Quantification of tissue shrinkage in canine small intestinal specimens after resection and fixation

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Abstract

The aim of this study was to quantify the longitudinal shrinkage of canine small intestinal specimens after resection and fixation in 10% formalin. Samples were obtained from 12 clinically normal dogs of medium to large breed via ventral midline coeliotomy and enterectomy. The length of each sample was measured before excision, immediately after excision, and after 24 h in 10% formalin. The results were interpreted with the use of single-sample *t*-tests of the average changes; *P*-values of less than 0.01 were considered significant. The samples indicated a significant decrease in length after resection and fixation. The mean shrinkage from the pre-excision state was 28.3% immediately after excision ($P < 0.0001$) and 26.3% after 24 h of fixation ($P < 0.0001$). There was a small but not significant increase in the length of the specimens between the 2nd and 3rd measurement points. Quantification of the longitudinal shrinkage of resected intestinal specimens may improve interpretation of the distance of surgical margins from abnormal tissue in histopathology reports and allow investigation of the margins required for the clearance of specific tumors.

Résumé

L'objectif de la présente étude était de quantifier le rétrécissement longitudinal de spécimens de petit intestin de chiens après résection et fixation dans de la formaline 10 %. Des échantillons ont été obtenus de 12 chiens cliniquement normaux de race moyenne à large via entérectomie et céliotomie via la ligne blanche ventrale. La longueur de chaque échantillon a été mesurée avant excision, immédiatement après l'excision, et après 24 h dans 10 % de formaline. Les résultats des changements moyens ont été interprétés au moyen de test de t *pour échantillon unique; les valeurs de* P *inférieures à 0,01 étaient considérées significatives. Les échantillons ont montré une diminution significative de leur longueur après la résection et la fixation. Le rétrécissement moyen par rapport à l'état pré-excision était de 28,3 % immédiatement après l'excision (*P *< 0,0001) et 26,3 % après 24 h de fixation (*P *< 0,0001). Il y avait une légère, mais non significative, augmentation de la longueur des spécimens entre la 2e et la 3e mesure. La quantification du rétrécissement longitudinal des spécimens d'intestin réséqués pourrait améliorer l'interprétation de la distance des marges chirurgicales de tissus anormaux dans les rapports d'histopathologie et permettre l'étude des marges requises pour l'enlèvement de tumeurs spécifiques.*

(Traduit par Docteur Serge Messier)

Introduction

The main goal of resection of localized malignant tumors is to clear the local neoplasm. This is sought by means of wide, tumor-free surgical margins. Local recurrence is more likely if the margins are contaminated and narrow, and the histopathological status and distance of the margins have been shown to correlate with disease-free interval and median survival time (1–7). There may be discrepancies in quantification and interpretation of the surgical margins between what the surgeon observes intraoperatively and what the pathologist reports after sample manipulation and processing. Previous studies have attempted to quantify the observed "shrinkage" of tissues and to identify its cause. Reports in the human literature have included studies on the shrinkage of skin, muscle, lung tissue, breast tissue, brain, soft tissues in the oral cavity, esophagus, stomach, small intestine, and large intestine (6, 8–17). A canine model has been used to assess shrinkage of the soft tissues of the oral cavity to quantify the margins of excision for human head and neck cancer, given the strong correlation between margin determination and local disease recurrence observed in humans (4). Together with knowledge of the histologic diagnosis, the status and distance of the surgical margins affect the indications for additional treatments such as revision surgery, radiotherapy, and chemotherapy (18). Knowledge of the extent of tissue shrinkage has been shown to be important in evaluating histopathology reports and planning the appropriate excision of tumors *in situ* (7,10,19).

The aim of this study was to quantify the longitudinal shrinkage observed with resection and fixation of samples of canine small intestine and thus improve the objective interpretation of the surgical margins reported after histopathological study of canine small intestinal tumors.

