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Anal submucosal injection: methotrexate concentration in rectal tumor tissue and serum after anal compared with parenteral injection

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As the results of adjuvant chemotherapy in the treatment of advanced rectal cancer are unsatisfactory, more effective regimens and routes of administration are being tried. Submucosal anal injection of methotrexate (MXT) has given encouraging results in the treatment of pelvic malignancies. This communication studies the MXT level in serum and rectal cancer tissue after either anal submucosal or parenteral MXT administration. Twenty four patients, mean age 46.4 years (16 men and eight women), with stage C rectal cancer were divided into two equal, age- and sex-matched groups. MXT (50 mg) was administered to each patient intravenously in one group and into the anal submucosa in the other. A blood sample was taken 30, 60 and 120 min after injection, and again after 24 h. A tumor sample was also taken each 30 and 60 min of injection. The serum and tissue MXT levels were determined using a radioimmunoassay kit. The serum MXT concentration was significantly higher after parenteral than after anal injection. Meanwhile, the concentration of MXT in tumor tissue was higher after anal administration. In conclusion, the anal route of administration of MXT, by inducing a high MXT concentration in the tumor tissue associated with a low serum level, might achieve satisfactory therapeutic results in advanced rectal cancer, with minimal side effects.

Key words: Anorectum, chemotherapy, methotrexate, rectal tumors.

Introduction

Early stages (Dukes A and B1 or stage 1) of rectal cancer generally are curable by surgery alone. Patients with full-thickness penetration of the tumor through the rectal wall (Dukes B2, stage 2) have a 5 year survival rate of 65–75%.[1–3] Regional nodal involvement (Dukes C, stage 3) has a lesser 5 year survival rate (40–50%). Patients of the latter category are at high risk of loco-regional recurrence and need adjuvant treatment.[4–7]

Adjuvant radiotherapy has been widely studied in rectal cancer.[8–10] Although it has effected a significant decrease in loco-regional recurrences, there was no improvement in the overall survival because systemic failure is not addressed by radiotherapy.[11,12] Adjuvant chemotherapy was used as a single agent or as a combination of multiple agents.[13–15] As long as the results of adjuvant chemotherapy remain unsatisfactory, there will be continued interest in developing more effective regimens and routes of administration aimed at increasing efficacy and diminishing side effects.[16–20]

A previous study has shown that injection of a radiopaque dye into the submucosa of the rectal neck (anal canal) led to visualization of the urinary bladder, uterus and vagina.[21] Thus anal cystography[22] and anal cystovaginohysterography[23] could be achieved by this route. Further studies have demonstrated that the urinary bladder after injection of misonidazole (a radiation sensitizer) into the anal submucosa showed a concentration of the drug as high as eight times the serum level.[24]

The submucosal anal route is being taken advantage of to administer drugs for pelvic malignancies and other pathologic conditions. It has been used with encouraging results for the treatment of advanced cancer of the bladder,[25] prostate[26] and uterus,[27] as well as in the treatment of chronic relapsing prostatitis.[28]

These studies called for the use of the submucosal anal route for chemotherapy administration in the treatment of advanced rectal cancer. Before embarking on the treatment, we compared the methotrexate (MXT) concentration in the serum and tumor tissue when the drug was injected into the anal submucosa to that when injected intravenously. The results of this study are presented herein. MXT was selected from the different chemotherapeutic agents as it has no histopathologic effects on the perianal tissues when injected into the anal submucosa; this was proved experimentally[24] and in humans.[25–28]

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Subjects and methods

Twenty-four subjects entered the study after giving informed consent. Sixteen were men and eight were women. Ages ranged from 23 to 74 years (mean 46.4 ± 16.6 SD years). All had stage T3 rectal cancer (Dukes C). Endorectal ultrasonography, CT scanning and biopsy were performed for diagnosis and staging of the tumor. Liver functions, chest X-ray and abdominal sonography were normal.

