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INFLAMMATORY RESPONSE IN THE FIXATION OF PROLINE MESH USING N BUTERYL 2 CYANOACRYLATE (HISTOACRYL ^{R}) IN ABDOMINAL WALL: EXPERIMENTAL STUDY

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The use of glue and particularly histoacryl in different branches of medicine and surgery has been explored. Its role and use in general and different branches of surgery is very wide. Its role to minimize the surgical procedure time and production of cosmetic effect is now accepted. This study was done to define and evaluate the tissue response to histoacryle as glue in fixation of anterior abdominal wall mesh in hernia repair i.e. its patho-histological effect. So we will be able to apply it in human safely. 30 mice were used and proline meshes were applied and fixed with histoacryl to the anterior abdominal wall. They were all sacrificed and the abdominal walls were fixed in formaline and histopatological and immunohistochemestry studies were done. We found that all our specimens had primary union healing with first intention in the skin incision and in the deeper tissue were the mesh lies no complications were found in our study in relation to the method of fixation.

Key words: Tissue adhesive, glue, histoacryle, hernia repair, experimental study

INTRODUCTION

Tissue adhesives (glue) offer significant potential advantage over traditional methods of wound closure [1,2]. There are different types of glue that are widely used in surgery and medicine; one of these tissue adhesives is a Buteryl 2 cyanoacrylate i.e. (Histoacryl) [3,4]. Histoacryl has been widely used in different areas in surgery and medicine; its application in surgery is very unlimited and broadly expanded in plastic surgery [5,6,7]. Its use in tissues and vessels in different systems in the body is proven to be one of the most significant methods in such field [8,9]. Its chemical component as a cyanide ring gives it the bactericidal activity as well [10].

The healing of clean uninfected surgical incision approximated by surgical sutures or glue, such healing is referred to as primary union or healing by first intention. The surgical incision causes the death of a limited number of epithelial cells and connective tissue. It also causes the disruption of epithelial basement membrane continuity, the narrow incision space immediately fills with clotted blood containing fibrin and blood cells; dehydration of the surface clot forms the well know scab that covers the wound [11]. The healing wound is a dynamic and changing process, the early phase is inflammation, followed by stage of fibroplasia, followed by tissue remodeling and scaring.

Collagen is the most common protein in the mammalian world; providing the extracellular framework for all multicelluler organisms, it constitutes about one third of body proteins [12]. Collagen also has a role in chemotaxis, platelet adhesion, aggregation [13], and attachment [14].

In skin the predominant type of collagen is type I, III. Also Fibronectins which are non-collagen's protein and form a family of glycoproteins that are closely related to each other structurally and antigenically. They have been described as forming a molecular glue (autobiological) since they are necessary for many (cell to cell) interaction as well as the adhesion of cells to surface such as collagen and fibrin and play a role in wound healing. [15] .

Fibronectins are present in plasma, in the interstitial fluid, on cell surface, on collagen fibers and in basement membranes.

Fibronectins functions are many and various in connective tissue. it has a high affinity for collagen., in cell binding: it is necessary for cell to cell interactions as well as cell to substance binding. Thus it is required for fibroblasts and macrophages to adhere to collagen and fibrin. In wound healing: it play a role in the healing of wound [16].

Fibronectin is abundantly present in normal humman skin it is located in the dermo-epithelial junctions area in the papillary and reticuler dermis epidermal appendages and in vascular and renal structures. In the epidermis of the skin there is no fibronectin [15,16].

Aim of the Study:

This is a study aimed in looking at the tissue response after making use of the Histoacryl as one of new modalities to fix the mesh to the anterior abdominal wall hernia repair so as to able to apply it on human safely after this study.

MATERIAL AND METHODS

This study was performed at the experimental surgical unit Department of surgery in the animal house and the pathology Department of Theodore Bilharz Research Institute.

30 white male mice weigh each 20 ± 2gm were anethesized in the operating theater room on bench with cone shape ether mask inhalation by volume of 10ml. After making sure the animal was sleep a skin incision was done from xephisternum to the symphysis pubis. Then splitting the skin from anterior abdominal wall. Then implanting a piece of Proline mesh inbetween. Macro pores knitted monofilament polypropylene mesh was used 2cm x 2cm piece (Elhicon-LTD UK.). After which we apply a few drops of (n. Byteryl-2 cyanoacryle, Hystoacryl- Brann AG-GMBH.) using a black needle attached to the tube of the glue to control amount used [15]. After that the skin edges were closed and opposed and glued together.