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Materials and methods

Twelve clinically normal adult dogs of medium to large breed (weighing 12 to 40 kg) were included in the study. The dogs were part of a terminal-use veterinary teaching program for enterectomy and were euthanized at the end of the study with an overdose of sodium pentobarbitone [Lethabarb; Virbac (Aust) Pty, Regents Park, New South Wales]. The dogs were premedicated with acepromazine (ACP 2; Ceva Delvet Pty, Asquith, New South Wales), 0.05 mg/kg body weight (BW), and methadone [Methadone injection; Parnell Laboratories (Aust) Pty, Alexandria, New South Wales], 0.5 mg/kg BW, administered subcutaneously 30 min before general anesthesia was induced by intravenous injection of thiopentone [Bomathal; Bayer Australia (formerly Bomac Animal Health Pty), Pymble, New South Wales], 8 to 10 mg/kg BW. Anesthesia was maintained with inhaled halothane and oxygen. Fluid (0.9% NaCl, 10 mL/kg BW per hour) was administered intravenously, and the depth of anesthesia was monitored by observations of the heart rate, respiratory rate, eye position, and jaw tone, as well as by pulse oximetry, noninvasive blood pressure measurement, electrocardiography, and capnography. Additional methadone analgesia was given intravenously when deemed necessary by the supervising veterinarian. The abdomen was clipped and prepared aseptically for ventral midline coeliotomy.

The sample was collected by exteriorization of a section of macroscopically normal jejunum. The tissue to be excised was minimally handled and observed for a period of minimal peristaltic motion. The resection positions were marked with a sterile pen perpendicular to the antimesenteric border. The samples ranged from 80 to 280 mm in length, the measurement (M1) being taken, with a millimeter ruler, along the lateral serosal surface at mid-distance between its mesenteric and antimesenteric borders. The intestine was resected at the premeasured points. The lumen was occluded by means of clamps positioned perpendicular to the antimesenteric border adjacent to the resection lines. The intestine was sharply excised with a scalpel blade along the predrawn lines. The length of the excised jejunum, excluding the mucosal eversion, was measured 30 and 60 s after excision (measurement M2) with the same method. The samples were placed in 10% formalin for 24 h to allow adequate tissue fixation. The lengths of the formalin-fixed samples were then measured again (measurement M3). The measurements were made by a single observer, who recorded the average of 2 measurements, to the nearest 1 mm, at each time point. The samples were laid flat without stretching for measurement, with the aim of obtaining specimens representative of those obtained in a clinical setting.

Results were interpreted with the use of single-sample *t*-tests of the average changes. *P*-values of less than 0.01 were considered significant.

Results

The resected specimens of small intestine showed a significant amount of longitudinal shrinkage and a marginal, but not statistically significant, increase in length between the end of the excision process and the end of the fixation period (M2–M3) (Table I). The mean reductions in length were 28.3% ($P < 0.0001$) between the pre-excision and postexcision measurements (M1–M2) and 26.3% ($P < 0.0001$) between the pre-excision and postfixation measurements (M1–M3). The mean change in length from postexcision to postfixation (M2–M3) was –3.0% ($P = 0.18$). The 95% confidence intervals for the mean percentage changes in length were 32.9% to 23.7% (M1–M2), 31.4% to 21.2% (M1–M3), and 1.6% to –7.6% (M2–M3).

Discussion

The aim of this study was to quantify the longitudinal shrinkage of samples of the normal canine small intestine, which might aid in the interpretation of the distance of surgical margins, explain discrepancies between surgical and histological reports on the macroscopic margins obtained during surgical resection of small intestinal tumors, and assist in prognostication based on the clearance of local neoplasia and the distance of clean surgical margins. For specimens in which no tumor cells are identified by a pathologist at the cut edge, a comment on the distance of tumor clearance is beneficial for determining prognosis (7,19). To the authors' knowledge no studies have quantified the contraction of canine small intestinal tissue after resection and fixation. Studies have demonstrated that quantifying tissue shrinkage from fixed-tissue specimens of cutaneous melanoma in humans allows the use of a formula to calculate before excision the required surgical margins as observed *in vivo* and the minimum safe margins of excision (10,20). Similarly, it is hoped that objective measurements of the shrinkage of canine small intestinal specimens may be used by pathologists to allow more precise recommendations on the macroscopic surgical margins required for the clearance of specific tumors.

