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Research Article

Adhesions, inflammatory response and foreign body giant cells infiltration of the topical hemostats TachoSil®, Hemopatch™ and Veriset™ – An Animal Study

Abstract

Background: When liver bleeding cannot be controlled by conventional methods, a topical hemostatic patch can be applied during surgery. In recent years new hemostats have become available. The aim of this study was to investigate the degree of adhesion and inflammation for three topical hemostatic patches, TachoSil®, Hemopatch™ and Veriset™.

Methods: In 60 adult male Sprague Dawley rats liver two lesions were induced with a scalpel. Each rat was treated with two of the three patches tested. After 1, 2 and 3 months the animals were euthanized and macroscopic evaluation of adhesions and histological assessment of inflammation and macrophage infiltration were performed.

Results: A significant higher ($p<0.05$) occurrence of foreign body giant cells (FBGCs) was found in Hemopatch™ and Veriset™, whereas both had a lower degree of inflammatory and macrophage infiltration compared to TachoSil®. No differences in the occurrence of adhesions were found.

Conclusion: Our study found evidence for difference in inflammation and formation of foreign body giant cells for the three hemostatic patches.

Introduction

Bleeding during hepatic surgery is associated with a higher risk of morbidity and mortality and cannot always be controlled by compression, ligature or other conventional procedures [1-4]. The occurrence of bleeding during liver resections and liver transplantations is minimized due to new methods of resection, such as segmented resections and proximal hemostasis [5,6]. Hemostatic sealants and topical patches are now also widely used. In Denmark, three patches are available for this purpose. TachoSil® (Takeda, Austria) is a topical hemostatic patch for mild or moderate bleeding based on human fibrin and thrombin on an equine collagen patch [7]. Hemopatch™ (Baxter AG, Austria) is made from bovine collagen with reactive pentaerythritol polyethylene glycol ether tetra-succinimidyl glutarate (NHS-PEG) on the active side, which initiates the organisms own clotting mechanisms [8,9]. Veriset™ (Covidien, USA) is a cellulose matrix with a non-specified polyethylene glycol (PEG) on the active side [10,11].

The risk of adhesions has proven to be lower by topical hemostatic patches compared to conventional methods of hemostasis [12,13]. However, biomaterials cause a foreign body response, which is the end-stage response of the inflammatory and wound healing responses, characterized by protein adsorption, macrophage adhesion and fusion of the macrophages into foreign body giant cells (FBGCs) [14]. FBGCs are found in larger numbers in adhesions, and indicative for adhesion formation [15].

The aim of our study was to compare three topical hemostatic patches with respect to occurrence of adhesion and inflammation, macrophage infiltration, and occurrence of FBGCs.

Materials and Methods

The study was designed as a randomised trial with 60 adult male Sprague Dawley rats. The rats were divided in three groups of 20 rats. Each rat received 2 patches on 2 separate

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liver lobes. The patches were divided through randomisation with all rats receiving 2 different patches. Two standardized lesions (diameter 1 mm) were made with a scalpel after surgical exposure of the liver on separate lobes, and a patch was applied on each location. One group was euthanized 1 months after surgery, a second group after 2 months and a third group was euthanized 3 months after surgery. One rat was euthanized preliminary due to post-operative complications, and not included in the study.

The study was approved by an ethical committee (Danish Animal Experiments Inspectorate, j.nr. 2012-15-2934-00129) and performed at approved animal facilities.

Anaesthesia

The rats had a mean weight of 266 ± 19.4 grams (mean ± SD) on the day of the operation. They were anaesthetized by SC injection of fentanyl 236 µg/kg, fluanisone 7.5 mg/kg and midazolam 3.75 mg/kg. The rat was placed on a heated operating table and an SC injection with 3 ml was given and Viscotears applied on the eyes to prevent dehydration, whereas oxygen was supplied via a nose mask.