The patients were divided into two equal age- and sex-matched groups. MXT (50 mg diluted in 5 ml saline) was given to each patient, into the anal submucosa in one group and intravenously in the other group. The technique of anal submucosal injection has been described elsewhere.[25–28] Blood (5 ml) was taken from each patient 30, 60 and 120 min, and 24 h after MTX injection in both groups. The serum was separated and kept at −20 °C until it was processed for MTX assay. A tumor sample was taken 30 and 60 min after the injection. Samples were 0.5 × 0.5 × 0.5 cm in volume and were obtained from the lateral edge of the tumor. The tumor sample, site- and size-matched in all the patients, was washed by saline to remove contaminating blood and kept in a vial at −20 °C until being assessed for MXT concentration.

The tumor tissue was minced thoroughly and subjected to sonication in 5 ml saline. The cell sonicate was centrifuged at 20 000 r.p.m. for 30 min at 4 °C and MXT level in the supernatant was used for the assay. The level of MXT/ml of tumor extract represented the MXT/g of tumor tissue. The serum and tissue MXT level was estimated using a radioimmunoassay kit (Syva, San Francisco, CA).

Technique of submucosal anal injection

Anesthesia was not required since the injection was performed in a painless area, i.e. the anal submucosa above the pectinate line. With the patient in the lithotomy or left lateral position, a 21-gauge needle was inserted into the perianal skin 1 cm lateral to the anal orifice (Figure 1). The needle was guided with a finger in the rectal neck so that its tip lay in the anal submucosa above the pectinate line and the MXT solution was slowly injected. Injection in the proper plane was associated with bulging of the anal mucosa. This mucosal bulge did not occur when MXT solution was injected into the perianal tissues; in such a case, the needle was re-positioned with the help of the finger in the rectal neck.

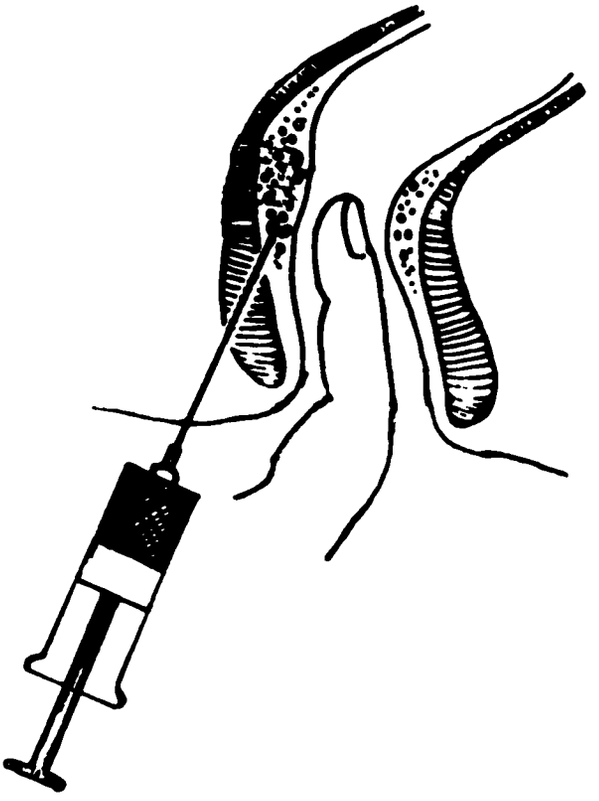

Figure 1. The technique of submucosal anal injection.

Results

All the subjects of the study were evaluable. No complications occurred from intravenous or anal submucosal injection of MXT. Figures 2 and 3 show MXT serum and tissue concentrations after anal submucosal and parenteral injection of 50 mg MXT. In both administrations, the mean serum MXT level was highest at the 30 min examination and diminished gradually to reach the lowest mean level after 24 h. Likewise, the mean MXT tumor tissue concentration was highest at the 30 min examination (Figures 2 and 3).

