Postoperative changes in high mobility group box 1 levels after colorectal cancer surgery

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Abstract

Objective: To investigate the potential use of high mobility group box 1 (HMGB1) as a marker for the surgical course following surgery for colorectal cancer (CRC).

Methods: Patients with advanced CRC undergoing open colorectal surgery who did not develop postsurgical complications were enrolled in the study. Blood samples were taken preoperatively and at 1 day, 1 week and 3 weeks after surgery for the measurement of the white blood cell count, serum C-reactive protein, serum amyloid A and HMGB1.

Results: Data from 21 patients were analysed. HMGB1 levels changed significantly during the surgical course, increasing from a preoperative median of 6.8 ng/ml to 12.1 ng/ml at 1 day postoperatively, and then decreasing to 8.1 ng/ml at 1 week postoperatively and 4.0 ng/ml at 3 weeks postoperatively. These changes were similar to but were not completely correlated with the changes seen in the other markers.

Conclusion: Serum HMGB1 may be a potential marker to monitor the surgical course in patients undergoing surgery for CRC, although further studies are warranted before it can be introduced into routine clinical practice.

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Introduction

Colorectal cancer (CRC) is prevalent worldwide and many patients with CRC undergo surgery.[1] Monitoring such patients for postsurgical complications is of importance.[2,3] A number of blood markers produced in response to invasive and inflammatory stimuli have been used to monitor the postsurgical course, including the white blood cell (WBC) count and acute phase reactants such as C-reactive protein (CRP) and serum amyloid A (SAA).[4–9] Although typically used to detect postsurgical inflammatory complications, changes in the levels of these markers can also reflect the degree of surgical invasive stress.[7]

High mobility group box 1 (HMGB1) is a non-histone nuclear protein found in systemic organs and tissues that when secreted also plays an important role as a pathophysiological mediator of inflammation.[10] Levels of HMGB1 have been reported to be increased not only in individuals with severe infections such as sepsis[11–13] but also in infection-free individuals with invasive conditions associated with oxidative stress and triggering of the immune response, including cancerous pathologies.[14–16]

To date there are limited studies investigating HMGB1 as a potential marker for detecting postsurgical complications. Suda et al.[15] reported on its use in monitoring patients after oesophagectomy for oesophageal cancer,[15] whereas Takahata et al.[16] studied changes in HMGB1 after gastrointestinal surgery in patients with various cancers of the gastrointestinal tract, including CRC.[16] The value of monitoring HMGB1 in patients undergoing surgery for CRC is still unknown, and the behaviour of HMGB1 levels in a normal surgical course in patients with CRC needs to be determined. The present study aimed to obtain data on the changes in HMGB1 levels in patients with advanced CRC following elective colorectal surgery without postoperative complications, with reference to concomitant changes in conventional markers such as WBC count, CRP and SAA.

Patients and methods

Patients

Patients with advanced CRC diagnosed by systemic imaging and histological examination who were scheduled to receive open colorectal surgery at Jichi Medical University, Tochigi, Japan, between March 2012 and August 2013 were enrolled in the study. Patients requiring emergency surgery and those who developed postoperative infections such as pneumonia or peritonitis or in whom the CRC lesions could not be resected were excluded from the study. Patients in whom blood samples could not be taken at the four time points (preoperatively, 1 day postoperatively, 1 week postoperatively and 3 weeks postoperatively) were also excluded.

All patients gave written informed consent and the study protocol was approved by the Ethics Committee of Jichi Medical University.

Measurement of HMGB1 levels

At the four time points, venous blood was collected from each patient into tubes containing ethylene diamine tetra-acetic acid dipotassium salt for the WBC counts and tubes without anticoagulant for serum separation. WBC counts were measured using a Coulter LH780 device (Beckman Coulter Corp., Tokyo, Japan). The blood collected for serum measurements was centrifuged at 3 500 r.p.m. for 15 min at room temperature. Serum CRP and SAA levels were measured using enzyme-linked immunosorbent assays (Eiken Chemical Corp., Tokyo, Japan) according to the manufacturer's instructions. Serum HMGB1 levels were also measured using an enzyme-linked immunosorbent assay (Shino-Test Corp., Kanagawa, Japan)

Table 1. Pre- and postoperative levels of markers in 21 patients undergoing colorectal surgery.

