

Effects of Decompressive Craniectomy with Mesh in the Level of Transforming Growth Factor Beta, Fibroblast Cell Count, Size of Fibrosis, and Clinical Adhesion of Peridural Tissues in Wistar Rat with Traumatic Brain Injury

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One option to control intracranial pressure (ICP) is by performing decompressive craniectomy (DC) before definitive treatment. A common problem after DC is adhesion. Separated injured tissue surfaces by using bioabsorbable membranes over a critical/certain period are predicted to prevent fibrin bridge formation and adhesion. This study is conducted to prove the effects of DC with mesh on TGF- β levels, fibroblast cell count, fibrosis size and clinical adhesions in wistar rat with traumatic brain injury. The current research uses animal model with randomized post-test only control group design. A total of 20 samples that met the eligibility criteria were included in the study and randomized. A total of 10 samples were grouped as a control group (standard DC) and 10 samples were grouped as a study group (DC with mesh). On day 7, we performed euthanasia and took peridural tissue for examination of TGF- β levels, fibroblast cell count, and fibrosis size. The mean results of TGF- β in the study group were lower than the control group (81.28 ± 17.48 pg/dl vs 93.83 ± 9.51 pg/dl) although this difference was not statistically significant ($p=0.062$). For the mean number of fibroblasts cells, there was a significant difference where the study group was lower than the control group (56.8 ± 20.59 cells/HPF vs 94.8 ± 18.56 cells/HPF) with $p < 0.001$. The mean size of peridural fibrosis also showed a significant difference where the mean size of peridural fibrosis in the study group was lower than the control group (1182.52 ± 347.05 μ m vs 1545.78 ± 378.28 μ m) with $p=0.038$. Only DC samples (without the use of mesh) had a relative risk 8 times greater for clinical adhesions compared to mesh (RR=8;95% CI, 1,215-52,693; $p=0,005$). DC with mesh significantly resulted in low fibroblast cells count, size of peridural fibrosis, and the risk of clinical adhesions in wistar rat with traumatic brain injury.

Keywords: Traumatic brain injury, mesh, TGF- β , fibroblast cell counts, fibrosis size, clinical adhesions, peridural tissue.

Intracranial hypertension was a major cause of complications and death among many problems because of TBI. One attempt to control intracranial pressure (ICP) in patients with TBI is by performing Decompressive Craniectomy

(DC)². In the last few decades, DC methods for TBI management have become the focus of attention.

Decompressive craniectomy frequently performed by a neurosurgeon in the treatment of persistently high ICP and having a midline

shift on a patient with traumatic brain injury. This surgical management also performed on ischemic stroke patients, hemorrhagic strokes and subarachnoid hemorrhage. The DC aims to reduce ICP by removing cranium bone so that there is more room for the swollen brain. It is an early action for life-saving which will be followed by a second operation of cranioplasty after the patient is stable¹⁰.

A common problem after DC is adhesion between soft tissues, especially between the duramater, the temporal muscle, and the galea. The adhesion formation due to difficulties during tissue dissection resulting in increased operating time and increased risk of dural perforation¹². At the time of cranioplasty, temporal muscle is separated from the duramater, sometimes it can be very difficult and takes a long time, the risk of cerebrospinal fluid effusion (CSF) with potential postoperative complications can occur.

By separating the injured tissue surfaces using bioabsorbable membranes over a critical/certain period, fibrin bridge formation and adhesion are predicted to be prevented. Septrafilm mesh contains Hyaluronic Acid carboxymethylcellulose (HA-CMC) is a bioabsorbable membrane that has been used for anti-adhesive. The combination of these 2 materials looks transparent, thin, adherent and absorbable membrane can be used as a mechanical separation device. In randomized clinical trials of animal model, it has been demonstrated that HA-CMC decreases incidence and severity of postoperative adhesion⁶.

In 1996, HA-CMC was combined as an anti-adhesion membrane in clinical trials of abdominal surgery, where there was a significant decrease in adhesion/postoperative adhesion in animal models and clinical studies of adult and child patients. HA-CMC membranes serve as the physical barrier that can decrease the activity/proliferation of fibroblasts, prevent fibrin deposition on serous surfaces and cellular elements during peritoneal repair⁶. HA also has anti-inflammatory activity and acts as a synthetic membrane to inhibit invasion of inflammatory cell inflammation and vascularization⁷.