Comparing the parenteral with the anal administration, the serum MXT concentration was significantly higher after parenteral than after submucosal anal injection (Figure 2). At the 24 h examination, the MXT serum level was negligible in the anal injection group but still high in the parenteral group. Meanwhile, the MXT tissue concentration after anal injection was significantly higher than after the parenteral administration at both the 30 and 60 min examinations (Figure 3). The determination of MXT concentration in the tumor tissue

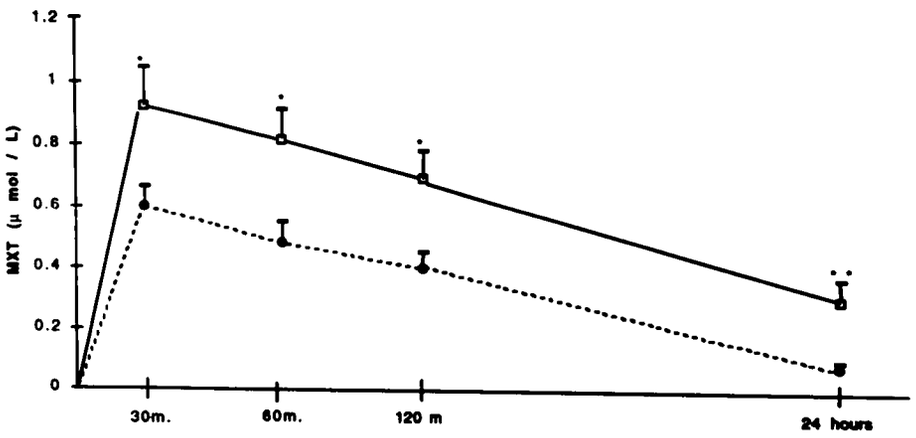

Figure 2. MXT serum concentrations after parenteral (⊟; 12 patients) and submucosal anal (●; 12 patients) injection of 50 mg MXT in 24 rectal cancer patients. m, min; $^{*}p < 0.05$, $^{**}p < 0.01$ ± SD.

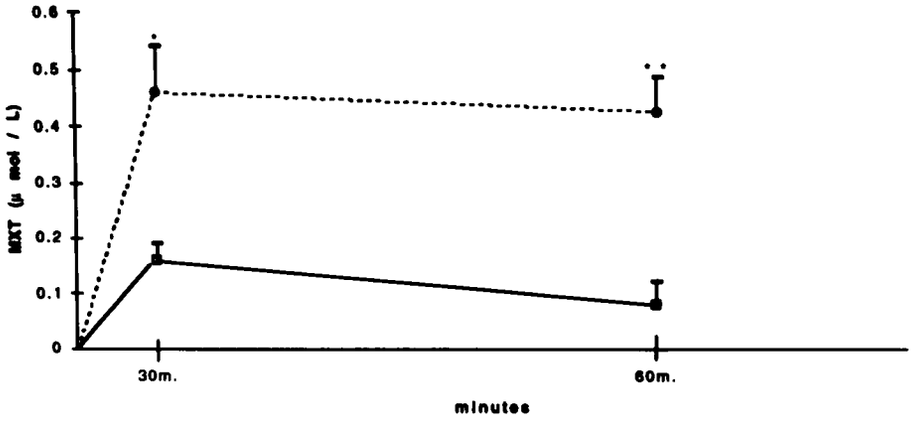

Figure 3. Concentrations of MXT in tumor tissue after parenteral (⊟; 12 patients) and submucosal anal (●; 12 patients) injection of 50 mg MXT in 24 rectal cancer patients. $^{*}p < 0.01$, $^{**}p < 0.001$ ± SD.

after 24 h of administration was not done as all the patients refused a third tumor biopsy to be taken.

Discussion

The present study demonstrates that MXT concentration in the tumor tissue was higher and the serum level lower after anal MTX administration than after parenteral administration. The concentration of MXT in tumor tissue was approximately three and five times higher after anal than after parenteral injection at the 30 and 60 min examinations, respectively. Meanwhile the MXT serum level was significantly lower after the anal route of administration than after the parenteral route.

For these reasons, the anal route of administration of MXT, as it induces a higher MXT concentration in the tumor tissue with a lower serum level, might achieve a satisfactory therapeutic result with minimal or no side effects when used as adjuvant therapy for advanced rectal cancer. This therapeutic effect seems to also apply to other chemotherapeutic agents provided they have been tested for their histopathologic effects on the anal and perianal tissues when they are injected via the anal submucosal route.

