Effect of intraoperative irrigation with alteplase on adhesion formation associated with intraperitoneal chemotherapy (experimental study)

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Summary

Objective: To examine the efficacy of intraoperative irrigation with alteplase, a tissue plasminogen activator, for the prevention of adhesion formation associated with intraperitoneal chemotherapy. *Material and Methods:* Rats, in which serosal injury was induced in the right uterine horn and ipsilateral parietal peritoneum, were randomly divided into four groups. Group 1 (n = 10) had intraoperative intraperitoneal irrigation with alteplase following the standard operation. Group II (n = 10) had irrigation with normal saline, while the rats in Group III (n = 10) and Group IV received no peritoneal irrigation. All rats, except for those in Group IV, received intraperitoneal (IP) paclitaxel plus carboplatin chemotherapy on the seventh postoperative day, and all rats were sacrificed seven days after chemotherapy. Total adhesion scores in the induced standard defects were calculated by evaluating percentage of adhesion formation, as well as the severity and degree of the adhesions. The scores were compared among the groups. *Results:* Comparison of the severity, percentage, degree and total score of adhesions among the groups demonstrated that subjects in Group I, where intraoperative alteplase irrigation was used, had fewer adhesion components (severity, percentage, degree) and a lower total adhesion score when compared to the other groups ($p < 0.05$). Adhesion components and the total adhesion score in Group IV, which did not receive chemotherapy, were found to be significantly lower when compared to Groups II and III ($p < 0.05$). *Conclusion:* Intraoperative Alteplase irrigation may reduce adhesion formation associated with intraperitoneal chemotherapy. Thus, intraperitoneal chemotherapeutic agents may be ensured to reach all peritoneal surfaces easily.

Key words: Intraperitoneal chemotherapy; Adhesion formation; Paclitaxel; Carboplatin; Alteplase.

Introduction

Recently, intraperitoneal chemotherapy has been reported to significantly increase survival rates when compared to intravenous chemotherapy in both ovarian and other cancers [1]. More specifically, it has been reported that intraperitoneal use of cisplatin plus paclitaxel combination in ovarian cancer significantly increases survival rates when compared to standard intravenous chemotherapy [2]. However, this route of chemotherapy administration is not perfect yet, and has some specific disadvantages, side-effects and complications. Intraperitoneal chemotherapy requires experience, causes port-associated complications and intraabdominal adhesions. The intraabdominal adhesion rate associated with intraperitoneal chemotherapy has been reported to be as high as 21.8% [3].

Experimental studies investigating the physiopathology of adhesion formation have shown that there is a direct relation between a decrease in fibrinolytic activity and an increase in adhesion formation [4]. In previous studies, tissue plasminogen activators (TPA), as fibrinolytic agents, have been used to prevent postoperative adhesion formation, and produced successful results [5, 6]. However, TPAs have not been used to prevent adhesion formation after intraperitoneal chemotherapy procedures.

The present study investigated the efficacy of alteplase, a TPA, in adhesion formation associated with intraperitoneal paclitaxel-carboplatin administration.

Material and Methods

Care and feeding of animals

The present study was carried out in the "Experimental Animals Study Unit of Firat University". The local ethics committee approved the study protocol (no: 24-2005). The study included 39 Wistar-Albino rats weighing between 200 and 300 grams. Before the operation, all rats were kept in an environment with 12-hour light and 12-hour dark periods and 50-60% moisture. Feed and water for all rats were given ad libitum. "Ethical guidelines for experimental studies on animals" as identified in the 1993 Helsinki declaration were followed throughout the study period.

Formation of groups and the experiment

The rats were randomly divided into four groups. Of these, the first three included ten rats each (Groups I, II, III), and the last one was comprised of nine rats (Group IV). The standard operation was performed in all groups. Following the standard operation, intraabdominal irrigation was conducted immediately after surgery using 1 mg Alteplase (Actilyse®, Boehringer Ingelheim) in 5 ml normal saline (NS) in Group I, and the same volume of NS without alteplase in Group II. No intraoperative irrigation was performed in Groups III and IV. The first three groups received intraperitoneal chemotherapy (paclitaxel; 3.5

Revised manuscript accepted for publication August 23, 2007

Table 1. — *Detailed presentation of distribution of components comprising the total adhesion scores by groups*.

Group I (n = 10)			Group II (n = 10)			Group III (n = 10)			Group IV (n = 9)		
Percent	Severity	Degree	Percent	Severity	Degree	Percent	Severity	Degree	Percent	Severity	Degree
1	1	1	2	3	2	3	3	3	3	2	3
0	0	0	1	2	2	2	2	2	2	3	2
2	1	1	2	3	2	2	3	2	3	3	3
1	1	1	2	2	3	2	2	2	3	3	3
1	2	1	2	2	3	2	1	2	3	3	3
2	1	1	2	2	2	2	2	1	2	2	3
1	1	1	2	2	2	2	2	2	2	2	3
1	2	1	2	2	2	2	2	2	3	3	3
1	1	1	2	2	2	2	1	2	3	3	2
1	1	2	2	2	2	2	2	3			

Percent: Percentage of adhesion formation, Severity: Severity of adhesion formation, Degree: Degree of adhesion formation.

mg/kg and carboplatin; 10 mg/kg) on the seventh postoperative day, whereas no chemotherapy was administered to Group IV. The study design is demonstrated in Figure 1.

