

Original Article

A new experimental model to allow use of clinical-scale endoscopes in small-animal tumor models

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ABSTRACT

Background: The evaluation of novel endoscopes may require testing in experimental tumor models, particularly when employing new biomarkers. Tumor models, however, exist almost exclusively in small animals. Therefore, we aimed to develop an experimental setting that allows the use of clinical-scale endoscopes in small animals.

Methods: In our approach, the proximal large bowel with intact blood supply is exposed on a movable and height-adjustable table. The endoscope's tip may be inserted into the bowel; the dark environment of the bowel lumen in vivo is simulated by mounting a light-tight curtain around the endoscope. Proof-of-principle experiments were done in Wag/Rij rats following cecal injection of the cell line R1H.

Results: Using high-definition television white-light endoscopy, narrow-band and autofluorescence imaging, and miniprobe-based confocal laser microscopy (CLM) marked differences were observed between normal mucosa and tumors. Depending on the techniques, mean examination times ranged from 3 to 10 minutes. Even after 90 minutes the colon displayed an intact blood supply, imaged by Evans blue injection and by CLM.

Conclusion: These experiments demonstrate that our model allows in vivo examination of small-animals by clinical-scale endoscopes. Therefore, it may be useful for evaluation, at various stages of GI carcinogenesis, of both new biomarkers and endoscopic technologies.

KEY WORDS

abdominal malignancies, basic science, colon cancer, high-tech endoscopes, small-animal tumor models

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Introduction

Endoscopy has made enormous progress in imaging of the gastrointestinal (GI) tract, ultimately leading to better detection of luminal lesions, esp. in one of the main areas such as screening colonoscopy for adenoma detection. Nevertheless, neoplasia miss rates have

repeatedly been reported, and the acceptance of classical endoscopy as diagnostic and screening tool has limited availability (1-4). Thus either better lesion detection by conventional endoscopy and/or the introduction of simpler and more patient-friendly techniques such as capsule endoscopy may lead to better outcomes. With simpler methods such as the colon capsule, a decrease in sensitivity has to be accepted (5). This raises the need for additional imaging technology using markers or other red flag techniques to overcome these limitations. Such new markers will need to be tested in an experimental live setting, as will new imaging technologies, in the various stages of colon neoplasia as well as in tumors elsewhere in the GI tract.

Animal testing is usually required for any new technology before being used in humans, particularly if they involve the topical or intravenous (i.v.) application of new substances to stain or highlight dysplastic or neoplastic lesions. However,

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tumor models have been preferentially developed in rodents (mice and rats), while new endoscopes, particularly prototypes, are manufactured exclusively with large diameters (~9-12 mm) appropriate for patients. Current attempts to address this issue include use of tumor models in larger animals and the development and use of small (i.e. rodent-scale) endoscopes but these have not yet progressed significantly. Dedicated small-animal endoscopes, for example, are often based on fiberoptic technology rather than on high-resolution video, the technology routinely used in clinical endoscopy (6,7).

The current study has therefore been undertaken to combine both considerations, small-animal tumor models and normal size clinical-scale endoscopes, in a single experimental setting and to thereby establish a practical method for evaluating new endoscopic techniques in small-animal models of colon carcinogenesis. The main aims of the study were to determine (i) the feasibility of the proposed method, and (ii) the duration of preservation of a vital colon mucosa with intact blood supply under the study conditions described.

Methods

Animal tumor model

The rhabdomyosarcoma cell line R1H, originally derived from the jaw musculature of a WAG/Rij rat, was provided by Dr. Annette Raabe, Department of Radiotherapy and Radio-Oncology, University Hospital of Hamburg-Eppendorf, Germany. Male WAG/Rij rats were purchased from Charles River WIGA (Deutschland) GmbH (Sulzfeld, Germany). All experiments were performed according to German law at the Preclinics – Gesellschaft für präklinische Forschung mbH (Potsdam, Germany).

For tumor formation, 50 µl of cell suspension of 2×10^7 R1H cells were injected at 4 sites into the cecal wall of 12-week-old male WAG/Rij rats (n = 6) following laparotomy under general anesthesia with isoflurane. Another male WAG/Rij rat did not receive tumor cell injections.