The route adopted by MXT to reach the rectal tumor from the anal submucosa requires to be clarified. Previous studies[21,29] have demonstrated that anorectal veins are arranged in two plexuses: submucosal and adventitial. The two plexuses are connected with each other by means of intercommunicating veins which pass from the submucosal to the adventitial plexus through the anorectal musculature (Figure 4). MXT injected into the anal submucosa seems to perfuse the tumor tissue through these plexuses at a concentration higher than that after parenteral injection. Meanwhile, MXT enters into the circulation. The anal route thus has a local effect on the tumor tissue and a systemic effect on the possibly circulating malignant cells.

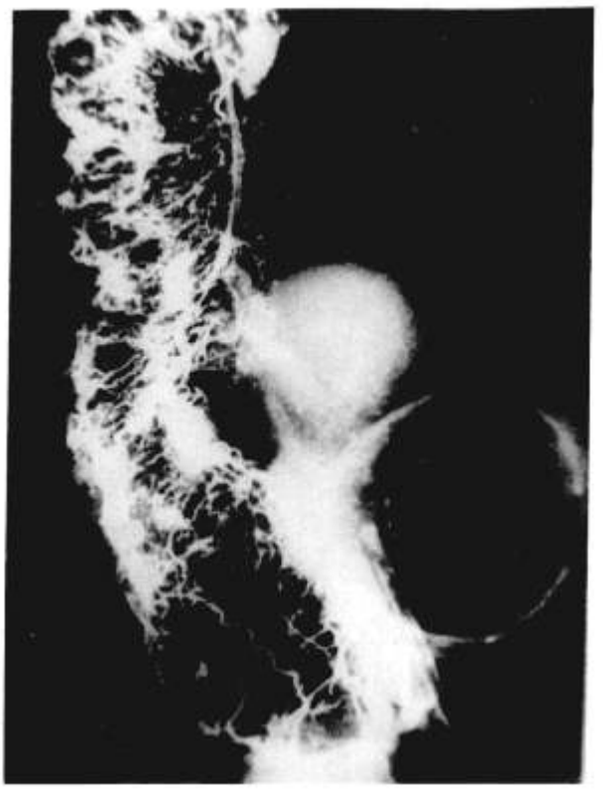

Figure 4. Inferior mesenteric vein of female cadaveric specimen injected with barium sulfate. The photograph shows the anorectal venous plexuses. The vagina, uterus and urinary bladder are also opacified (from Shafik[29]).

In conclusion, the anal route could thus be optimal for cytotoxic drug administration in rectal cancer.