Marker	Preoperative	1 day postoperatively	1 week postoperatively	3 weeks postoperatively	Statistical significance[a]
WBC count, $\times 10^3/\mu l$	6.1 (5.4–7.3)	10.3 (8.5–12.3)	5.3 (4.2–6.9)	5.1 (4.3–6.2)	$P < 0.01$
CRP, mg/dl	0.3 (0.1–1.3)	2.5 (0.5–7.6)	1.4 (0.7–2.3)	0.1 (0.1–0.3)	$P = 0.03$
SAA, μg/ml	24.3 (10.6–124.6)	159.9 (50.0–356.5)	49.1 (25.1–154.9)	10.0 (5.8–18.7)	$P < 0.01$
HMGB1, ng/ml	6.8 (4.9–11.0)	12.1 (7.3–16.3)	8.1 (5.4–13.0)	4.0 (2.8–7.6)	$P < 0.01$

Data presented as median (interquartile range).
WBC, white blood cell; CRP, C-reactive protein; SAA, serum amyloid A; HMGB1, high mobility group box 1.
[a]Using repeated measures analysis of variance.

according to the manufacturer's instructions.[17] Coefficient variations in the measurements for each marker were <5%.

Statistical analyses

Results were given as the mean ± SD or the median and interquartile range. Repeated measures analysis of variance with multi-comparison tests was used to analyse the changes in each marker. Relationships between the changes in the different markers were analysed using the Pearson correlation test. WBC counts and CRP, SAA and HMGB1 levels were log-transformed before analysis.

All statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). A *P*-value < 0.05 was considered to be statistically significant.

Results

A total of 23 patients were initially eligible for the study; however, two patients developed postoperative complications, leaving a total of 21 patients for inclusion in the data analysis. Of these, 17 were male and four were female, with a mean ± SD age of 69.5 ± 10.4 years. All the patients recovered with an unremarkable surgical course. The changes in the levels of each marker during the colorectal surgical period are shown in Table 1.

There was a significant change in the WBC count over the surgical period studied (overall *P* < 0.01) (Table 1). The level was significantly increased 1 day postoperatively compared with the preoperative level (*P* < 0.01), but by 1 week postoperatively had significantly decreased from the level at 1 day postoperatively (*P* < 0.01). The level at 3 weeks postoperatively remained similar to that at 1 week postoperatively.

The levels of CRP also showed a significant change during the surgical period studied (overall *P* = 0.03) (Table 1). The level at 1 day postoperatively was significantly increased compared with the preoperative level (*P* = 0.02). The level then decreased, with the level at 1 week postoperatively being lower than at 1 day postoperatively, but this difference was not significant. By 3 weeks postoperatively the level had significantly decreased compared with both the level at 1 day postoperatively (*P* < 0.01) and that at 1 week postoperatively (*P* < 0.01).

With regard to SAA, there was again a significant change during the surgical period studied (overall *P* < 0.01). The level at 1 day postoperatively was significantly increased compared with the preoperative level (*P* = 0.04). The level then decreased, with

Table 2. Correlations between changes in the levels of high mobility group box 1 (HMGB1) and those of other conventional markers before and after colorectal surgery.

	Change in HMGB1					
	Presurgery to 1 day postoperatively		1 day postoperatively to 1 week postoperatively		1 week postoperatively to 3 weeks postoperatively	
	Correlation coefficient	Statistical significance	Correlation coefficient	Statistical significance	Correlation coefficient	Statistical significance
Change in WBC count	0.33	NS	0.68	$P < 0.01$	0.18	NS
Change in CRP	0.33	NS	0.41	NS	−0.15	NS
Change in SAA	0.28	NS	0.51	$P = 0.01$	0.37	NS

HMGB1, high mobility group box 1; WBC, white blood cell; CRP, C-reactive protein; SAA, serum amyloid A.
NS, not statistically significant using Pearson correlation test ($P \geq 0.05$).

the level at 1 week postoperatively being lower than at 1 day postoperatively, but this difference was not significant. The level at 3 weeks postoperatively was significantly decreased compared with both the level at 1 day postoperatively ($P < 0.01$) and that at 1 week postoperatively ($P < 0.01$).

The levels of HMGB1 also showed a significant change during the surgical period studied (overall $P < 0.01$). The level at 1 day postoperatively was significantly increased compared with the preoperative level ($P = 0.02$). The level then decreased, with the level at 1 week postoperatively being lower than at 1 day postoperatively, but this difference was not significant. The level at 3 weeks postoperatively was significantly decreased compared with the preoperative level ($P < 0.02$), the level at 1 day postoperatively ($P < 0.01$) and that at 1 week postoperatively ($P < 0.01$).