Intraoperative endoscopy

At 23 days after the cell injection, the animals underwent a second laparotomy under inhalation anesthesia with isoflurane. The cecum was then opened longitudinally. The cut was made at the side contralateral to the insertion of the mesenteric blood vessel, in order to preserve an unhindered blood supply during the entire procedure. With the now exposed luminal surface upwards, the cecum was positioned on a specially designed height-adjustable table above the animal's chest; the table also had a measuring line alongside

the exposed bowel.

The endoscope was fixed in an upright clamp with a screw mechanism to allow adjustment of the distance between the endoscope tip and the bowel (Fig 1). In order to simulate the dark conditions of in vivo endoscopy, an opaque curtain was fixed around the circumference of the distal portion of the endoscope. Relative movement of the endoscope and the bowel was achieved by slow and controllable movement of the whole animal, placed on a movable hoist on top of which was the small table for the exteriorized bowel, a unit designed and built by E.F (Fig 1).

Following the procedure all animals were sacrificed immediately using T61 (Intervet, Germany).

Endoscopes and confocal laser microscopy

For endoscopy, a Lucera CF-H260AZL/I[®] colonoscope attached to the EVIS Lucera Spectrum[®] video system (Olympus, Tokyo, Japan) was employed, providing 1080i high-definition television (HDTV) imaging and two additional light observation modes, that is, narrow-band imaging (NBI) and autofluorescence imaging (AFI). For confocal laser microscopy (CLM) a Leica animal Z-probe attached to a laser scanning unit (Cellvizio[®]-Lab system; Mauna Kea Technologies, Paris, France) was used. Leica FCM 1000 IC software was employed for image documentation. Immediately before the CLM examination, 0.1 ml of a 5% fluorescein solution (Alcon Pharma GmbH, Freiburg, Germany) was injected into the rat's tail vein.

Results

Preparation of the animal model

Sufficient inhalation anesthesia of an individual animal, opening of the abdominal cavity, longitudinal exposure of the cecum and cleansing of stool required a mean time of 11 minutes (SD ±4 min). The integrity of the blood flow was determined by i.v. administration of Evans blue dye (Sigma-Aldrich, Munich, Germany) through the tail vein of a tumor-free WAG/Rij rat, revealing an apparently intact blood supply for the maximum tested time of 90 minutes (Fig 2). The adequacy of the blood supply was also proven by CLM imaging after i.v. administration of fluorescein in all animals. Moreover, no notable bleeding occurred in any of the animals.

The opened cecum, placed on a small table located above the rat's chest, offered an area of approximately 4 × 2 cm of exposed mucosa for further endoscopic evaluation. All animals that had undergone prior injection of the R1H cell line displayed tumor nodes of 3–8 mm diameter at the injection sites.

Assessment of NBI, AFI, and CLM in rat colon tumors

To test the applicability of a clinical-scale endoscope to inspect the rat cecum, a colonoscope was placed close to the mucosa. Conventional white-light endoscopy showed that about two thirds of the cecum could be observed while keeping a fixed position, with slight movements