Research on the use of anti-adhesion materials during DC operation has not been done in Bali or Indonesia. For that reason, researchers wanted to conduct experimental research on Wistar

rat whether the use of mesh as anti-adhesion in DC can reduce adhesion with low TGF- β levels, fibroblast cell count and peridural fibrosis.

METHODS AND MATERIALS

This current study is a laboratory experimental study using an animal model with randomized post-test control group design. We conducted this study in February-Augustus 2017 in the Pharmacologic Laboratory of Medical Faculty of Udayana University and Laboratory of Veterinary Medicine Faculty of Udayana University. The inclusion criteria of wistar rats are male, healthy, age 6-8 weeks, body weight 150-200 gram. Drop out criteria are severe illness or death which is determined by a veterinarian. Randomization was done to allocate samples to each group, 10 samples to study group and 10 samples to control group.

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted No:363/UN.14.2/KEP/2017. The study was done by trained staff and all animal model got comfort environment.

A traumatic brain injury of the animal model was made first by injection of an anesthetic agent to reach pain-free of animal model, using ketamine 44-100 mg/body weight intramuscularly. We used *weight drop model* technique to induce traumatic brain injury. The feathers on the rat's head were shaved and cleaned with 70% alcohol. A linear incision was done on the rat head in the midline, then a 75-gram iron cylinder was dropped once from 10 cm height, 90-degree angle. The energy of the impact was 0.01 Joule.

The models were prepared to undergo the DC procedure, the duramater was opened with microscissor leaving the cortical intact. In control group, at the end of the procedure, the temporalis muscle, fascia, and skin were sutured without mesh, whereas, in the study group, the mesh was placed proportionately to the bone flap above the dura mater without suturing, and the temporalis muscle, fascia, and skin were sutured layer by layer.

The TGF- β level was measured using ELISA, fibroblast cells were counted using immunohistochemistry method, peridural fibrosis was evaluated based on its size (using

immunohistochemistry method) and clinical adhesion (the adhesion between duramater and temporalis muscle, facia or the skin).

The data for transforming growth factor beta (TGF-β), fibroblast cells count, and fibrosis size in the peridural area were tested for normality using Shapiro-Wilk. The results came out the data were normally distributed. Independent t-test was used to evaluate the level of transforming growth factor beta (TGF-β), fibroblast cells count, and fibrosis size in the peridural tissue between study and control group, confidence interval 95% (p<0,05). Fisher Exact was used to evaluate

the difference in clinical adhesion between both groups.

RESULTS

The mean of TGF-β level in study and control group were 81,28±17,48 pg/dl and 93,83±9,51 pg/dl respectively. Based on analysis results, an independent t-test showed p-value 0,062, therefore we concluded that the mean TGF-β level after day 7 in the study group was lower compared to control group and the difference was statistically not significant (Table 1).

Table 1. Mean difference of TGF-β level in both group

Subject	n	Mean ± SD (pg/dl)	Mean difference	95% CI	p
Control group	10	93,83 ± 9,51	12,55	(-0,67-25,77)	0,062
Study group	10	81,28 ± 17,48			

SD= standard deviation; p= p-value; n= sample number

Table 2. Mean difference of fibroblast cells count in both group

Subject	n	Mean ± SD (cells/HPF)	Mean difference	95% CI	p
Control group	10	94,8 ± 18,56	38	(19,58-56,42)	<0,001
Study group	10	56,8 ± 20,59			

SD= standard deviation; p= p-value; n= sample number

Table 3. The difference in mean size of fibrosis in both groups

Subject	n	Mean ± SD(im)	Mean Difference	95% CI	p
Control group	10	1545,78 ± 378,28	363,26	(22,2-704,32)	0,038
Study group	10	1182,52 ± 347,05			

SD= standard deviation; p= p-value; n= sample number

Table 4. The differences in clinical adhesion risk in both groups

Group		Clinical Adhesion				p	RR	95% CI	
		Positive		Negative				Lower	Upper
		n	%	n	%				
Group	Control	8 ^a	80	2	20	0,005* ^b	8	1,215	52,693
	Study	1 ^a	10	9	90				
Total	9	45	11	55					

RR: Relative risk; p= p-value; n= sample number; CI= confidence interval; a= expected count <5; b= Fisher test; *significant p-value<0,05

We obtained an average fibroblast cells count in the control group that is 94.8 ± 18.56 cells/HPF while in the study group was 56.8 ± 20.59 cells/HPF. The significance value of the independent t-test showed the p -value < 0.001 . It can be concluded that there is a significant difference of mean fibroblast cell counts between the control group and the study group where the average fibroblast cell counts in the study group are lower than the control group on day 7 (Table 2). Fig. 1 showed the microscopic view of fibroblast cells.