The correlations between the changes in the levels of HMGB1 and other markers during the colorectal surgical period are shown in Table 2. In the phase from presurgery to 1 day postoperatively, there was a mildly positive but insignificant correlation between the changes in HMGB1 and the other three markers. In the phase from 1 day postoperatively to 1 week postoperatively, the changes in HMGB1 were significantly and positively correlated with changes in the WBC count and SAA levels, while there was a mildly positive but insignificant correlation between the changes in HMGB1 and CRP levels. In the phase from 1 week postoperatively to 3 weeks postoperatively, there was a mildly positive but insignificant correlation between the changes in HMGB1 and SAA levels, while there were no apparent correlations between the changes in HMGB1 and the changes in the WBC count and CRP levels.

Discussion

In the present study, the serum levels of HMGB1 significantly changed over the course of a normal surgical period in patients with advanced CRC undergoing elective colorectal open surgery, with the highest levels being seen from 1 day postoperatively to 1 week postoperatively. The changes in HMGB1 appeared to be similar but not identical to the changes in other conventional markers. These results help to clarify the behaviour of HMGB1 levels during a normal colorectal surgical period and further support the consideration of HMGB1 as a marker for monitoring the surgical course in patients

undergoing surgery for CRC, with further studies being required on the similarities and differences between various markers before the use of HMBG1 levels in clinical practice.

The HMGB1 level is elevated as a systemic reaction to surgical invasive stimuli and tissue damage.[13–16] In particular, a significant change in HMGB1 level in cancer patients has been reported after oesophagectomy[15] and gastrointestinal surgery.[16] In the present study, the main cancer lesions were resected and postoperative complications did not occur, suggesting that the main effect on HMGB1 levels after resection of cancers is due to surgical invasiveness. In patients who underwent oesophagectomy without complications, the peak HMGB1 level was observed 2–3 days postoperatively, with the level then gradually decreasing until 1 week postoperatively,[15] whereas the peak HMGB1 level was seen 3 days postoperatively followed by a gradual decrease until 1 week postoperatively in patients undergoing surgery for gastrointestinal tract cancer.[16] These results together with those of the present study suggest that the trends in the changes to HMGB1 levels may be consistent, even though the degree of surgical invasiveness and the cancer patient populations are different. Confirmation of these findings would be of value in considering the possible use of HMGB1 for monitoring the surgical course in patients with CRC.

The correlations seen between the changes in HMGB1 and those in other conventional markers are also of interest. Given that the conventional markers are more typically used to monitor inflammatory conditions, they would not be expected to tightly correlate with HMGB1 under conditions with no apparent postsurgical inflammation.[10] The changes seen in the conventional markers measured in the present study can be explained by their different responses to invasive stress.[4] The WBC count is known to show a rapid increase and then decrease in the early stages of a postsurgical course,[4]

which was observed in the present study. With regard to CRP, the narrow dynamic range of CRP is a disadvantage when observing the changes during the surgical period; the overall changes in CRP were barely significant in the present study. In contrast, SAA has a wide dynamic range,[18] but the large inter-individual differences in SAA may make it difficult to compare the surgical course between individuals. In the present study, mildly positive but insignificant correlations between the changes in HMGB1 and those of the conventional markers in the early phase of the surgical course suggests that the change in HMGB1 may have a different origin to that of the other markers in the immediate postoperative period. Mild-to-moderate positive correlations between the changes in HMGB1 and those of the conventional markers, especially the WBC count and SAA levels, in the recovery stage (1 day to 1 week postoperatively) suggest similarity in the origin of the change in HMGB1 with that of the other markers at this stage. In the stable stage (1 week to 3 weeks postoperatively), an insignificant but mildly positive correlation was found between the changes in HMGB1 and SAA. Throughout the entire surgical course, the changes in HMGB1 were closest to the changes in SAA out of the conventional markers studied, although this correlation was not very strong. Clarification of the similarities and differences among these markers merits further investigation for establishing the application of HMGB1 in the monitoring of patients with CRC undergoing surgery.

The present study has a number of limitations. The sample size was relatively small. The study was preliminary in that it did not compare the HMGB1 level among patients with and without postsurgical complications. The patient population could not be assessed in terms of the extent of surgical invasiveness, as this is not evaluated in routine clinical settings. These points should be addressed in future studies.

In conclusion, in the present study serum HMGB1 levels significantly changed during the normal surgical period in patients with advanced CRC who received elective colorectal open surgery and were free of post-surgical complications. The changes in HMGB1 were similar to, but not completely correlated with, those of other conventional markers. These results suggest the potential use of HMGB1 levels to monitor the surgical course in patients undergoing surgery for CRC, although further studies are warranted before it can be introduced into routine clinical practice.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

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