Original article

Effects of super-extended paraaortic lymphadenectomy (PAL) on biological responses in totally gastrectomized patients with T3 or T4 gastric cancer

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Abstract:

Background. In Japan, much attention has recently been paid to super-extended paraaortic lymphadenectomy (PAL) for the treatment of advanced gastric cancer. However, it has been reported that PAL is associated with increased morbidity and mortality, as compared to conventional extended lymphadenectomy (D2 or D3). Therefore, an analysis of the effects of PAL on perioperative changes in the biological responses of patients is essential for determining the potential utility of this procedure.

Methods. The current non-randomized prospective study included evaluations of perioperative changes in parameters of surgical stress (series I; serum levels of antidiuretic hormone, interleukin-6, trypsin, and phospholipase A₂) and immunocompetence (series II; phytohemagglutinin- and concanavalin A-induced blastogenesis, activity of natural killer cells and the ratio of CD4 cells to CD8 cells) in patients with advanced gastric cancer (T3 or T4), comparing groups treated with D3 plus PAL ($n = 12$) and D3 ($n = 13$), and a control group with early gastric cancer ($n = 16$) treated with D1 lymphadenectomy (perigastric N1 nodes) between April 1995 and April 1997.

Results. The duration of surgery and the amount of blood lost were longer and greater in the D3 plus PAL group than in the D3 and D1 groups. D3 plus PAL and D3 were associated with significant postoperative increases in parameters of surgical stress, as well as with significant postoperative immunosuppression, compared to results with D1. However, there were no significant differences in the respective parameters between the D3 plus PAL and D3 groups.

Conclusions. Our results indicate that there are no essential differences in patients' biological responses between D3 plus PAL and D3 lymphadenectomy. It appears that PAL-associated morbidity can be minimized by very careful manipulation during the dissection of paraaortic lymph nodes.

Key words: gastric cancer, total gastrectomy, super-extended paraaortic lymphadenectomy, surgical stress, immunocompetence

Introduction

In recent years, extended lymphadenectomy (D2 or D3) has been included by Japanese surgeons in the treatment of advanced gastric cancer, and improved survival has been reported as a consequence [1–2]. Some surgeons in western countries have also described the benefits of extended lymphadenectomy [3–5], but others have not endorsed this procedure because of an apparent association with increased morbidity and mortality [6–9].

Some Japanese surgeons have examined the utility and benefits of the addition of super-extended paraaortic lymphadenectomy (PAL, dissection of lymph nodes around the abdominal aorta; no. 16 nodes) to D2 or D3 lymphadenectomy. Such radical surgery might be expected to improve survival over that obtained after extended lymphadenectomy. Recent reports of retrospective studies of PAL have indicated improved postoperative survival [10–12]. However, increased morbidity and mortality associated with PAL have also been reported [10–12]. Reasons for the increased morbidity and details of the effects of PAL on biological responses have not yet been provided. Therefore, we initiated a prospective controlled study ($n = 70$ patients) to compare morbidity, mortality, and survival in patients with advanced gastric cancer (T3 or T4) treated by extended (D3) and by super-extended PAL. The study was conducted from May 1990 to April 1997.

During the last 2 years of this period, we compared the perioperative changes in parameters of surgical stress and immunocompetence in 25 patients with advanced gastric cancer treated by D3 plus PAL, D3, with

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16 patients with early gastric cancer who had undergone D1 lymphadenectomy as controls.

Patients and methods

Patients

The eligibility requirements for inclusion in the prospective controlled study conducted from May 1990 to April 1997 study were: advanced gastric cancer (T3 or T4) age under 75 years and no manifest disorders in other organs and no simultaneous or metachronous primary malignancies in organs other than the stomach.

After laparotomy, we also excluded patients with peritoneal seeding or liver metastasis, as well as patients who had macroscopic metastasis to no.16 lymph nodes. Patients who had T3 (serosal invasion) or T4 (invasion of adjacent structures) gastric cancer and who underwent potentially curative total gastrectomy were entered into the study after extensive dissection of group 1 (N1), group 2 (N2), and group 3 (N3) lymph nodes; namely, the D3 procedure.

The extent of lymphadenectomy was as follows: D1, complete removal of N1 nodes; D2, complete removal of N1 and N2 nodes; D3, complete removal of N1, N2, and N3 nodes; and D3 plus PAL, complete removal of N1, N2, N3 nodes plus paraaortic lymphadenectomy.

