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Can Anti–Adhesive Efficacy of Sodium Hyaluronate and Carboxymethylcellulose Membrane Be Improved Further by Colchicine and Collagenase?

Sodyum Hiyaluronat ve Karboksimetilselluloz Membranın Anti-Adezif Etkinliği Kolşisin veya Kollojenaz ile Artırılabilir mi?

ABSTRACT

Aim: The aim of this study was to determine the impact of collagenase and colchicine in the prevention of postsurgical adhesion formation in a rat uterine horn model.

Materials and Methods: Forty female Wistar albino rats were divided into four equal groups including controls, seprafilm, colchicine-seprafilm and collagenase-seprafilm groups. After laparotomy, a standard 0.5-cm injury was made to the antimesosalpingeal side of the right proximal uterine horn by unipolar cautery. After hemostasis, 0.5 cm seprafilm or colchicine 2 mg-0.5 cm seprafilm or collagenase 1µg – 0.5 cm seprafilm was applied to the site of injury. The abdominal incision was then sutured en bloc and the rats were observed for 20 days. Then, all rats were sacrified and second look laparotomy was performed. Macroscopic adhesion score of each rat was noted and histopathologic scoring was made according to Kanbour-Shakir criteria.

Results: All of the five scores of the histologic parameters were lowest in the colchicine-seprafilm group. The total histologic score of colchicine-seprafilm group was significantly lower than controls, seprafilm and collagenase-seprafilm groups ($p<0.05$).

Conclusions: The results of this rat model suggested that local use of colchicines in the abdominal operations prevents formation of postsurgical adhesions based on both visual assessment and histological analyses. The promising result obtained from this experimental study needs further investigation.

Key words: Collagenase, Colchicine, Seprafilm, Postoperative Adhesions, Rat, Uterine Horn.

ÖZET

Amaç: Cerrahi sonrası intraperitoneal adezyonların oluşumunu önlemede kollajenaz ve kolşisinin etkinliklerini deneysel hayvan modelinde histopatolojik değerlendirme ile araştırmaktır.

Gereç ve Yöntem: Çalışmada 40 adet dişi Wistar Albino cinsi rat kullanıldı. Ratlar, kontrol, seprafilm, kolşisin-seprafilm, kollajenaz-seprafilm olmak üzere dört gruba ayrıldı. Laparotomi ile sağ proksimal uterin horn antimezosalpingeal yüzde bisturi aracılığı ile 0.5 cm'lik bir kesi oluşturuldu. Takiben kesi üstüne deneklerin grubuna uygun olarak 0.5 cm'lik Seprafilm; 2 mg Kolşisin emdirilmiş 0.5 cm'lik Seprafilm; 1µg kollajenaz emdirilmiş 0.5 cm'lik Seprafilm yerleştirilerek batın kapatıldı. Kontrol grubuna herhangi bir ajan uygulanmadı. Ratlar 20 gün sonra sakrifiye edilerek makroskopik ve histopatolojik değerlendirmeleri yapıldı. Histopatolojik inceleme Kanbour-Shakir kriterlerine göre yapıldı.

Bulgular: Histopatolojik olarak değerlendirilen beş parametrenin toplamı ile elde edilen histolojik toplam skorun kolşisin-seprafilm grubunda en düşük olduğu görüldü. Kolşisin-seprafilm grubunun total histolojik skoru ile kontrol, seprafilm ve kollajenaz-seprafilm gruplarının ortalama histolojik skorları arasında istatistiksel olarak anlamlı ölçüde farklılık bulundu ($p<0.05$).

Sonuç: Batın içi operasyonlarda kolşisinin uzun süre lokal olarak etki etmesini sağlayacak bir metod ile kullanımı, cerrahi sonrası adezyon oluşumunu hem makroskopik hem de histolojik olarak azaltmaktadır. Bu deneysel çalışmadan elde edilen umut verici sonuçların araştırılacağı daha geniş kapsamlı çalışmalara ihtiyaç vardır.

Anahtar kelimeler: Kollajenaz, Kolşisin, Postoperatif Adezyon, Rat, Seprafilm, Uterin Horn

INTRODUCTION

Peritoneal adhesions continue to be an unresolved problem. Pelvic adhesions following gynecologic surgery may cause significant morbidity; bowel obstruction, chronic pelvic pain, infertility and ectopic pregnancy. In 56% of

the patients with small bowel obstruction, history of a gynecological operation exists and hysterectomy carries a 2.8% future risk of small bowel obstruction (1,2).