The mean size of fibrosis on the sample speridural tissue 7 days after DC with mesh was $1182,52 \pm 347,05\text{im}$ and after standard DC was $1545,78 \pm 378,28\text{im}$. Statistical analysis showed was $p < 0.05$. This p -value means that there was a significant difference in the size of fibrosis between the control group and the study group, where the mean fibrosis size in the study group was lower than the control group (Table 3). The size of fibroblast cells in both groups was shown in fig. 2.

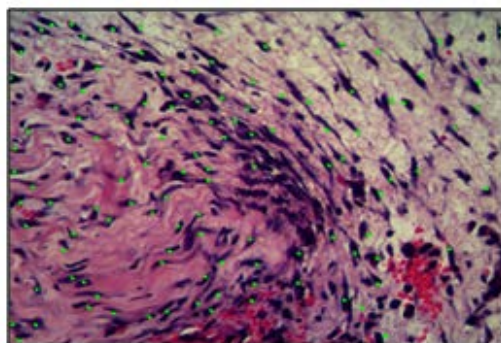
Table 4 showed the incidence of clinical adhesions in the study group was lower than

the control group with $RR = 8$. The results of Fisher's test analysis showed that $p < 0.05$, it can be concluded that the standard DC samples (without mesh placement) had a relative risk 8 times greater for clinical adhesion compared to mesh ($RR=8$; 95% CI, 215-52,693; $p=0.005$).

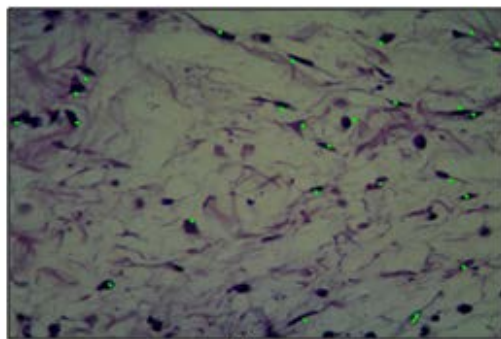
Analysis with 2x2 tables was done based on the division of each variable (group samples and clinical adhesions). Based on the analysis, there were 50% cells having an expected count value of less than five, therefore the 2x2 table was not feasible to be tested by the Chi-Square test. We decided to use the Fisher test, instead.

The new findings (research novelty) of this study are:

1. This study was the first study which examines DC with mesh to reduce peridural fibrosis in wistar rat with traumatic brain injury.
2. It was also found that DC with mesh can reduce adhesion of peridural tissue as evidenced by lower of fibroblast cells counts, the size of fibrosis, and clinical adhesions in the peridural tissues in rat with the traumatic brain injury. Mesh as an anti-adhesion membrane in DC serves as a physical or mechanical barrier between the duramater and

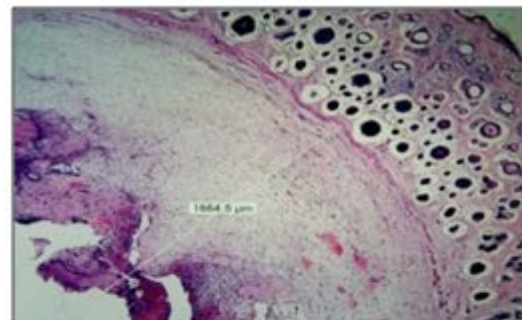


(a)



(b)

Fig. 1. (a) Number of fibroblasts in the control group (K5) and (B) study group (P4). (H-E 400X)]



(a)



(b)

Fig. 2. (A) Size of fibrosis in control group (K5) and (B) study group (P4). (H-E 40X)]

the tissue above it. Mesh acts as a barrier to the migration of inflammatory cells and fibroblast cells and indirectly reduce TGF- β levels, decreasing fibroblast/proliferation activity, reducing fibrin and collagen deposition on the surface of peridural tissue resulting in lower fibrosis and reduced the risk of adhesion.