Between April 1995 and April 1997, 25 patients were entered in the study and they were allocated, by alternate order at the time of operation, into two groups after their informed consent had been obtained preoperatively. We report here the findings for these 25 patients. Twelve patients were selected for the D3 plus PAL dissection and the other 13 received D3 dissection. Sixteen patients who underwent total gastrectomy with D1 lymphadenectomy, without splenectomy and cholecystectomy, for the treatment of early gastric cancer during the same period were selected as control patients. Therefore, this was a non-randomized pro-

spective study. The biological responses that we examined were related to surgical stress (series I) and immunocompetence (series II), as shown in Table 1.

Perioperative management and surgical procedures

All patients received endotracheal inhalation anesthesia combined with epidural anesthesia. The epidural analgesia was provided before surgery and maintained for 72 h postoperatively as analgesia. Postoperatively, continuous intravenous fluid resuscitation was given to maintain urine output (>1.0 ml/kg per h) and appropriate systolic blood pressure (within 15% of the preoperative level) with the aid of intravenous administration of dopamine, ranging between 0 and 5 μ g/kg per min.

During surgery, all 25 patients (D3 and D3 plus PAL) underwent splenectomy and cholecystectomy for the complete removal of the lymph nodes at the splenic hilum and in the hepatoduodenal ligament. In the PAL group, lymph nodes 16a1, 16a2, 16b1, and 16b2 were dissected out (Fig. 1) [13]. The region of dissection of nodes was intended to include the lateroaortic, preaortic, inter-aorticocaval, precaval, and laterocaval spaces, while the nodes of the retroaortic and retrocaval spaces were not removed.

Determination of perioperative biological responses

Surgical stress (series I)

Venous blood was collected from each patient on the day before surgery and 6, 24, and 72 h after surgery. Serum samples were stored at -30°C until just prior to analysis. Perioperative changes in serum levels of antidiuretic hormone (ADH), interleukin-6 (IL-6), trypsin, and phospholipase A₂, as parameters of the extent of surgical stress, were analyzed perioperatively. The principles of the analyses and the commercially available kits utilized were: radioimmunoassay (AVP-RIA Kit; Mitsubishi Chemical, Tokyo, Japan) for ADH

Table 1. Grouping and background factors of patients with advanced gastric cancer ($n = 25$) examined between April 1995 and April 1997

Series Group		No. of patients	Males/female	Age (years; mean $\pm$ SD)	Duration of surgery (min; mean $\pm$ SD)	Amount of blood lost (g; mean $\pm$ SD)	No. of patients transfused	Mean no. of nodes dissected			
								16a1	16a2	16b1	16b2
I	D3 + PAL	6	2 / 4	61 $\pm$ 4	303 $\pm$ 39 ^a	727 $\pm$ 230 ^a	3	0.8	7.0	5.6	3.8
	D3	6	4 / 2	57 $\pm$ 9	268 $\pm$ 41 ^b	526 $\pm$ 154 ^b	2	—	—	—	—
	Controls D1	9	6 / 3	59 $\pm$ 7	186 $\pm$ 51 ^c	256 $\pm$ 102 ^c	0	—	—	—	—
II	D3 + PAL	6	4 / 2	64 $\pm$ 13	291 $\pm$ 41 ^d	771 $\pm$ 191 ^f	3	2.0	3.8	5.4	3.4
	D3	7	4 / 3	59 $\pm$ 12	245 $\pm$ 45	530 $\pm$ 184 ^g	2	—	—	—	—
	Controls D1	7	4 / 2	63 $\pm$ 10	197 $\pm$ 60 ^e	247 $\pm$ 91 ^h	0	—	—	—	—

$P < 0.01$ (a versus c, b versus c, f versus h, g versus h); $P < 0.05$ (d versus e, f versus g). PAL, Paraaortic lymphadenectomy; Controls, patients with early gastric cancer; D3, conventional extended lymphadenectomy; D1, complete removal of all N1 nodes
Locating nodes 16a1, a2, b1, b2, see Fig. 1

Fig. 1. Location of nodes around the abdominal aorta (AA; no. 16; reprinted from ref. 13 with permission). *16a1*, Lymph nodes in the aortic hiatus; *16a2*, lymph nodes around the AA from the upper margin of the celiac trunk to the lower margin of the left renal vein (LRV); *16b1*, lymph nodes around the AA from the lower margin of the LRV to the upper margin of the inferior mesenteric artery (IMA); *16b2*, lymph nodes around the AA from the upper margin of the IMA to the aortic bifurcation

[14]; chemiluminescent enzyme immunoassay (CLEIA; Fujirebio, Tokyo) for IL-6 [15]; radioimmunoassay (RIA Trypsin Kit; Hoechst Japan, Tokyo) for trypsin [16], and radioimmunoassay (Shionolia; Shionogi, Osaka, Japan) for phospholipase A_2 [17].