Inflammatory response following peritoneal trauma results in exudation of proteinous exudate rich in fibrinogen and inflammatory cells. By the day 3 or 4, formation of fibrin matrix follows which will consolidate into fibrin polymers or the fibrin matrix will dissolve and reabsorbed by fibrinolytic system. Insufficient fibrinolysis will result in organization and formation of fibrinous bands. With ingrowths of cellular elements such as fibroblasts that secretes collagen into fibrinous bands, consolidation and adhesion formation are completed in nearly 7 days (3,4).

Despite the intensive research, no satisfactory preventive measure has been validated so far in adhesion prevention. Tissue opposition and tissue separation techniques found limited use in clinical practice. However, none had unequivocal success over others. Sodium hyaluronate and carboxymethylcellulose membrane (HA/CMC) (Seprafilm®) is the most studied one and is one of the three adhesion barrier approved by FDA. It has satisfactory biocompatibility, adherence to tissue, low side effect and optimal reabsorbing time at surgical surface but high cost and difficult handling. Despite its accepted efficacy, adhesions could not be completely eliminated (5,6).

Colchicine, an effective drug used in familial Mediterranean fever (FMF) has been used previously as an anti-adhesive agent successfully in a few experimental studies (7-10). Anti-adhesive efficacy is probably related to its antimitotic, anti-inflammatory and also collagenolytic properties.

Clostridium peptidase A is a collagenase that degrades collagen and extracellular matrix proteins, derived from the bacteria Clostridium histolyticum and is a member of the metalloproteinase enzyme family. The collagenolytic property of Clostridium peptidase A and other matrix metalloproteinases are under investigation for their potential role in adhesions prevention (11,12).

In this experimental study our goal was to find out if the anti-adhesive effect of the HA/CMC membrane could be further potentialized by other agents that are proposed to have antiadhesive; colchicine and collagenase.

MATERIALS AND METHODS

The study was conducted in Duzce University School of Medicine Experimental Animal Laboratory under approval and supervision of Duzce University School of Medicine Experimental Animal Care and Protection Ethics Committee (07.03.2008 / 100-3).

Forty female non-pregnant Wistar albino rats weighting 185-215 g were randomly assigned to four groups with 10 rats in each group. The animals were kept under standard laboratory conditions, 20-25°C; humidity %50-60; 12:12 hour light/dark cycle and free access to food and water ad libitum.

Surgical Procedures

All surgical procedures were performed by the same surgeon. Anesthesia was induced by subcutaneous ketamine HCL at 50 mg/kg (Ketalar flk, 500 mg/10 mL, Eczacıbası; Istanbul, Turkey). After shaving of the lower abdominal area followed by antisepsis with iodine, laparotomy was carried out with 3 cm midline vertical abdominal incision. Transverse 0.5 cm hysterotomy incision was applied to the right uterine horn anti-mesosalphingeal border with no:15 scapel. Homeostasis was carried out only with compression and washing out of incision. After application of anti-adhesives to incision, fascia and skin were closed with 2/0 chromic catgut sutures. No antibiotics were used. Follow up and care for incision maintained for 21 days. After 21 days second-look laparotomy was performed and adhesions were evaluated with a surgeon blinded to the groups. The uterus and ovaries were removed enblock, fixed in %10 formol and embedded in paraffin blocks. 3-4 μm tissue sections from blocks were obtained and stained with hematoxylin and eosin. Evaluation for collagen formation was done after staining sections with standardized Masson trichrome.

Experimental groups

Forty rats were assigned to four groups with ten rats in each group as follows: In group 1 (control group): hysterotomy was done and laparotomy was closed without any application of

anti-adhesive; In group 2 (HA/CMC group) 0.5 cm2 sodium HA/CMC membrane (HA/CMC-Seprafilm®) was applied to cover the lesion. In group 3 (HA/CMC + colchicine group) 0.5 cm2 sodium HA/CMC impregnated with 2mg colchicine (Colcemide solution10μg/ml, Biological Industries) was applied to cover the lesion. In group 4 (HA/CMC + collagenase group) 0.5 cm sodium HA/CMC impregnated with 2 mg collagenase (Collagenase, lypholized, prepared from Cl. Histolyticum) was applied to cover the lesion.