The animals were allowed to live in cages. Water and proper food were allowed as well. We sacrifice 3 mice on the 1st day post operative, 3 mice on the 3rd day post operative 3 mice on the 7th day post operative and 3 mice were scarified on the 14th day post operative. The remaining 12 mice were kept alive from start of operation till the 35th day post operative i.e. 5 weeks then we sacrifice them.

So we had 30 mice for the experiment, 6 died during the whole period {4 with wound dehiscence in the 1st week and 2 due to unknown reason in the weeks after}, 24 were scarified and autopsied. On day 1,3,7,14 and 35 post operative as shown in (Table 1). On the remaining [24] mice non-of them showed wound dehiscence clinically.

Table (1): ***Showed the number of mice and the time of their sacrification.***

Post operative day	*Scarified mice*	*Deaths*
1st	3	0
3rd	3	4
7th	3	2
14th	3	0
35th	12	0
Total	24	6

The only explanation to what happened i.e. the cause of death of those animals was due to deheacince due to; at the beginning of the experiment the animals were living in cages together and they use to eat the material on the skin of the abdominal wall from each other that is why we kept them on separate cages after day 5 where we noticed the loss of six of them.

The sample obtained was the whole abdominal wall (Skin, muscles and peritonium) and the upper part where marked from the lower part. The animals were sacrificed by putting them down mercifully and suddenly instantaneously to grantee no misconduct.

The skins of the abdominal wall of the mice with the proleine mesh were fixed in 10% buffered formaline. They were processed and embedded in paraffin blocks.

Histopathological examination:

Serial sections 5µm in thickness was stained with hematoxyline and Eosin, and Masson Trichrome stain for light microscopy examination. 10µm thick Sirius red stained skin sections were examined for evaluation of collagen content (It is a very specific stain for collagen fibers) was performed basically according to what was described before [16].

Immunohistochemistry:

The immunohistochemical reactions used were the streptavidin-biotin method rabbit antihuman fibronectin antigen (DAKO). LSAB 2 kit alkaline phosphatase Universal K676.

All specimens were fixed in 10% formalin and embedded in paraffin. Sections 4µm. thick were mounted on poly –L lysine coated slides, dewaxed in xylene and rehydrated to water through graded alcohol's. The fibronectin antigen was activated by micro heating as described by [17-31]. After antigen activation, sections were coated to room temperature, washed in phosphate buffered saline (pH. 7.2) and incubated with 10% normal goat serum, for 30 minutes to decrease non specific back ground staining. Subsequently sections were allowed to stand over night at 4Co in a 100 fold dilution of antibody anti fibronectin Developed in rabbit (Sigma) in phosphate

buffered saline at pH 7.6 supplemented with 1% bovine serum albumin. The same buffer without primary antibody was applied to negative controls.

After incubation the slides were washed with PBS. The further process was a biotin-strept avidin standard procedure using (the Universal DAKO L SAB2 kit alkaline phosphatase K676).

Immunohistochemical assesment was performed semiquantitativly . The intensity of staining was scored from zero(0) to (3) i.e.(+/- ; Weak =1+, Moderate = 2+, Strong = 3+, Intense = 4+).

RESULTS

By light microscopy we examine the skin section (stained by H & E, Masson Trichrome, Sirius red, fibronectin) for infection, inflammation, dehiscence, ulceration, bleeding, collagen deposition, fibrosis. edema, and neo vascularization shown in (Table 2).

We did not have any infected specimens and there were no bleeding or ulceration and no deheaicence in the remaing 24 animals after 5th post opeartive day

The different stains and the collagen and fibronectin deposition in different intensity is shown for all the specimens in (Table 3).

Table (2): ***Showed the relation ship between the time (day of operation) and the histopathological changes occuring in the inscision***

post operative day	*Granulation tissue*	*Vascularity*	*Edema*	*Collagen deposition*
1st	--	--	++++	--
3rd	+	+	+	+
7th	++	++	++	++
14th	+++	+	+	+++
35th	++++	--	--	++++

+ mild. ++ moderate. +++ marked. ++++ sever.

