-I     | Grade-II      |
| Grade-I        | 8       | 0           |               |
| Grade-II       | 72.7)   | .0)         |               |
| Grade-I        | 1       | 3           | 13            |
| Grade-II       | 8.3)    | 27.3)       | 68.4)         |
| Grade-I        | 4       | 0           | 6             |
| Grade-II       | 50.0)   | .0)         | 31.6)         |
| Grade-II       | 41.7)   | 63.6)       | 10.5)         |
| Grade-I        | 1       | 2           | 10            |
| Grade-II       | 12.5)   | 18.2)       | 52.6)         |
| Grade-II       | 33.3)   | .0)         |               |
| Grade-I        | 2       | 7           |
| Grade-II       | 16.7)   | 18.2)       | 36.8)         |
| Grade-I        | 11      | 19          | 11            |
| Grade-II       | 12      | 19          |               |
| Total          | 8       | 12          | 11            |

It shows that in the control group the grade 1 tears had grade 1 adhesion in 3 animals, grade 2 in 4 animals and grade 3 in only 1 animal. While in the grade II tears grade 1 adhesion was present in only 1 animal, grade 2 in 5 and grade 3 in 4 and grade 4 adhesions were seen in 2 animals. In the fibrin glue group, in the grade 1 tear, 8 animals showed no adhesions while 3 showed grade 1 adhesions. In the grade II tear 13 animals showed grade 1 adhesions while 6 animals showed grade 2 adhesions. In the splenorrhaphy group, in the animals with grade I tear, 7 showed grade 2
adhesions while 2 showed grade 3 & 2 showed grade 4 adhesion. In the animals with grade II tear 2 animals showed grade 2 tear while 10 showed grade 3 and 7 showed grade 4 adhesions. In each of the 3 groups there was higher grade of adhesion in higher grade of tears and the difference was statistically significant (P<0.05).

Table 2 - Correlation between Different Interventions and Presence of Healing

| SPECIES | GROUP            | Control | Fibrin glue | Splenorrhaphy | Total |
|---------|------------------|---------|-------------|---------------|-------|
| Rabbit  | Healing Yes      | 10      | 10          | 10            | 20    |
|         |                  | 100.0%  | 100.0%      | 100.0%        |       |
| Total   |                  | 10      | 10          | 20            |       |
| Rat     | Healing No       | 3       | 0           | 0             | 3     |
|         |                  | 15.0%   | 0%          | 0%            | 5.0%  |
|         | Healing Yes      | 17      | 20          | 20            | 57    |
|         |                  | 85.0%   | 100.0%      | 100.0%        |       |
|         |                  |         |             |               | 95.0% |
| Total   |                  | 20      | 20          | 20            | 60    |
|         |                  | 100.0%  | 100.0%      | 100.0%        |       |

It shows that in rabbits all of the 20 rabbits (100%) in whom splenorrhaphy were done & in whom fibrin glue was applied showed healing. There was no difference between the two groups (P>0.05). In rats, 3 of the 20 rats (15%) in the control group without intervention showed no healing while all of the 20 rats (100%) in the fibrin glue & splenorrhaphy group respectively healed. Even in rats the difference between the 3 groups was not statistically significant as far as healing was concerned (P>0.05).

Table 3: Comparison of healing, resorption of fibrin glue and adhesions in different studies using sealants for splenic trauma

| Studies         | Healing                  | Complete resorption of fibrin glue | Types of adhesions                  |
|-----------------|--------------------------|-----------------------------------|-------------------------------------|
| Kuzu et al      | 15/15 rabbits            | 7-10 days                         | Minimal omental adhesions           |
| Kram et al      | 6/6 dogs                 | 14-42 days                        | Filamentous omental adhesions       |
| Schelling et al | 12/12 dogs               | >30 days                          | Filamentous omental adhesions       |
| Present study   | 19/20 rats and 10/10 rabbits | >10 days                         | Minimal omental & peritoneal adhesions |

In the present study, healing was seen in all cases in the fibrin glue group who survived. All studies using sealants for superficial splenic trauma showed healing. In the splenorrhaphy group healing was seen in all cases. No animal studies for healing after splenorrhaphy are published in literature to the best of our knowledge so no comparison can be done. This study also shows splenorrhaphy can be used as a method of splenic salvage in superficial splenic injury i.e. grade I and II tears without any undue risk of re-bleeding in well selected patient. In the present study partial resorption of the sealant was seen at 10th day. All studies using sealants for splenic trauma showed resorption over a period of time and depended on the amount of glue used.

Discussion

This was an experimental study done in albino rats & rabbits. Iatrogenic splenic injury of grade I & II (superficial lacerations) according to modified Shackford’s grading were made then splenic salvage was done by suture splenorrhaphy and local application of fibrin glue. The rats were sacrificed on day 10 and observed for healing, presence of hemoperitoneum, abscess cavity, adhesions & mortality and the cause of mortality was found out.
Difference in resorption time has been seen between autologous and synthetic compounds, i.e. synthetic compounds take comparatively more time for resorption. The Fibrin glue group showed significantly lower grades of adhesions than the control group and splenorrhaphy group in the present study and this difference was statistically significant. This finding is similar to the results seen in studies done by Kram[51], Schelling[53] and Kuzu et al[48] using sealants for splenic trauma in which minimal and low-grade adhesions were seen. In the control group the adhesions were of a higher grade and more in number. It was seen that higher grades of adhesions were seen with higher grade of splenic tear. This may be due to increased tissue response due to increased surface area of the spleen damaged.

**Table 4: Present Study**

| Factor        | Fibrin glue | Splenorrhaphy |
|---------------|-------------|---------------|
| Healing       | 30/30       | 30/30         |
| Hemoperitoneum| 1/30        | 1/30          |
| Adhesions     | No or Lesser grade | Higher grade |
| Abscess       | 1/30        | 5/30          |
| Deaths        | 1/30        | 2/30          |

Present study shows that the healing is same in both the groups. The amount and grade of adhesions in the present study was significantly higher in splenorrhaphy group than those in the fibrin glue group. No other experimental study to the best of our knowledge has compared these two splenic salvage techniques.

**Conclusion**

Splenorrhaphy and fibrin glue application had no significant difference in healing and hence, are equally effective techniques of splenic salvage in rats and rabbits in grade I and II tear (Shackford's classification). Splenic salvage should be practiced in splenic tears grade I and II and splenectomy should be avoided in such cases. Fibrin glue is an effective and reliable technique for iatrogenic superficial splenic tears. Non-operative management had increased risk of hemoperitoneum formation in rats as compared to the operative salvage techniques; however there was no statistically significant difference between mortality in non-operative and splenic salvage techniques. Splenorrhaphy had significantly more and higher grades of adhesions than fibrin glue application in both rats and rabbits. The grade of adhesions increased with increasing grade of tear in both rats and rabbits. And there was no significant difference in abscess formation, mortality rates in the different groups in rats and rabbits.

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