Adhesion Evaluation

Adhesions were graded according to the clinical adhesion scoring system as described by Linsky (Table-1). Macroscopic evaluation of the adhesion were as follows: Grade 0 represented no adhesion, grade 1 represented adhesion covering %25, grade 2

Table-1. Comparison of groups for histological parameters involved in adhesion pathogenesis

Histologic parameter	Control	HA/CMC	Colchicine+HA/CMC	Collagenase+HA/CMC
Inflammation	1.00±0.53	0.63±0.51	0.89±0.78	1,50±0.53
Foreign body reaction	0	0	0.11±0.33	**0.75±0.88**
Fibroblastic activity	2.50±0.53	2.75±0.46	**1.56±0.72**	2.25±0.46
Collagen formation	2.38±0.51	2.38±0.51	**1.44±0.52**	2.13±0.35
Vascular proliferation	2.63±0.74	3.00±0.75	2.33±0.79	2.88±0.35
Total score	8.50±1.30	8.75±1.48	**6.33±2.34**	9.50±1.19

Total score Colchicine+HA/CMC vs. control; $p<0.05$
Total score Colchicine+HA/CMC vs. HA/CMC; $p<0.05$
Total score Colchicine+HA/CMC vs. Collagenase+HA/CMC; $p<0.05$

Table-2. Comparison of groups for adhesion scores.

Macroscopic parameter	Control	HA/CMC	Colchicine+HA/CMC	Collagenase+HA/CMC
Adezyon thickness-tenacity	1.50 ±0.75	**0.75±0.88**	1.22±0.83	1,50±0.53
Adhesion resistance	1.25 ±0.46	**0.50±0.53**	0.89±0.60	1.25±0.46
Total score	2.75 ±1.16	**1.25 ±1.38**	2.11 ±1.36	2.75 ±0.88

Total score Control vs. HA/CMC; $p<0.05$
Total score Control vs. Colchicine+HA/CMC; $p>0.05$
Total score Control vs. Collagenase+HA/CMC; $p>0.05$
Total score Colchicine+HA/CMC vs. HA/CMC; $p>0.05$
Total score Collagenase+HA/CMC vs. HA/CMC; $p>0.05$

represented adhesion covering %50 and grade 3 represented adhesion covering the full surface of lesion. Tenacity of the adhesions was evaluated as follows: grade 0 adhesion falling apart without resistance upon traction, grade 1 representing a medium level of resistance that could be separated with moderate traction and grade 2 adhesion representing high resistance that could be separated only with sharp dissection. Total adhesion score were calculated as the sum of the scores of adhesion extent and resistance.

Histopathological evaluations were performed by a pathologist blinded to groups. Preparations were evaluated under light microscopy for inflammation, fibroblastic activity, foreign body reaction, collagen synthesis and vascular proliferation (Figure 1-3). These parameters were evaluated semi quantitatively with adhesion histologic scoring system as Kanbour-Shakir et al. described.

Statistical Evaluation

Groups were compared by Kruskal-Wallis, Tukey HSD test. Data were presented as mean values and standard deviations (mean ± sd). Level of the statistical significance was set as $p<0.05$.

RESULTS

Interventions were well tolerated by the animals and only two mortalities occurred; one in HA/CMC + collagenase group (group 4) because of endometrial abscess and another died due to focal necrotizing infection in uterine serosa. Relaparotomy was performed in 38 rats 3 weeks after the initial surgery.

Histopathological results

The histopathological results are shown in Table 1. Considering inflammation, HA/CMC group had the lowest score among all groups but neither the HA/CMC group nor others had significantly less inflammation score than control group.