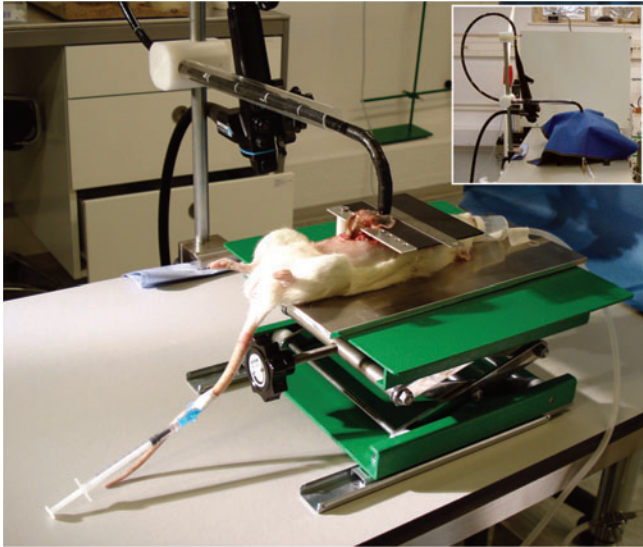


Figure 1 Preparation of the rats for intraoperative endoscopy, and the experimental setup. Laparotomy was performed in WAG/Rij rats which were under inhalation narcosis. The opened cecum was positioned with the lumen upwards on a specially designed small table above the animal's chest; the table also had a measuring line alongside the exposed bowel. This setup was positioned on a height-adjustable hoist, itself placed on slides, thus allowing movement of the exposed animal bowel relative to the endoscope. The various endoscopes were fixed in an upright clamp with a screw mechanism that permitted adjustment of the distance between the endoscope tip and the opened bowel. Intravenous injection was done via a cannula placed in the animal's tail vein. A larger GI lumen is simulated by mounting a roughly circular curtain around the endoscope tip (small picture).

of the endoscope tip allowing gradual inspection of the entire surface. Normal mucosa and tumor sites were clearly distinguishable in all animals. The NBI mode enhanced the visibility of the blood vessels and of the superficial mucosal structure, displaying a regular structure in untransformed areas. In contrast, tumor areas showed a lack of structural organization. AFI clearly discriminated normal mucosa and tumor, with sharp borders between the purplish untransformed areas and the greenish tumors. Subsequent CLM revealed distinctly different patterns for normal mucosa, which displayed a honeycomb-like regular structure, and for tumors, which showed an irregular structure (Fig 3). Examination times depended on the individual techniques: requiring 3 min (SD \pm 30 s) for NBI and AFI and 10 min (SD \pm 2 min) for CLM.

In addition, the procedure was terminated in one animal by exposing the stomach and the esophagogastric junction in a similar manner to the bowel; accessibility was proven, but no further experiments with regards to blood supply or prior tumor injection were performed in these areas (Fig 4).

Discussion

The objective of the current study was to establish a practical and reliable method for evaluation of new endoscopic imaging techniques, including biomarkers as well as clinical scale endoscopes, in small-animal tumor models. Rodent structures which can be imaged by the method described include the colon, small bowel, stomach, and esophagogastric juncture. Extension of this method to capsule endoscopes and other such devices is readily achievable as well.

Our experimental system requires two notable components: first, a small table positioned over the animal adjacent to the laparotomy wound to expose the animal's bowel properly; second, a curtain placed around the tip of the endoscope in order to achieve light-tight examination conditions simulating those in the GI lumen in vivo. The assembly allowed relative movement, vertically and in

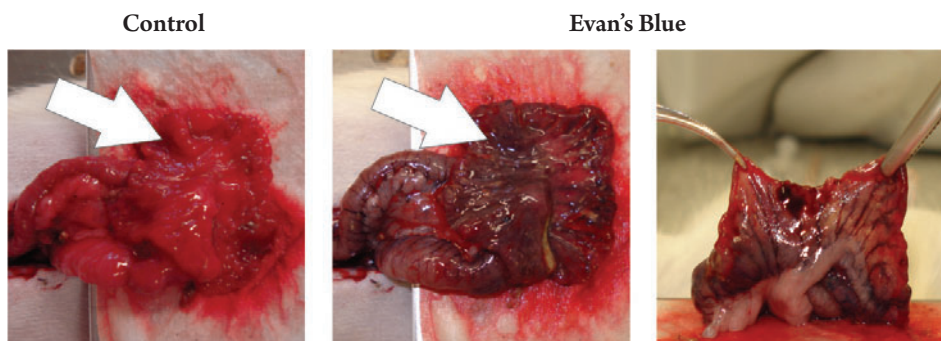


Figure 2 Integrity of the intestinal blood supply. Preservation of the intestinal blood supply was tested by tail vein injection of Evan's blue dye which resulted in a blue coloration. The arrows mark the lumen of the exposed bowel, and the outside of the bowel is shown in the panel on the right.