Table (3): ***Demonstrate the different staines for collagen fibers by Masson Trichromes, Sirius red and the immunohistochemical stain for fibronectin***

Post operative day	*Masson Trichrome*	*Sirius red*	*Fibronectin*
1st	--	--	--
3rd	+	+	+
7th	++	++	++
14th	+++	+++	+++
35th	+++	+++	+++

+/- week reaction (1+) mild intensity. (2++) moderate intensity (3+++) marked intensity, by using light microscopy collagen fibrils appear as (blue fibrils, by using Masson Trichrome) as (red fibrils, by using Sirius red stain) and the Fibronectin fibers as (brown fibrils)

Table (4): ***shows a key for the eleven figures we had***

Post op. day	*Hematoxyline & Eosin*	*Masson Trichrome*	*Sirius Red*	*Iimmunohistochemistry*
1st	NA	NA	NA	NA
3rd	**Fig. 1** (x200). Aactive granulation tissue formation with increase vascularity,	**Fig. 2** (NA)	**Fig. 3** (x100). Mild + for collagens in the upper dermis the skin	**Fig. 4** (NA)
7th	**Fig. 5** (x200). Giant cell granuloma at the line of repair	**Fig. 6** (x200), Showed the proline mesh (Arrow) collagen are seen as green bundles.	**Fig. 7** (x200) Moderate ++ for collagen stained red in the dermis and epidermis of the skin	**Fig. 8** (x100) Mild Intensity + of the tiny fibronectin fibrils stained pink
14th	**Fig. 9** (x200) Collagen deposition in the upper dermis of the skin	**Fig. 10** (NA)	**Fig. 11** (x400) Marked +++ for collagen stained red in the dermis and epidermis of the skin	**Fig. 12** (x400) Mmoderate intensity ++ of tiny fibrionectin fibrils stained pink inbetween the inflammatory cells.
35th	**Fig. 13** (NA)	**Fig. 14** (NA)	**Fig. 15** (x400) Marked +++ for collagen stained red in the dermis and epidermis of the skin	**Fig. 16** (x400) Marked intensity +++ of fibronectin bundles in the epidermis of the skin, and at the edge of the gap

NA: Not avialable

Day 1 post operative:

Examining the sections stained by ***H&E and Masson Trichrome within 24*** hours of injury, we found neutrophils appear at the margin of incision moving toward the fibrin clot. Spurs of epithelial cells from the adjacent epidermis migrate into the wound and insinuate themselves between the inert dermis and the clot.

Day 3 post operative:

Examining the *H&E and Masson Trichrome* stained slides the neutrophils have been largely replaced by macrophages. Granulation tissue progressivly invade the incision space (Fig. 1,2).

Sirius red stained slides showed collogen fibers prtesent in the margins of the skin incisions first and bridged the incisions latter on (Fig. 3).

With well approximated wound by 48-72 hours a continuos layer of epidermal cells covers the surface, an acute inflammatory reaction occurs, and the fibrinous exudate helps to cement the cut margins of the wound together. Overlying the area there is a crust or scrab of dried clot.

By i*mmunohistochemestry* stained sections we found weak postive pink color stained fibronectin fibers i.e. (+/-)

Day 7 post operative

We found that the incisional space is filled with granulation tissue, edema is present, neovascularization is maximal in the area around the mesh .By *examining H&E and Masson Trichrome* slides the wound area is invaded by fibroblastic cells and capillary buds growing in the edges of cut surface (Fig. 5,6).

Sirius red sections examination revealed that collagen fibrils become more abundant and begain to bridge the icisions. The epidermis recover its normal thikness. Soon after the granulation tissue appears collagen formation commences. At first type III collagen predominates and formation of reticulin fibres. A type I collagen predominates and adult type collagen fibers are formed (Fig. 7). *The immunohistochemestry* showed mild increase in the intensity of fibronectin fibers (Fig. 8).

Day 14 post operative:

The leukocytic infilterate, oedema and increased vascularity have largely disappeared. This was shown by the *H&E and Masson* Trichrome stained sections (Fig. 9).

We found there is continued accumulation of collagen, and prolieferation of fibroblast within the icisional scar which appeared as much darker red color in Sirius red sections (Fig. 11).

Dark pinkish color showed as moderate (2+) by the immunstain showing high collagen deposition within the incisional scar (Fig. 12).