All rats were sacrificed on the seventh day following intraperitoneal chemotherapy by cervical dislocation, and a relaparotomy was conducted. The percentage of adhesion formations in the abdomen and the areas where standard operations were performed, and the severity and degree of the adhesions were assessed, and the total adhesion score was calculated for each subject (Figure 2). Linsky's scale was modified and used as the scoring system [7]. The adhesion percentage was calculated as, no adhesion: 0 point, adhesions in 1-25% of the defect: 1 point, adhesions in 25-75% of the defect: 2 points, and adhesions in 75-100% of the defect: 3 points. Evaluation of the severity of adhesions was based on vascularization and thickening of the adhesion. According to that, no adhesion: 0 point, film-like adhesion with no vascularization: 1 point, adhesion with thin vessels and medium thickness: 2 points, and thick adhesion with marked vascularization: 3 points. The degree of adhesions was assessed depending on the endurance of the adhesion, so that no adhesion: 0 point, adhesion that could be separated by slight traction: 1 point, adhesion that could be separated by mild traction: 2 points, adhesions that required sharp dissection and severe traction: 3 points. Of these three adhesion components, the highest one was included for the evaluation. All visual scoring procedures were carried out by two independent researchers.

Standard Surgical Operation

All rats were anesthetised with xylazine (10 mg/kg) and ketamine (40 mg/kg) after six hours of fasting. Under sterile conditions and using sterile techniques, the intraperitoneal area was accessed through a 5 cm midline incision, and the standard operation was performed. In the standard operation, a 2-cm long by 1-cm wide area on the peritoneum of the right paracolic gutter was scraped using a bistoury to induce peritoneal injury, and a standard serosal incision of about 1 cm was made on the right uterine horn. A buffer was applied to the induced defects for two to three minutes to control bleeding. Layers of abdomen were sutured with 4/0 vicryl, and the skin with 4/0 silk.

Statistical analysis

The total score of each subject was calculated by adding all components (percentage, degree, severity). The scores obtained were compared among the groups. Kruskal Wallis and Mann-Whitney U-tests were employed as the statistical method, where appropriate. Level of significance was set at $p < 0.05$.

Results

Throughout the study period, there was no complication associated with surgery or intraperitoneal chemotherapy. No deaths nor decrease in the activity of rats were recorded during the study. Consistency between the inde-

Figure 1. — Study design.

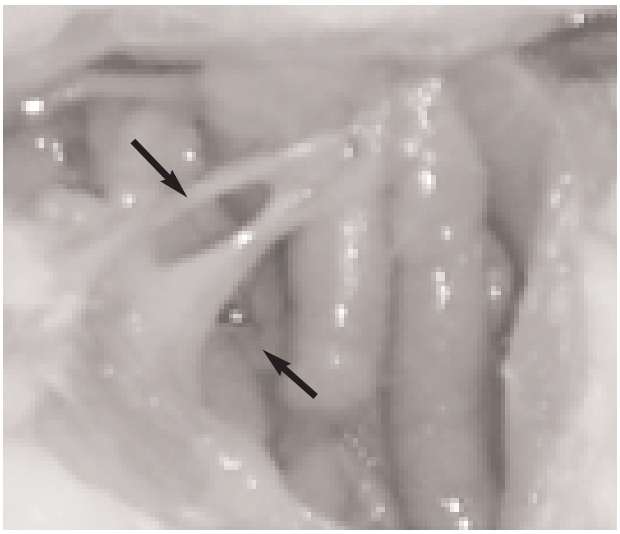

Figure 2. — The appearance of adhesions between the peritoneum and intestines.

pendent researchers who evaluated adhesion percentage, the severity and degree of adhesions was found to be 100%.

Percentage of adhesion formations, severity and degree of adhesions formed, and the calculated total adhesion scores in each group are presented in Table 1. Comparison among groups showed that each component of the total score, as well as the total adhesion score were significantly lower in Group I when compared to Groups II, III, and IV ($p < 0.05$) (Table 2). Both the components of the total score and the total adhesion scores were found to be significantly lower in Group IV when compared to Group II and Group III ($p < 0.05$). No significant difference was found between the score components and the total adhesion scores between Group II and Group III.

Table 2. — *Distribution of total adhesion scores by groups*.

	Percentage of adhesion formation	Severity of adhesion formation	Degree of adhesion formation	Total screening of adhesion formation
Group I	1.1 ± 0.5 [a]	1.1 ± 0.5 [d]	1 ± 0.4 [g]	3.3 ±1.2 [j]
Group II	1.9 ± 0.3 [b]	2.2 ± 0.4 [e]	2.2± 0.4 [h]	6.3 ± 0.6 [k]
Group III	2.1 ± 0.3 [b]	2.0 ± 0.6 [e]	2.1± 0.1 [h]	6.2 ± 1.2 [k]
Group IV	1.8 ± 0.8 [c]	2.6 ± 0.5 [f]	2.7± 0.4 [i]	8.1 ± 0.9 [l]

Different upper symbols in the same column shows statistical significance.