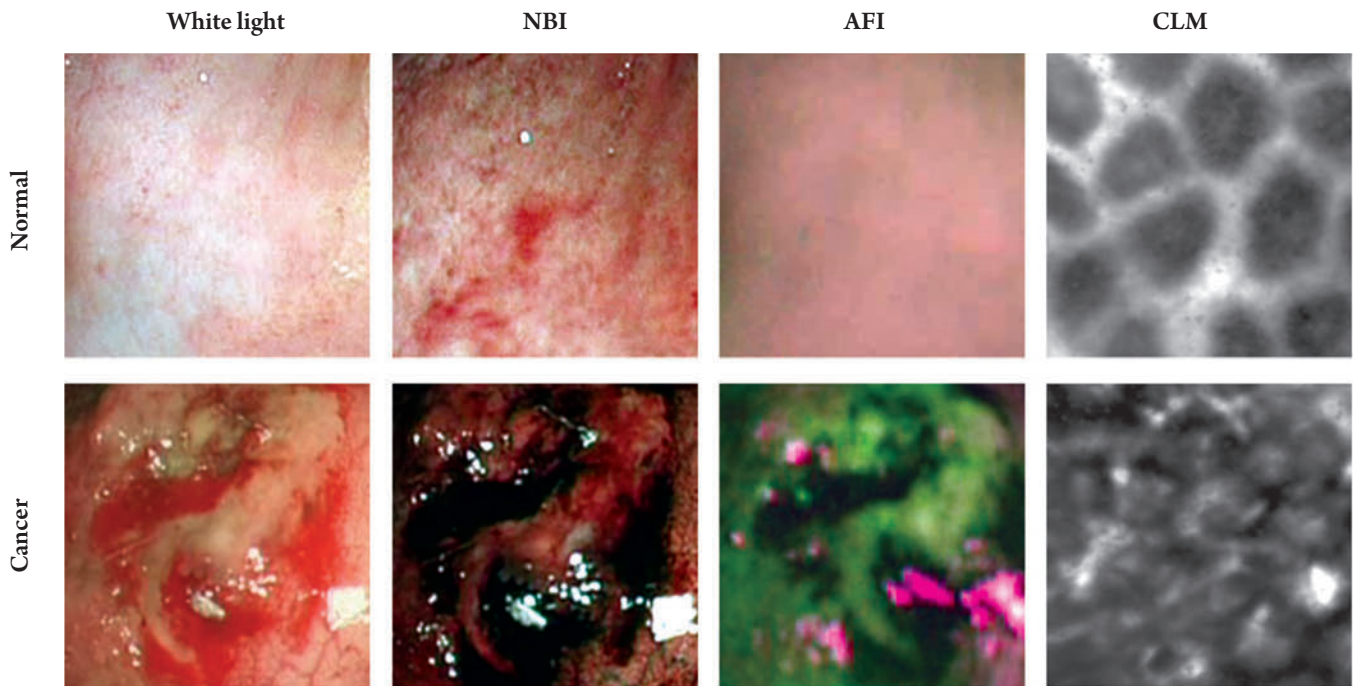


Figure 3 Endoscopic evaluation of non-transformed mucosa and of cancer manifestations in the cecum. All tested endoscopic technologies clearly distinguished normal mucosa (upper panels) from cancerous lesions (lower panels). In addition to conventional white light endoscopy, narrow-band imaging (NBI) enhanced the structure of the mucosal surface, whereas autofluorescence imaging (AFI) displayed a clear discrimination between normal mucosa (purple) and tumor nodes (green). CLM revealed a sharp difference between the honeycomb pattern of normal mucosa and the unstructured and darker appearance of the rhabdomyosarcoma nodes.

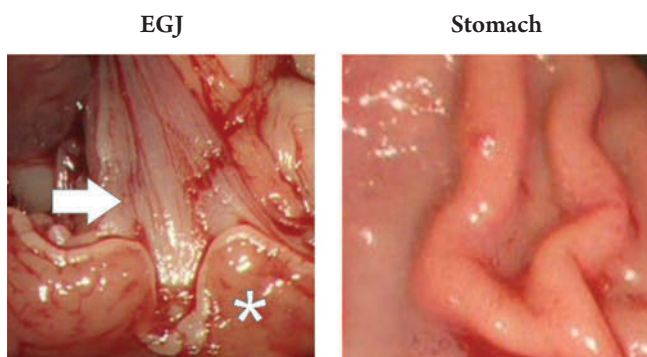


Figure 4 Exposure of the esophagogastric junction (EGJ) and gastric mucosa accessibility of the esophagogastric junction and of the stomach was proven in one animal (arrow: distal esophagus; star: gastric cardia), no further experiments with regards to blood supply or prior tumor injection were performed.

all lateral directions, between the fixed endoscope and the exposed bowel by movement of the whole animal on a movable table. This setup proved to be adequate and easy to use, requiring little time and effort for appropriate positioning.