Day 35 post operative

We found the scar comprises a cellular connective tissue devoid of inflammatory infiltrate covered now by intact epidermis in the remaining 24 cases.i.e. marked reactions (3+) (Fig. 15,16)

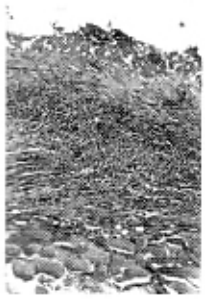

Fig. (1)

Fig. (2)

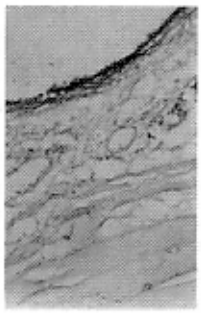

Fig. (3)

Fig. (4)

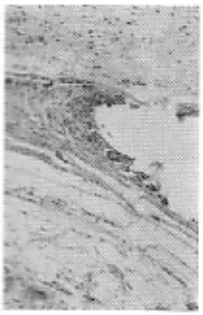

Fig. (5)

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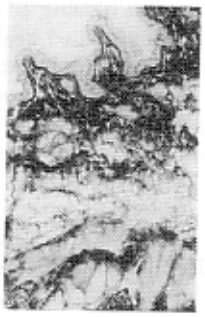

Fig. (11)

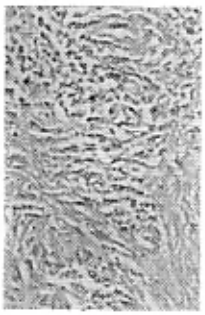

Fig. (12)

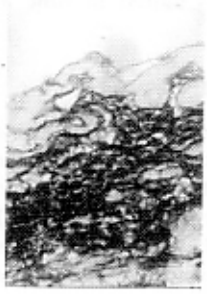

Fig. (13)

Fig. (14)

Fig. (15)

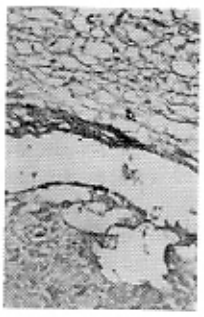

Fig. (16)

DISCUSSION

It was belived that tissue adhesive offer significant potential advantage over traditional methods of wound closure. A new n-butyl 2- cyanacrylate adhesive formation was utelized for the closure of abdominal wounds after general laparascopic gastero intestinal surgery [3].The use of tissue adhesives as an alternative to or replacement of sutures in wound closure has long been an area of intrest. One of this tissue adhesives is a cyanoacrylate [18]. No exprimental or clinical stuides have shown that this glue has any carcinogenic or mutagenic properties [10]. Also cyanoacrylate tissue glue has been claimed to have the advantage of being haemostatic, bacteriostatic and easy to use [19]

It is reported that the introduction of cyavoacrylate tissue adhesives appear to be an ideal techniques for laceration closure in children because they are easy and rapid to apply, are relatively painless, eliminate the need for suture removal, and provide an acceptable cosmotic result [22]. In our work, and from the above results we found that all the incisional scars we did and the use of glue to fixition of proline mesh, they all had primary union or healing by first intension, no cases showed infection, and these was going with the result done before [3,21]. In this work no cases showed bleeding or ulceration on microscopic level, these results are going with the previous results [22], which reported that this glue is usefull in avoiding bleeding. In our expermient the healing of our wound obtained were by granulation tissue called healing by primary intention. The six deaths we had at 3rd and 5th post operative days showed no infection but wound deheacince and loss of glue material and we atributed this to what we said befor as the animals were living togather they used to eat the wound and the forign material and as soon as we discovered this we kept the animals in separate cages and we had no deaths after that. We also had no infection and no bleeding and no ulcerations what so ever in our wound and inbetween the layersas well.

Also in this work, we found the union in our exprimental cases are similar to that happen in human healing incision healed by primary union in time and process of healing [11].

CONCLUSION

From our results we conclude: that cyanoarylate tissue adhesive effectively closes The anterior abdominal wall wounds and is able to fix the mesh to the muscler layer of the anterior abdominal wall without no complication. This relatively fast and safe method could effectively be utelized for general abdominal wound closure and for mesh fixation in inguinal hernia repair.

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