Fibroblastic activity score of the HA/CMC +

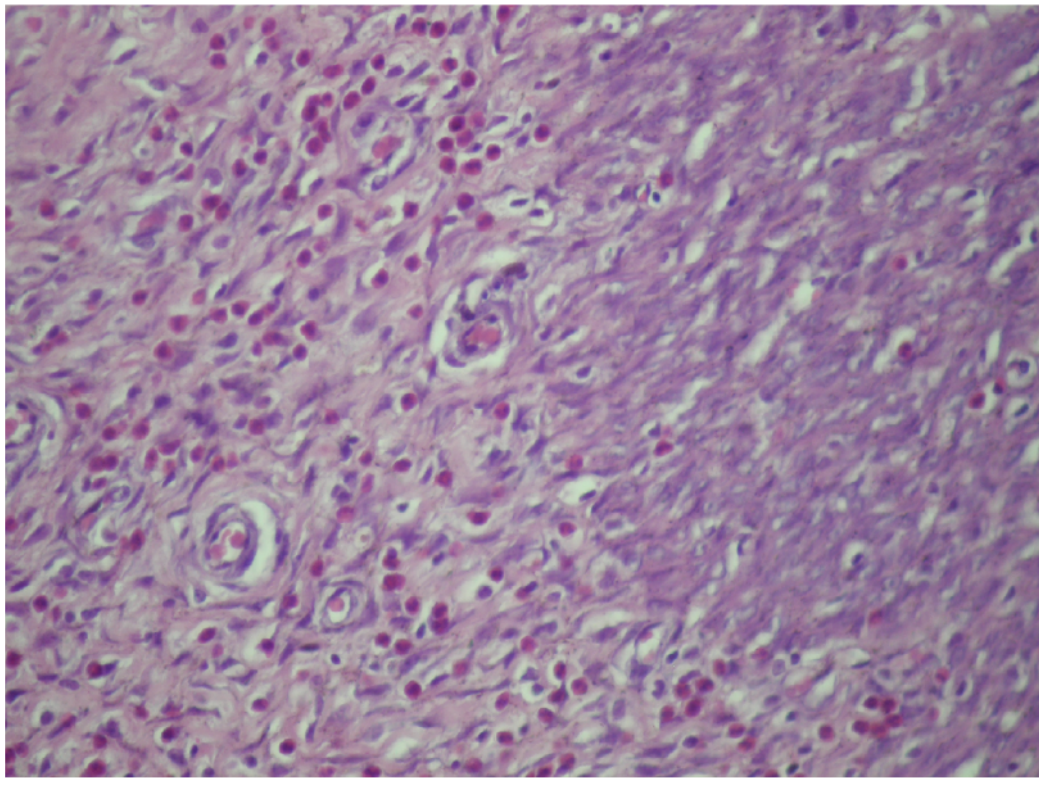

Figure 1: Grade-2 Mixed inflamation (H&EX200)