To the authors' knowledge, no papers have been published stating the required margins of excision for the commonly encountered canine small intestinal tumors. The currently recommended, anecdotal margin is 5 cm for canine small intestinal neoplasms (21). The data on the shrinkage of normal canine small intestinal samples provided by this study may allow further investigation of the surgical margins required for the clearance of specific small intestinal tumors. This information may be especially beneficial for certain areas of the canine gastrointestinal tract in which wide margins can be obtained only at the expense of increased morbidity as a result of the excision of anatomic structures that might otherwise have been preserved. In a series of 20 dogs with small intestinal neoplasms the tumor was in the descending duodenum in 6 and in the terminal portion of the ileum in 4 (22). This series suggests that a substantial proportion of canine small intestinal tumors may occur in locations that require more than routine excision and end-to-end anastomosis. Preservation of structures such as the ileocolic junction and major or minor duodenal papillae would avoid the increased morbidity associated with biliary diversion techniques and pancreaticoduodenectomy. Further investigation into the effects of excision and fixation on the shrinkage of specimens from other areas of the canine gastrointestinal tract (esophagus, stomach, large intestine) would be required. The present study was only preliminary, as it was limited in the number and type of specimens collected owing to the constraints of the teaching environment and ethics approval.

The results obtained in the current study are consistent with those previously reported in the human literature in that the observed decrease in length of gastrointestinal specimens after resection and

Table I. Lengths of canine small intestinal specimens at various times and changes in length during the intervals

	Length (mm)			% reduction in length		
Sample number	Before excision (M1)	Immediately after excision (M2)	After 24 h of fixation[a] (M3)	M1–M2	M1–M3	M2–M3
1	150	100	101	33.3	32.7	−1.00
2	80	55	65	31.3	18.8	−18.2
3	105	75	80	28.6	23.8	−6.7
4	105	80	90	23.8	14.3	−12.5
5	200	170	165	15.0	17.5	2.9
6	100	80	80	20.0	20.0	0
7	120	90	95	25.0	20.8	−5.6
8	120	75	80	37.5	33.3	−6.7
9	170	115	110	32.4	35.3	4.3
10	140	110	102	21.4	27.1	7.3
11	100	64	64	36.0	36.0	0
12	280	180	180	35.7	35.7	0
Extremes				15.0, 37.5	14.3, 36.0	−18.2, 7.3
Mean ± standard error				28.3 ± 2.1	26.3 ± 2.3	−3.0 ± 2.1
P-value				< 0.0001	< 0.0001	0.1801

[a] In 10% formalin.

before placement in formalin ranged from 11.4% to 37.8% (6,16). Continued shrinkage after formalin fixation resulted in an overall reduction of 18.0% to 51.6% in those studies (6,16), whereas in the present study there was a small but not statistically significant increase in specimen length between the postexcision and postfixation measurement points. In the present study the time between excision and immersion in fixative was 30 to 60 s, similar to that described by Weese et al (6) but different from the 2 to 5 min reported by Wang et al (16) and the 10 to 20 min used by Goldstein et al (19). The degree of specimen shrinkage in those 3 studies was similar. Goldstein et al (19) stated that most of the observed shrinkage occurred within the first few minutes but gave no further details. Rapid transfer of the resected portion of intestine into fixative was chosen in our study as it was thought to be more easily reproducible in a clinical setting. In this study the samples were kept in formalin for 24 h, similar to the 12 to 48 h reported in comparable studies (6,16). Investigation of the effect on specimen shrinkage of time to fixation and duration of fixation may be warranted. The shrinkage is thought to be related to unopposed contractility of myofibrils and release from the surrounding supporting structures and connective tissue (4). Sui et al (14) hypothesized that there is irreversible change to the muscle fibers immediately upon resection of portions of the esophagus but provided no evidence to support this statement. The current study presents the results as percentage decreases in intestinal length, as they were found to be statistically significant regardless of the absolute length of the specimen. Although this may not be true for all lengths of intestinal specimens, the lengths of the samples collected in this study were considered to be representative of those expected to be resected with a variety of canine small intestinal tumors.

The measurement method chosen for this study was considered to be readily repeatable for surgeons, not requiring special instrumentation or technique. It also reflected the situation at surgery, when the intestine cannot always be straightened before resection, and could therefore easily be used in clinical practice. However, the samples in the present study were from macroscopically normal sections of jejunum; further studies are warranted before this method can be used in a clinical setting on diseased portions of intestine. Indeed, the effect of intestinal disease on the degree of shrinkage would need to be assessed. Sui et al (14) reported 10% shrinkage of carcinoma tissue within resected esophageal specimens compared with 32% to 39% shrinkage of the normal surrounding esophageal tissue. However, since the margins sought for clearance of specific gastrointestinal tumors by definition involve portions of healthy intestine, the results of the present study may be used when making recommendations as to required macroscopic length of resection.