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References

1. Massimo L. Adjuvant therapy of colorectal cancer. *Dis Colon Rectum,* 1994; **37**: S86–91.
2. Heald RJ, Karanjia ND. Results of radical surgery for rectal cancer. *World J Surg* 1992; **16**: 848–57.
3. McDermott FT, Hughes ES, Phil E, *et al.* Local recurrence after potentially curative resection for rectal cancer in a series of 1008 patients. *Br J Surg* 1985; **72**: 34–7.
4. Michelassi F, Vannucci L, Ayala JJ, *et al.* Local recurrence after curative resection of colorectal adenocarcinoma. *Surgery* 1990; **108**: 787–93.
5. Philipsen SJ, Heilweil IL, Quan SH, *et al.* Patterns of pelvic recurrence following definitive resections of rectal cancer. *Cancer* 1984; **53**: 1354–62.
6. Rich T, Gunderson LL, Lew R, *et al.* Patterns of recurrence of rectal cancer after potentially curative surgery. *Cancer* 1983; **52**: 1317–29.
7. Jones DJ, Zaloudik J, James RD, *et al.* Predicting local recurrence of the rectum after pre-operative radiotherapy and surgery. *Br J Surg* 1989; **76**: 1172–5.
8. Mohiuddin M, Marks G. Patterns of recurrence following high-dose preoperative radiation and sphincter-preserving surgery for cancer of the rectum. *Dis Colon Rectum* 1993; **36**: 117–26.
9. Pahlman L, Glimelius B. Pre- or postoperative radiotherapy in rectal and rectosigmoid carcinoma. *Ann Surg* 1990; **211**: 187–95.
10. Reis Neto JA, Quilici FA, Reis JA, Jr. A comparison of non-operative vs preoperative radiotherapy in rectal carcinoma: a 10-year randomized trial. *Dis Colon Rectum* 1989; **32**: 702–10.
11. Farley PC, Colorectal cancer: are adjuvant therapies beneficial. *Postgrad Med J* 1988; **84**: 175–9.
12. Balsev I, Redersen M, Teglbjaerg PS, *et al.* Post-operative radiotherapy in Dukes' B and C carcinoma of the rectum and rectosigmoid. A randomized multi-center study. *Cancer* 1986; **58**: 22–8.
13. Buroker TR, Moertel CG, Fleming TR, *et al.* A controlled evaluation of recent approaches to biochemical modulation of enhancement of 5-fluorouracil therapy in colorectal carcinoma. *J Clin Oncol* 1985; **3**: 1624–31.
14. Windle R, Bell PR, Shaw D. Five year results of a randomized trial of adjuvant 5-fluorouracil and levamisole in colorectal cancer. *Br J Surg* 1987; **74**: 569–72.
15. Canobbio L, Nobile MT. Phase II study of sequential methotrexate and 5-fluorouracil combination in the treatment of advanced colorectal cancer. *Cancer Treat Rep* 1986; **70**: 419–23.
16. Poplin EA, Kraut M. A dose intensive regimen of 5-fluorouracil for the treatment of metastatic colorectal carcinoma. *Cancer* 1991; **67**: 376–71.
17. Fiore J, Kemeny N, Raymound V. Phase II trial of Esorubicin in patients with advanced colorectal carcinoma. *Cancer Treat Rep* 1986; **70**: 1241–5.
18. Fujimoto S, Takahashi M. A clinical pilot study combining surgery with intraoperative hyperthermochemotherapy to prevent local recurrence of rectal cancer. *Ann Surg* 1991; **213**: 43–6.
19. Goldberg JA, Wilmott KN. Regional chemotherapy for colorectal liver metastasis: a phase 2 evaluation of target hepatic 5-fluorouracil colorectal liver metastasis. *Br J Surg* 1990; **77**: 1238–40.
20. Sugarbaker PH. Intraperitoneal chemotherapy for treatment and prevention of peritoneal carcinomatosis and sarcomatosis. *Dis Colon Rectum* 1994; **37**: S115–22.
21. Shafik A, Mohi M. A new concept of the anatomy of the anal sphincter mechanism and the physiology of defecation. XXIV. Hemorrhoidal venous plexuses: anatomy and role in hemorrhoids. *Coloproctology* 1985; **7**: 291–6.
22. Shafik A. Anal cystography. New technique of cystography. *Urology* 1984; **23**: 313–6.
23. Shafik A, Mohi M. Pelvic organ venous communications. Anatomy and role in urogenital diseases. A new technique of cysto-vagino-hysterography. *Am J Obstet Gynecol* 1988; **159**: 347–51.
24. Shafik A, El-Merzabani MM, El-Aaser AA, *et al.* Anal submucosal injection. A new route for drug administration in pelvic malignancies. I. Experimental study of misonidazole distribution in serum and tissues, with

special reference to urinary bladder. *Invest Radiol* 1986; **21**: 278–81.
25. Shafik A, Haddad S, Elwan F, *et al.* Anal submucosal injection. A new route for drug administration in pelvic malignancies. II. Methotrexate anal injection in the treatment of advanced bladder cancer. Preliminary study. *J Urol* 1988; **140**: 501–5.
26. Shafik A. Anal submucosal injection: A new route for drug administration in pelvic malignancies. V. Advanced prostatic cancer: results of methotrexate treatment using the anal route. Preliminary study. *Eur Urol* 1990; **18**: 132–6.
27. Shafik A. Anal submucosal injection: a new route for drug administration in pelvic malignancies. IV Submucosal anal injection in treatment of cancer of uterine cervix. Preliminary study. *Am J Obstet Gynecol* 1989; **161**: 69–72.
28. Shafik A. Anal submucosal injection: a new route for drug administration. VI. Chronic prostatitis: a new modality of treatment with report of 11 cases. Preliminary study. *Urology* 1991; **37**: 61–4.
29. Shafik A. A concept of the anatomy of the anal sphincter mechanism and the physiology of defecation. *Dis Colon Rectum* 1987; **30**: 970–82.

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