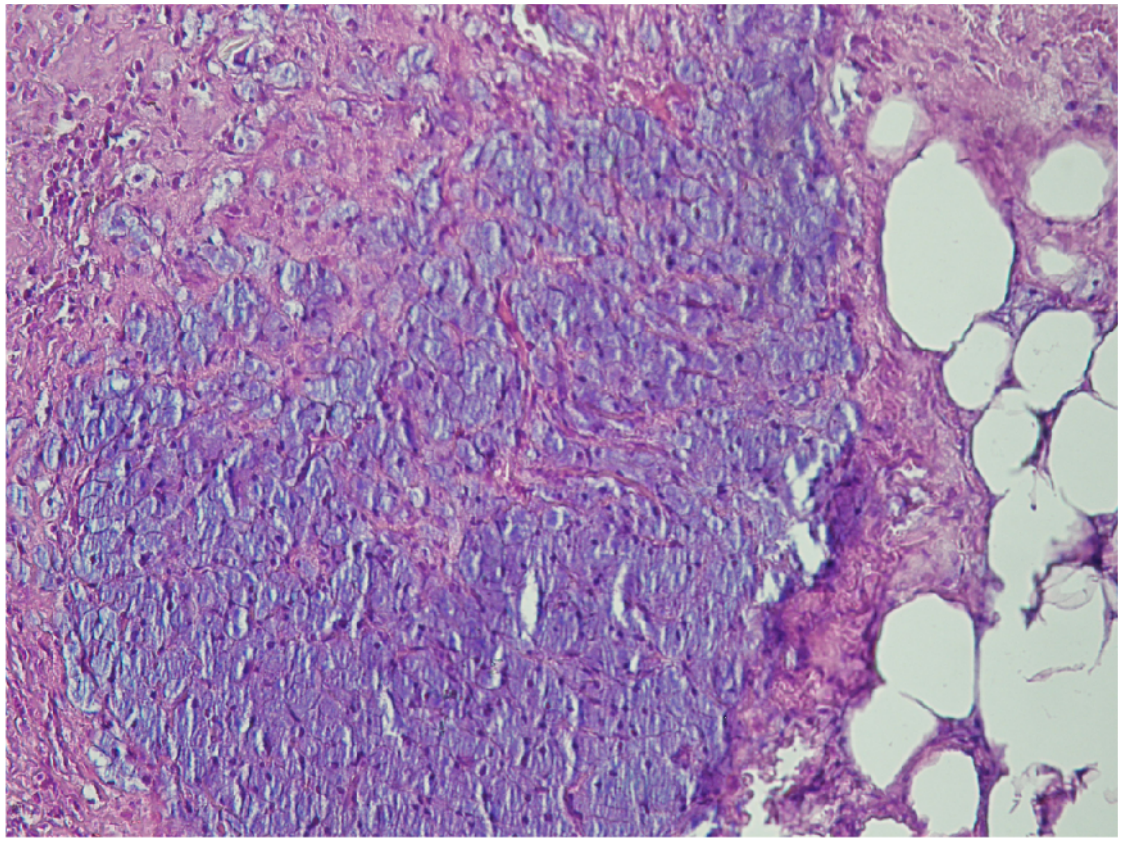

Figure 2: Foreign body reaction-(H&EX200)

colchicine group was significantly lower than the control and the HA/CMC groups ($p<0.05$ and $p<0.001$ respectively). There was no significant difference between HA/CMC + colchicine and HA/CMC + collagenase groups.

Foreign body reaction was not observed in control and the HA/CMC groups. Foreign body reaction in HA/CMC + Collagenase group was significantly

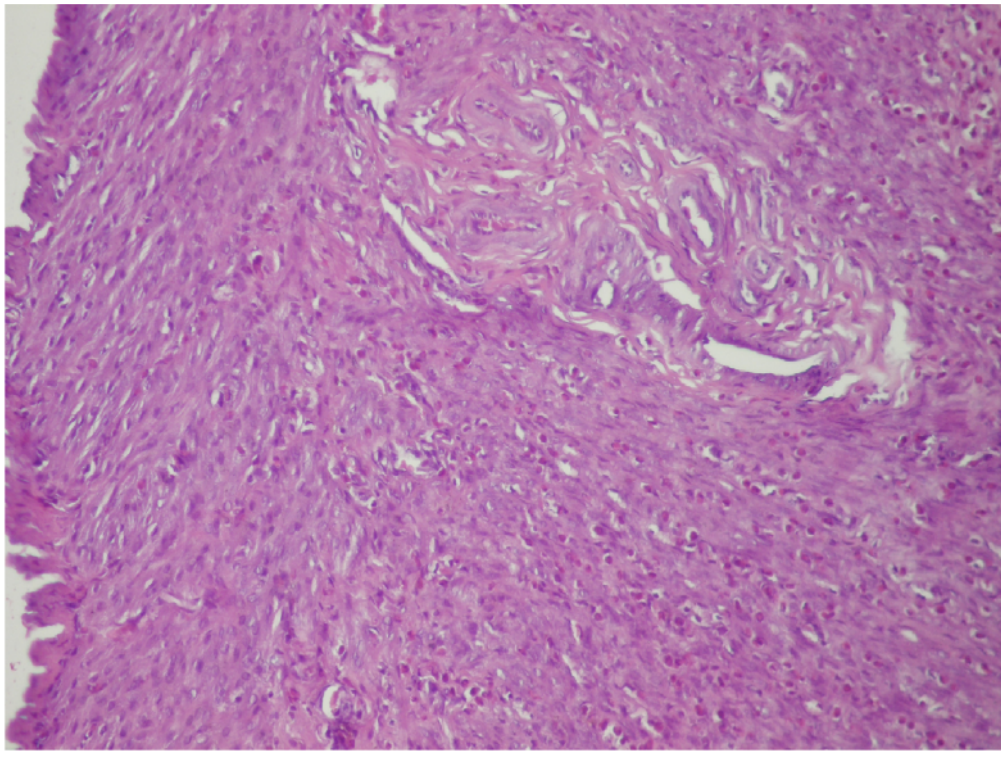

Figure 3: Distinctive collagen filament increase (H&EX100)

severer than all other groups.