Immunocompetence (series II)

Mitogen-induced blastogenesis. Peripheral blood mononuclear (PBM) cells were suspended in RPMI-1640 medium (GIBCO, Grand Island, NY, USA) that contained 10% heat-inactivated fetal calf serum (GIBCO). Suspensions of cells, supplemented with phytohemagglutinin (PHA; Difco, Detroit, MI, USA; 15 μ g/ml) or with concanavalin A (Con A; Difco; 15 μ g/ml), were cultured at 37°C. After 64-h culture, 3 thymidine (0.25 μ Ci) was added to each culture. After a further 8-h incubation, the 3 thymidine uptake was determined with a liquid scintillation counter [18].

Activity of NK cells. The activity of NK cells was determined by monitoring the release of radioactivity from 51 Cr-labelled K562 target (T) cells. PBM cells, suspended in RPMI-1640, were used as effector (E) cells (E:T = 20:1). Released radioactivity was measured in a "g" scintillation counter [18]. The activity of NK cells was calculated as (experimental counts per min — spontaneous counts per min) / (maximum counts per min — spontaneous counts per minute) $\times$ 100.

T-Lymphocyte surface markers. The CD4 and CD8 subpopulations were quantitated by direct immunofluorescence using commercially available fluorescein-conjugated monoclonal antibodies specific for CD4 (helper/inducer T lymphocytes; OKT4; Ortho, Raritan, NJ, USA) and specific for CD8 (suppressor/cytotoxic T lymphocytes; OKT8; Ortho). Flow microfluorometry was performed with a laser flow meter. The CD4 and CD8 populations are presented as percentages and the ratio (CD4/CD8) was calculated [18]. Parameters of

immunocompetence were analyzed preoperatively and 2 and 4 weeks postoperatively.

Statistical analysis

Data values are presented as means $\pm$ SD. The statistical significance of differences between groups was determined by the Mann-Whitney *U*-test or by Fisher's exact test where appropriate. A *P* value of less than 0.05 was considered significant.

Results

The grouping and background factors of the patients examined are shown in Table 1. There were no differences in sex and age among patients in the various groups in the two series. In both series, the duration of surgery and amount of intraoperative blood lost in the D3 plus PAL group were longer and greater, respectively, than those in the D3 group. The duration of surgery and amount of blood lost in the D3 plus PAL and D3 groups were significantly longer and greater than those in the D1 group. In the D3 plus PAL group, the mean number of dissected paraaortic lymph nodes in patients in series I and II was 17.2 and 14.6, respectively. The mean number of nodes dissected at each location around the abdominal aorta is shown in Table 1.

Figures 2 and 3 show perioperative changes in parameters of surgical stress. Compared with preoperative values in each group, serum levels of ADH in the D3 plus PAL group were significantly elevated 6h after operation, and serum levels of IL-6 increased significantly in all groups postoperatively (Fig. 2). Significant increases in postoperative serum levels of trypsin and phospholipase A_2 were observed in the D3 plus PAL

Fig. 2. Perioperative changes in serum levels of antidiuretic hormone and interleukin-6. *a* and *b*, Significant differences compared to preoperative levels in each group *D3*, Conventional extended lymphadenectomy (see text); *PAL* super-extended paraortic lymphadenectomy; *D1*, complete removal of N1 nodes

Fig. 3. Perioperative changes in serum levels of trypsin and phospholipase A2. For *a* and *b*, see legend to Fig. 2. **a* and **b*, Significant differences between each group at the same postoperative time point

and D3 groups but such marked increases were not observed in the D1 group (Fig. 3).