The newest endoscopic imaging strategies may require topical or i.v. administration of substances to label neoplastic cells or to mark cells and tissues, for example, the use of fluorescein in confocal laser microscopy (8-10). The evaluation of such substances may need testing in animals with sustained intestinal vitality and intact blood supply. Our data, using Evans blue dye for macroscopic inspection and fluorescein for CLM (no CLM signal at all can be detected if fluorescein is not delivered by the bloodstream), suggest that the blood supply remains intact for at least 90 minutes when our setup is used. Therefore, it may be suggested that this system offers a feasible technology to test labeled (e.g. fluorescently marked) biomarkers. Given the short examination times for each technique (3-10 min) our model allows for evaluation of several methods in the same animal. In addition, the model includes a very precise

matching of imaging site and the site of histological analysis by using a measuring device alongside the exposed bowel. Such a feature is particularly important in studying subtle and perhaps macroscopically imperceptible lesions and/or using so-called “endoscopic histology” techniques such as CLM that sample only a very small area.

A disadvantage of our model may be that sequential examination of the same animal during various stages of tumor development is not possible since intraoperative endoscopy can only be performed once. However, sequential series of animals at different time intervals after tumor induction may largely solve this problem. Furthermore, in the same animal, precise identification of the same site for follow-up endoscopy is difficult or even impossible in any case.

In order to assess the new endoscopic technologies, comparisons within defined disease stages of colon carcinogenesis are desirable. However, such conditions can hardly be found in humans. Moreover, the comparison of various techniques within an individual patient may be hard to accomplish, as it may require a switch of endoscopes or administration of several marker substances. Therefore, tumor models resembling carcinogenesis in humans offer a valuable tool for preclinical testing of endoscopes and imaging technology. Several tumor models, including knockdown of tumor suppressor genes, chemically induced cancers, and orthotopic xenotransplantation of human colon cancer cell lines have been developed (11,12). However, these models have been primarily established in rodents that to date cannot be examined using clinical-scale endoscopes. Our approach provides an opportunity to employ these models to test such endoscopes. Thus there is no requirement either for dedicated small-animal endoscopes that are not adaptable to the full range of available image-transmission technologies (since they are fiberoptic-based) or for the time-consuming adaptation to rodents of a particular clinical-scale endoscope to rodents (6,7). As our experimental setting requires opening of the intestinal lumen it may not be used to evaluate risks of the endoscopic examination per se such as perforation. However, it may help to reveal unwanted side effects of new agents and/ or devices in terms of local tissue damage.

In the current proof-of-principle study, we also aimed to assess whether our approach might be used to discriminate normal from malignant tissue. Therefore, we tested conventional white-light endoscopy, NBI, AFI, and also CLM, after intracecal injection of a sarcoma cell line. Our results show that all these techniques clearly distinguish areas of normal mucosa from tumors, emphasizing that this approach could be used at various stages of colon carcinogenesis. However, our data are

based on a rhabdomyosarcoma cell line as this model had been previously established and, therefore, offered an immediate and well defined condition for the primary test of our method. Moreover, as this cell line was originally derived from WAG/Rij rats, as used for our experiments, no particular considerations were necessary regarding the immune status of the animals. Nonetheless, it must be stated that due to the use of this cell line the extrapolation of our results regarding tumor imaging to the conditions of colon cancers is limited. In contrast to previous observations in humans (13-15) with AFI we observed a purple signal in normal areas whereas tumors appeared green. Potential inter-species differences must therefore be considered in evaluating new endoscopic technologies in non-human systems.

In summary, we have described a novel, practical method for evaluation of new endoscopes and endoscopic imaging technologies for the diagnosis of various GI cancers and their precursors. Further studies of this method are currently underway.

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