Surgery

A 3 cm longitudinal midline incision was made from the *Proc. xiphoideus* and the abdominal wall was opened by blunt dissection. The liver lobe was held with cotton tip and a standardized lesion was made with an approximate diameter of 1 mm, ensuring to perforate the liver capsule and liver parenchyma. This always yielded a bleed, which is necessary for the patches to function. A patch measuring 20x20 mm was applied using the manufacturer's instructions. In most cases this was enough to ensure hemostasis, but in some of the rats, application was difficult. The patch either slipped off or did not cover the lesion. In these cases the patch was reapplied. The abdominal wall was closed with a continuous suture with 4-0 Vicryl suture (Ethicon, Belgium) and the skin was closed with 6-10 clips (Visistat® Weck skin stapler 35w, Teleflex Medical, USA).

Postoperative procedures

The rats were placed in individual cages for three days, and treated with buprinorphine (Temgesic, 40 µg/kg SC) for 72 hours postoperatively every nine hours. They had access to standard rodent chow, water and Diet Gel® (Clear H_20, USA). Hereafter they were placed in home cages, with four rats in each cage.

Necropsy

The rats were euthanized in pairs in a CO_2 chamber which was gradually filled with oxygen 1 l/min and carbon dioxide 5 l/min. and necropsy was performed. The degree of adhesion was scored according to Zühlke et al. [16], including dissemination from liver and patch area to the abdominal wall or other organs. The liver was extracted and preserved in a formalin solution.

Histology

The livers were processed and stained with Haematoxylin Eosin and Sirius Red. The slides were scanned with a high-resolution scanner from Hamamatsu (NanoZoomer 2.0-HT C9600, Photonics, Japan), 20x magnification, and analysed using the software specific NPD-viewer (Viewing software, NDP.view2 U12388-01, Japan).

Occurrence of inflammation (plasma cells and lymphocyte infiltration) and macrophages in the inner and outer layer of the patch was scored and presence of FBGCs was graded. The occurence of fibrosis and neavascularization was included as well. Odds Ratios (OR) were calculated using the score for TachoSil as a reference. The OR for Hemopatch and Veriset was calculated as the deviation from the score of TachoSil.

Statistical analysis

We used non-parametric statistics for binary data. The statistical analysis was performed with Stata® 13.1 (StataCorp, USA). The tests used were $\chi 2$, Fisher's exact test and logistic regression. All data was converted to binary data to make the data easily interpretable and presentable.

Results

The degree of adhesion, including dissemination from liver and patch area to the abdominal wall or other organs is presented in table 1. No significant differences were found.

Occurrence of inflammation (plasma cells and lymphocyte infiltration) and macrophages in the inner and outer layer of the patch was scored and presence of FBGCs was graded, and presented as Odds Ratios with TachoSil as a reference. The results are presented in tables 2,3, together with the occurrence of fibrosis and neovascularization.

No differences were found between the groups euthanized after 1, 2 or 3 months, and consequently the results from the groups were pooled. TachoSil® had significantly higher inflammation scores than Hemopatch™ and Veriset™ for both inner and outer layer. Also, TachoSil® had a significantly higher occurrence of macrophages than Hemopatch™ and Veriset™ for the inner layer, while no significance was found for outer layer. FBGCs in the inner layer were found more often in Hemopatch™ and Veriset™ than in TachoSil®. No significant differences between the patches were found for FBGCs in the outer layer.

Table 1: The degree of adhesion, including dissemination from liver and patch area to the abdominal wall or other organs.

Adhesion score	Characteristics
0	No adhesions
1	Filmy adhesions separable by blunt dissection
2	Stronger adhesions where blunt dissection is possible or partly sharp dissection is necessary. Minimal vascularisation
3	Strong adhesions where lysis is only possible by sharp dissection. Clear vascularisation
4	Very strong adhesions where lysis is only possible by sharp dissection. Organs strongly attached with severe adhesions and damage to organs hardly preventable

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Table 2: Odds Ratio (OR) of inflammation (plasma cell and lymphocyte infiltration), macrophage infiltration and the occurrence of foreign body giant cells (FBGCs), as well as OR scores for fibrosis and neovascularisation with TachoSil as reference.