Changes in perioperative immunocompetence are shown in Figs. 4 and 5. PHA- and Con A-induced blastogenesis decreased markedly 2 weeks after surgery. However, these postoperative decreases were significantly greater in the D3 plus PAL and D3 groups than in the D1 group (Fig. 4). As shown in Fig. 5, the activity of NK cells decreased in every group, with the decrease being greatest in the D3 plus PAL group. Although the changes were not significant, the CD4/CD8

ratio in the D3 plus PAL and D3 groups increased 2 weeks after surgery, returning to preoperative levels 4 weeks after surgery. These changes were due to an increase in the CD4 population and a decrease in the CD8 population 2 weeks after surgery, and to a subsequent decrease in the CD4 population and an increase in the CD8 population 4 weeks after surgery. No similar changes were observed in the D1 group.

In general, although the perioperative changes in the parameters related to surgical stress and to immunocompetence in the D3 plus PAL and D3 groups were

Fig. 4. Perioperative changes in phytohemagglutinin (PHA)- and concanavalin (Con A)-induced blastogenesis. For a and b, see legend to Fig. 2

Fig. 5. Perioperative changes in activity of natural killer (NK) cells and CD4/CD8 ratio. For a and *a, see legend to Figs. 2 and 3

markedly greater than those in the D1 group, there were no major differences in the various parameters between the D3 plus PAL and D3 groups.

Discussion

Seventy patients were entered in our prospective study of D3 plus PAL ($n = 35$) and D3 ($n = 35$)

lymphadenectomy between 1990 and 1997. Although, to date, there is no significant difference in survival between the two groups, survival seems to be better in the D3 plus PAL group (52.8%) than in the D3 group (38.4%). With respect to morbidity, the incidence of postoperative complications in the D3 plus PAL group was higher than that in the D3 group. Four patients had prolonged retention of intra-abdominal fluid, which occurs specifically after PAL, because of

the PAL-induced disruption of the retroperitoneal lymphatic vessels (lymphorrhea). One of the four patients underwent a second laparotomy for drainage of the fluid.

One patient in each group died of postoperative complications.

In the non-randomized prospective study of the 25 patients enrolled between April 1995 and April 1997 ADH was chosen as a classical parameter of stress-induced neuroendocrinological disorders [19]. IL-6 appears to play a central role in immune host-defense mechanisms [20] and its level provides a measure of surgical stress- and/or inflammation-induced cytokines. Postoperative increases in circulating levels of ADH and IL-6 have been well characterized [19,20]. Trypsin [21] and phospholipase A2 [22] are both secreted from pancreatic cells and are also shed into the circulation in conditions of surgical stress, sepsis, and multiple organ failure. Furthermore, there is evidence that surgical stress results in a reduction in postoperative immunocompetence that is associated, for example, with a decrease in the activity of NK cells [18,23] and mitogen-induced blastogenesis [18].

The duration of surgery and the amount of blood lost were longer and greater in the D3 plus PAL group than in the D3 and in the D1 group, and patients treated with perioperative allogeneic blood, which is immunosuppressive [18], were mostly in the PAL group. Therefore, the surgery-induced biological responses seemed to be most marked in the D3 plus PAL group. The serum level of ADH increased most markedly in the PAL group, while that of IL-6 increased more in the D3 than in the D3 plus PAL group. D1 lymphadenectomy for the treatment of early gastric cancer does not involve the dissection of lymph nodes along the splenic artery and the pancreas is kept intact. Thus, as anticipated, there were no major increases in serum levels of the pancreas-associated enzymes trypsin and phospholipase A2 in the D1 group, while there were significant increases in these enzymes in the D3 plus PAL and D3 groups. Although, compared to D1, D3 plus PAL and D3 induced significant postoperative increases in serum levels of indicators of surgical stress, as well as significant postoperative immunosuppression, there were no major differences in terms of the various parameters between the D3 plus PAL and D3 groups.

These results indicate the absence of fundamental differences in postoperative biological responses between conventional extended D3 and D3 plus super-extended PAL. However, the postoperative decrease in the activity of NK cells was significantly greater in the D3 plus PAL group than in the D3 group. This may be associated with increased lymphorrhea from PAL-induced disrupted retroperitoneal lymphatic vessels.

In the near future, a prospective randomized study by Japanese surgeons may reveal that PAL improves survival in advanced gastric cancer, and indications for PAL may be emphasized more strongly for the active and more effective treatment of advanced gastric cancer. Our present study indicates that the PAL-associated increased morbidity, suggested in previous studies by others [10–12] and ourselves, may be reduced to approach the morbidity associated with D3 by very careful manipulation during the dissection of paraaortic lymph nodes, namely, by fine and thorough ligation of the retroperitoneal tissue to prevent lymphorrhea.

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