References

1. Abrahams NA, Halverson A, Fazio VW, et al. Adenocarcinoma of the small bowel: A study of 37 cases with emphasis on histologic prognostic factors. Dis Colon Rectum 2002;45:1496–1502.
2. Barbour AP, Rizk NP, Gonen M, et al. Adenocarcinoma of the gastroesophageal junction: Influence of esophageal resection margin and operative approach on outcome. Ann Surg 2007;246:1–8.
3. Fulcher RP, Ludwig LL, Bergman PJ, et al. Evaluation of a two-centimeter lateral surgical margin for excision of grade I and grade II cutaneous mast cell tumors in dogs. J Am Vet Med Assoc 2006;228:210–215.
4. Johnson RE, Sigman JD, Funk GF, et al. Quantification of surgical margin shrinkage in the oral cavity. Head Neck 1997;19:281–286.
5. Tilney HS, Rasheed S, Northover JM, et al. The influence of circumferential resection margins on long-term outcomes following rectal cancer surgery. Dis Colon Rectum 2009;52:1723–1729.
6. Weese JL, O'Grady MG, Ottery FD. How long is the five centimeter margin? Surg Gynecol Obstet 1986;163:101–103.

7. Wibe A, Rendedal PR, Svensson E, et al. Prognostic significance of the circumferential resection margins following total mesorectal excision for rectal cancer. Br J Surg 2002;89:327–334.
8. Dauendorffer JN, Bastuji-Garin S, Guero S, et al. Shrinkage of skin excision specimens: Formalin fixation is not the culprit. Br J Dermatol 2009;160:810–814.
9. Eid I, El-Muhtaseb MS, Mukherjee R, et al. Histological processing variability in the determination of lateral resection margins in rectal cancer. J Clin Pathol 2007;60:593–595.
10. Golomb FM, Doyle JP, Grin CM, et al. Determination of pre-excision surgical margins of melanomas from fixed-tissue specimens. Plast Reconstr Surg 1991;88:804–809.
11. Hudson-Peacock MJ, Matthews JNS, Lawrence CM. Relation between size of skin excision, wound and specimen. J Am Acad Dermatol 1995;32:1010–1015.
12. Lum H, Mitzner W. Effects of 10% formalin fixation on fixed lung volume and lung tissue shrinkage: A comparison of eleven laboratory species. Am Rev Respir Dis 1985;132:1078–1083.
13. Mouritzen DA. Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations. J Hornfirsch 1979;20:1501–1506.
14. Sui KF, Cheung HC, Wong J. Shrinkage of the esophagus after resection for carcinoma. Ann Surg 1986;203:173–176.
15. Stickland NC. A detailed analysis of the effects of various fixatives on animal tissue with particular reference to muscle tissue. Biotech Histochem 1975;50:255–264.
16. Wang L, Shen J, Song X, et al. A study of the lengthening and contractility of the surgical margins in digestive tract cancer. Am J Surg 2004;187:452–455.
17. Yeap BH, Muniandy S, Lee SK, et al. Specimen shrinkage and its influence on margin assessment in breast cancer. Asian J Surg 2007;30:183–187.
18. Withrow SJ. The evolution of veterinary surgical oncology. Clin Tech Small Anim Pract 1998;13:1–3.
19. Goldstein NS, Soman A, Sacksner J. Disparate surgical margin lengths of colorectal resection specimens between in vivo and in vitro measurements; The effects of surgical resection and formalin fixation on organ shrinkage. Am J Clin Pathol 1999;111:349–351.
20. Silverman MK, Golomb FM, Kopf AW, et al. Verification of a formula for determination of pre-excision surgical margins from fixed-tissue melanoma specimens. J Am Acad Dermatol 1992;27:214–219.
21. Argyle DJ, Saba C. Gastrointestinal tumors. In: Argyle DJ, Brearley MJ, Turek MM, eds. Decision Making in Small Animal Oncology. Ames, Iowa: Wiley-Blackwell, 2008:217–237.
22. Gibbs C, Pearson H. Localized tumors of the canine small intestine: A report of twenty cases. J Small Anim Pract 1986; 27:507–519.