		Hemopatch[a]			Veriset[a]		
		OR[b]	[95% CI][c]		OR[b]	[95% CI][c]	
Inflammation	Inner	0.34*	0.13	0.85	0.29*	0.11	0.72
	Outer	0.27*	0.09	0.76	0.13*	0.04	0.43
Macrophages	Inner	0.25*	0.09	0.69	0.17*	0.06	0.49
	Outer	0.44	0.13	1.48	0.25*	0.06	0.99
FBGCs[d]	Inner	0.20*	0.07	0.55	0.32*	0.07	0.86
	Outer	0.78	0.24	2.52	3.79	0.71	20.14
Fibrosis	Inner	0.14	-0.14	0.43	0.14	-0.15	0.42
	Outer	0.04	-0.39	0.46	-0.54*	-0.97	-0.11
Neovascularisation	Inner	0.66	0.26	1.64	0.44	0.17	1.12
	Outer	2.14	0.80	5.71	1.61	0.60	4.32
Remaining Patch		1.05	0.14	7.85	0.16*	3.79	78.26
Patch infiltration		1.04	0.20	5.50	0.27	0.07	1.08
FBGCs[d] in patch		9.16*	3.17	26.42	0.52	0.19	1.42
Patch folding	Right and Left lobe	5.92*	1.17	30.04	1.39	0.00	0.12
	Left lobe	11.77*	1.32	105.01	1.89	0.01	22.79

All data has been calculated for groups A, B and C combined.
***Significant p-value < 0.05.**
[a]All results in the table are calculated with TachoSil as reference.
[b]OR: Odds Ratio.
[c]95% CI: 95% Confidence Interval.
[d]FBGCs: Foreign Body Giant Cells.

Table 3: Number of patches divided between the 60 rats. Each rat was treated with 2 patches and divided in 3 groups which were euthanized after 1, 2 and 3 months. Each rat received one patch on their right liver lobe and one on the left liver lobe.

	TachoSil		Hemopatch		Veriset	
	Right Lobe	Left Lobe	Right Lobe	Left Lobe	Right Lobe	Left Lobe
Rats treated with the patches for 1 month	n=5	n=8	n=8	n=6	n=7	n=6
Rats treated with the patches for 2 month	n=8	n=5	n=5	n=8	n=7	n=7
Rats treated with the patches for 3 month	n=7	n=6	n=5	n=7	n=8	n=7

Discussion

The pathophysiology of adhesion formation is not fully understood. It is believed that the formation of fibrin, an essential part of the coagulation cascade, acts as a scaffold for collagen deposition and neovascularisation. If the fibrin is not lysed within 5-7 days of surgery the temporary matrix persists and gradually becomes an organized peritoneal adhesion [17]. The three patches caused a similar degree of adhesion, so the different components of the patches, human fibrin and thrombin on an equine collagen in TachoSil®, NHS-PEG in Hemopatch™ and PEG in Veriset™ have no influence on adhesion formation.

According to Arung et al. [17,18], adhesion formation is the result of an inflammatory response to tissue injury. Our study did not find a positive association between markers of inflammation and the formation of adhesions, even in a patch yielding a high amount of inflammation. According to Nohuz et al. [12], TachoSil® is preventative in formation of adhesions in a rat model, where TachoSil® is tested against electrocoagulation. We could not confirm this finding, since TachoSil® caused a similar level of adhesions as Hemopatch™ and Veriset™. However, results from rat studies cannot be necessarily extrapolated to humans due to the many factors that play a role in adhesion formation [19].

Hemopatch™ and Veriset™, both patches containing PEG, caused higher presence of FBGCs than TachoSil®. Anderson et al. [14], state that particles of non-specified polyethylene from prosthesis and other biomaterials induce FBGC-formation, and explains this as the result of frustrated phagocytosis. FBGCs help degrading foreign bodies by releasing enzymes and reactive oxygen intermediates. Nagelschmidt et al. [20], showed that an intraperitoneal PEG-solution, given after a laparoscopic procedure, reduces the formation of adhesions, suggesting that PEG containing patches should cause fewer adherences. FBGCs are found in larger numbers in adhesions, and are indicative for adhesion formation [15], so it would be expected that Hemopatch™ and Veriset™ had lower levels of FBGCs if the PEG in these patches would reduce the formation of adhesions. The FBGC-reaction to PEG, and the importance of FBGCs in adhesion formation is an interesting area for further investigation.

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