Discussion

Two important findings were obtained at the end of the study. The first is that intraperitoneal paclitaxel plus carboplatin combination significantly increased adhesion formation in experimental conditions, and the second is that intraoperative intraabdominal irrigation with alteplase, a TPA, significantly reduced adhesion formation associated with surgery plus intraperitoneal chemotherapy.

The purpose of intraperitoneal chemotherapy in ovarian cancer is to ensure direct penetration of the drug to the tumor tissue at a high concentration [3, 8]. This has been shown in clinical studies through the significant differences between survival rates in intravenous and intraperitoneal chemotherapy procedures. To ensure easy circulation of the intraperitoneal fluid, and thus the chemotherapeutic agents in the intraperitoneal area is the cornerstone for the rationale of this procedure. Thus, apart from the known complications of adhesion formation (like need for a reoperation, pain, ileus, etc.), lack of adhesions associated with intraperitoneal chemotherapy theoretically means that that the efficiency of the drug will increase [9]. Our study is an important preliminary study to eliminate chemotherapy-associated adhesion formation, which is one of the main obstacles for intraperitoneal chemotherapy administration.

Although previous clinical studies reported significant levels of adhesion formation in cases who received intraperitoneal chemotherapy [3], the intraperitoneal adhesions in these cases might have been associated with surgery and probably directly with cancer, besides chemotherapy. There are many studies in the literature which cover all these three conditions [3, 4, 8]. The rats used in our study did not have cancer, and therefore adhesion formation associated with cancer has not been discussed. It is obvious that further studies are needed at this point. However, the design of our groups clearly demonstrated the effect of intraperitoneal chemotherapy added to surgery on adhesion formation. When Groups III and IV were compared, it was observed that although standard surgery was performed in both groups, carboplatin plus paclitaxel-combination chemotherapy significantly elevated adhesion scores in Group III. This finding is consistent with the literature. Likewise, it was demonstrated in previous studies that use of intraperitoneal paclitaxel reduced adhesion formation, whereas cisplatin and carboplatin increased it [10-12]. When our findings are interpreted in light of these previous studies, it can be stated that the increase in adhesion formation might be associated with carboplatin.

TPAs have been used in many studies to prevent postoperative adhesions and produced successful results. Studies that comprehensively investigated the physiopathological events related with postoperative intraabdominal adhesion formation reported that this process was initiated by fibrin clots, or more specifically, “fibrin gel matrix”, originating from fibrinogen [13]. Peritoneal trauma and ischemia are the starting points for tissue factor expression and adhesion formation, and the peritoneum responds fairly rapidly to such events as trauma and ischemia [14]. Fibrin is broken down by fibrinolytic enzymes like plasmin in the healthy intraabdominal environment, in the presence of inflammation. However, these fibrinolytic enzymes become inactive, which leads to local reproduction of fibroblasts and formation of permanent adhesions [15]. The direct relation between the decrease in fibrinolytic activity and the increase in adhesions has been also demonstrated experimentally [16]. In light of this information, it is possible to say that intraoperative irrigation with alteplase prevents adhesion formation by increasing fibrinolytic activity. Even though

this mechanism proposed for alteplase concerns postoperative adhesion formation, there is also peritoneal irritation associated with intraperitoneal chemotherapy, and the mechanism by which alteplase reduces adhesion formation may hold true for the latter case, as well [17]. In our study, alteplase was utilized in light of the literature information cited above, and significantly reduced adhesion formation.

In previous studies, the degree of peritoneal reactive angiogenesis reached a maximum on postoperative days [8-12]. In this period, expansion of the adhesions is restructured and reduced, whereby angiogenesis reaches a maximum [16, 17]. Therefore, it can be speculated that, theoretically, it may be more appropriate to use agents employed to prevent adhesion formation during the increase in peritoneal neo-angiogenesis. As fibroblasts differentiate in mesothelial cells on the eighth day, use of agents before this day may be thought to affect fibroblast differentiation, and could be effective against adhesion formation [18]. That is why TPA was used during the operation in the present study, that is on day 0. There are two reasons why chemotherapy was administered on the seventh day in our study. The first is that the critical steps in adhesion formation reach maximum levels in this period; and the second is that chemotherapy administration was delayed to avoid any complications like incision opening [11, 16].

In conclusion, the fact that subjects irrigated with alteplase had lower adhesion scores in comparison to other groups demonstrates that use of intraoperative alteplase in the primary surgery is quite effective. Although our study is experimental, the results obtained clearly demonstrate that intraperitoneal irrigation with alteplase during primary surgery can reduce adhesion formation. This effect is fairly important in terms of the chemotherapeutic agent coming into contact with more peritoneal surfaces. The doses reported in our study did not cause any bleeding complications. However, intraoperative intraperitoneal alteplase doses that would not cause such complications in humans need to be determined.

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