DISCUSSIONS

The TGF- β induces proliferation, differentiation, and cell angiogenesis. It has chemotactic property to macrophages and fibroblasts. This molecule is the most potent molecule for macrophages. In addition to macrophages signaling to the injury site, it also stimulates the synthesis of various growth factors and inhibits the production of hydrogen peroxide which is cytotoxic to the fibroblasts proliferation. The results of fibroblasts stimulation will cause chemotaxis, proliferation, and stimulation of the extracellular matrix molecules such as type I collagen, and inhibition of proteolytic enzymes that function to degrade the newly formed connective tissues³. Specifically, TGF- β inhibits matrix degradation by decreasing metalloprotease secretion. Furthermore, TGF- β increases the secretion of protease inhibitors, such as plasminogen activator inhibitor-1. As the final results, these mechanisms will lead to the formation of adhesion as a result of increased production of extracellular matrix and decrease of matrix degradation, combined with decreased fibrinolytic activity⁵.

Luo *et al.* (2015), conducted research on the effects of rapamycin (RPM) in preventing the proliferation of fibroblasts, the formation of peridural fibrosis, and inflammatory activity by default in vitro and in vivo in mice that was performed laminectomy by calculating fibroblasts, Rydell's assessment, histological analysis, measurement of hydroxyproline levels, interleukin-6 (IL-6) and expression of TGF- β mRNA. The expression of TGF- β and IL-6 in the RPM group was significantly lower than control ($p=0.002$). TGF- β and IL-6 mRNA analyses were performed 4 weeks after laminectomy with a real-time PCR (RT-PCR) Quantitative technique in BioRad MYIQ2 (USA). In this study, the decrease in IL-6 and TGF- β regulation shows the efficacy of RMP in preventing inflammation⁹.

Fibroblasts are cells that produce collagen fibers, reticulum, elastin, glycosaminoglycans, and glycoproteins from intercellular substances. In adults, fibroblasts in the tissues experiencing changes. Mitosis is only visible if a person needs additional fibroblasts, i.e. if the connective tissue is injured. Fibroblasts are more active in synthesizing matrix components in response to wounds by proliferating and enhancing fibrinogenesis. Fibroblasts become the main agents in the wound healing process¹¹.

Research by Chen *et al.* (2014), focusing on the effect of HA preventing fibrosis formation and post-laminectomy adhesion using rabbits as a model, showing fibroblast cells count was significantly lower in the study group compared to the control group (3078 ± 313.68 vs 3742 ± 455.65 , respectively $p=0.042$). Fibroblasts may play an important role in the formation of peridural fibrosis derived from the perivertebral muscles and blood flow. Blocking the migration of fibroblasts into the surgical field is a measurable measure theoretically to prevent peridural fibrosis⁴.

Kelten *et al.* (2016) reported the use of pentoxifylline for inhibition of peridural fibrosis in Post-Laminectomy mice obtained similar results with this study. Evaluation of epidural fibrosis in 2 groups according to the macroscopic assessment and light microscope revealed that the formation of epidural scarring was lower in the study group compared with the control group ($p<0.001$) and the fibroblasts counts also decreased significantly in the pentoxifylline group study ($p<0.05$)⁸.

Oxidized regenerated cellulose is a degraded barrier which first used in clinical practice. It was a modification of its surgical precursor, which has long been used as a hemostasis agent. Mesh is designed to be placed above or between traumatized surfaces. Various studies have been conducted to evaluate the efficacy of ORC in preventing the formation of postoperative adhesion. A meta-analysis of 11 randomized control trial (RCT) trials with laparoscopy showed that ORC barrier proved safe and significantly reduced the incidence of de-novo adhesion compared with no use of the ORC barrier. Whereas in the laparotomy procedure, the study of a meta-analysis of several RCTs also showed a significant decrease in adhesion formation with the use of ORC compared to no barrier¹.

Examination of TGF- β levels, fibroblast cell count, fibrosis size and clinical adhesion in this study were limited only 7 days postoperative DC with mesh, so it is necessary to check also on day 3, day 7, and day 28 post-operative DC with mesh to know the difference at all stages of wound healing.

CONCLUSIONS

The TGF- β levels in the rat peridural tissue on the 7th day postoperative DC with mesh was lower than standard DC postoperatively, but not statistically significant. The number of fibroblast cells counts in mice peridural tissue on the 7th day postoperative DC with mesh was lower than standard DC postoperatively. The size of fibrosis in the rat peridural tissue on the 7th day postoperative DC with mesh was lower than standard DC postoperatively. The incidence of clinical adhesion in the rat speridural tissue on day 7 postoperative DC with mesh lower than standard DC postoperatively.

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