Collagen formation was lowest in HA/CMC + colchicine group and reached statistical significance compared to control and HA/CMC groups ($p<0.001$, $p<0.05$ and $p<0.05$ respectively). There was no significant difference between HA/CMC + colchicine and HA/CMC + collagenase groups.

HA/CMC + colchicine group had the lowest score considering vascular proliferation but the results did not reach statistical significance.

Total score of the five histopathologic parameters was significantly lower in the HA/CMC + colchicine group than control, HA/CMC groups and HA/CMC + collagenase groups ($p<0.05$).

Macroscopic Evaluation

HA/CMC group had less adhesion scores compared to controls but adhesion scores of HA/CMC + colchicine and HA/CMC + collagenase groups were higher than HA/CMC group and was not different than controls (Table 2).

DISCUSSION

In this study we found that the total macroscopic adhesion score of HA/CMC group was lower than HA/CMC + Colchicine and HA/CMC+Collagenase groups although the difference was not significant. HA/CMC membrane group was the only group to show significantly smaller total adhesion score compared to control group. Neither colchicine nor collagenase have contributed to the anti-adhesive efficacy of HA/CMC membrane.

Histological total score was significantly lower in HA/CMC + Colchicine group compared to other groups but this was not reflected in macroscopic scores since adhesion scores was not significantly different than the control or NA/CMC groups. HA/CMC group had the lowest inflammation score (not significant) but total histologic score was not different than controls. Also histologic scores of the HA/CMC + Collagenase group was not different than the control group.

HA/CMC is an inert bioabsorbable anti-adhesive membrane composed of sodium hyaluronate and carboxymethylcellulose. It becomes a hydrophilic gel in 24-48 hours upon application on tissues and remains there for 7 days. Mechanism of action is not completely known but it most likely acts as a lubricating physical barrier rather than acting on molecular mechanisms in adhesion formation. Previously Gago et al. have suggested that products of peritoneal and adhesion fibroblasts and mesothelial cells of HA/CMC treated group (mRNA of matrix transforming growth factor-β1, type I collagen, metalloproteinase-1, matrix metalloproteinase-2) were not different than controls (13). They concluded that ability of HA/CMC membrane to reduce the postoperative adhesions is most likely dependent on its tissue separation without interfering with biologic processes involved in adhesion.

Our histologic results partially support Gago et al. since we found that HA/CMC membrane group had

no effect on fibroblast proliferation, collagen formation and total histological scores compared to control group. HA/CMC membrane group had reduced low inflammation score but this has not reached statistical significance. Using an adjunctive which can decrease collagen formation or that has collagenolytic property such as colchicine and collagenase to increase the efficacy of HA/CMC membrane seemed reasonable to us. However, despite the lowest total histologic scores of HA/CMC + Colchicine group, this was not reflected in macroscopic adhesion scores. Fibroblastic activity and the collagen formation were decreased significantly with addition of colchicine to HA/CMC membrane. This was consistent with collagenolytic and antifibroblastic activity of colchicine. But colchicine failed to increase the anti adhesive effect of HA/CMC membrane macroscopically as collagenase did not.

Previous experimental studies suggesting the anti-adhesive effect of colchicine mostly used systemic route (oral or intramuscular) (8-10). The pioneer study showing the anti-adhesive effect of colchicine used it by intraperitoneal injections for 3 weeks (7). But we used colchicine and collagenase solution only once at a time with impregnating it into HA/CMC membrane. This may have reduced the persistency of colchicine during the inflammatory and collagen formation periods of adhesion formation. Another possible mechanism may be the incompatibility of HA/CMC with colchicine since foreign body reaction was detected with impregnation of colchicine to HA/CMC but that wasn't the case with HA/CMC membrane group or control group. This incompatibility may also apply to collagenase since bacterial collagenase alone has been previously used successfully as an anti-adhesive by directly applying it on to intestines in one study (11).

CONCLUSIONS

In conclusion our results showed that the addition of neither colchicine nor collagenase have further improved the anti-adhesive effectiveness of the most commonly used physical barrier; NA/CMC membrane. Different doses, routes and times of administration of these combinations should be evaluated.

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