A New Tube Gastrostomy Model in Animal Experiments

Deneysel Yeni Bir Tüp Gastrostomi Modeli

Deneysel Tüp Gastroskopi Modeli / Tube Gastrostomy Model in Animal Experiments

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Özet

Amaç: Orogastrik yol, deney hayvanlarına orogastrik tüp veya cerrahi olarak açılan bir osteotomi aracılığıyla su vermek amacıyla sıklıkla kullanılan yoldur. Biz bu çalışmada hareketleri dizginlenmemiş deneysel rat modellerinde yeni ve basit tüp gastrostomi modelini sunmayı amaçladık. Gereç ve Yöntem: Bu deney on iki erkek Sprague-Dawley ratının rastgele olarak kontrol grubu (grup C, n: 6) ve tüp gastrostomi grubu (group TG, n: 6) olarak iki gruba ayrılmasıyla oluşturuldu. Silikon bir foley kateterden(6 French) basit bir gastrostomi tüpü türetildi. TG grubunda bir kesi yapıldı ve mide görüntülendi. Peritonyum açılışında orta hatta 1 cmlik bir kesi yapıldı. Askı dikişler atıldı ve ön gastrik duvar kaldırıldı. Sonrasında gastrik duvar açıldı. Cihaz mideye yerleştirildi ve cilt altından tünel içinden ilerletilip yan karın duvarına 2/0 dikiş ile tespit edildi. Bulgular: Prosedür deneyin onuncu gününde sona erdirildi. Grup C'de mortalite gözlenmedi. Ratlar günlük olarak takip edildi ve herhangi bir kendi kendine zarar verme, ısırık izi, ağızdan beslenmeye direnç gösterme, kaçmaya çalışma gibi anormal davranış gözlenmedi. Deney sonunda TG grubunda alanin transaminaz düzeyindeki artışta ($p<0.05$) ve vücut ağırlığı ve proteinindeki azalışta ($p<0.05$) istatistiksel anlamlı farklılık gözlendi. Grup C'de ise üre seviyesindeki artış ($p<0.05$) istatistiksel olarak anlamlı idi. Aynı zamanda Grup C'deki aspartat transaminaz, albumin, vücut ağırlığı ve total protein seviyesindeki düşüş de ($p<0.05$) istatistiksel olarak anlamlı idi. Preoperatif dönemden deneyin sonuna kadar olan dönemde gastrostomi ve laparotomi gruplarındaki glikoz ($p=0.047$) ve aspartat transaminaz ($p=0.050$) düzeylerindeki düşüş ve tartı kaybı ($p=0.034$) istatistiksel olarak anlamlı idi. Tartışma: Biz, yeni geliştirdiğimiz tüp gastrostomi modelinin orogastrik yolun kullanılamadığı deneysel modellerde iyi bir alternatif olacağına inanmaktayız.

Anahtar Kelimeler

Hayvan Deneyleri; Deneysel Dizayn; Gastrostomi

Abstract

Aim: The orogastric route is the most preferred application method in the vast majority of the animal experiments in which application can be achieved by adding the material to the water of the experiment animal, through an orogastric tube or with a surgically managed ostomy. Material and Method: This experiment was constructed with twelve male Sprague-Dawley rats which were randomly assigned to one of two groups consist of control group (group C, n: 6) and tube gastrostomy group (group TG, n: 6).A novel and simple gastrostomy tube was derivated from a silicone foley catheter. Tube gastrostomy apparatus was constituted with a silicone foley catheter (6 French). In the group TG an incision was performed, and the stomach was visualized. A 1 cm incision was made in the midline and opening of the peritoneum. Anchoring sutures were placed and anterior gastric wall was lifted. The gastric wall is then opened. The apparatus was placed into the stomach and pulled through from a tunnel under the skin and fixed to the lateral abdominal wall with a 2/0 silk suture. Result: The procedure was ended in the 10th day of experiment. No mortality was observed in group C. The rats were monitored daily and no abnormal behavior consists of self harming incision site, resistance to oral intake or attending to displace. There was statistically significant difference in increasing alanine transaminase level ($p<0.05$) and decrease in the total protein and body weight ($p<0.05$) at the group TG at the end of experiment. There was significant increase in urea levels in Group C ($p<0.05$) at the end of experiment. The statistically significant decrease was observed in the same period in group C between aspartate transaminase, albumin, total protein, and body weight ($p<0.05$). Glucose ($p=0.047$) and aspartate transaminase ($p=0.050$) level decrease changes and weight loose ($p=0.034$) from preoperative period to the end of the experiment between gastrostomy and laparotomy groups were statistically significantly. Discussion: Here in we presented a novel and simple tube gastrostomy model in an experimental rat model in which rats had unrestrained movements. We believe that this new constructed tube gastrostomy model may be an alternative route for the experimental models in which orogastric route is unavailable to use.

Keywords

Animal Experimentation; Experimental Designs; Gastrostomy

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Aim

The orogastric route is the most preferred application method in the vast majority of the animal experiments in which the application or the effect of a new drug on the gastrointestinal system or experiments based on nutrition support or nutrition solutions are investigated. Application of the research material to the gastrointestinal tract may be available by adding the material to the water of the experiment animal, through an orogastric tube or with a surgically managed ostomy [1, 2]. Here in we presented a novel and simple tube gastrostomy model in an experimental rat model in which rats had unrestrained movements.

Material and Method

The study was performed at the Laboratory Animals Care Unit in accordance with the guidelines for the care and use of laboratory animals established by the Animal Ethics Committee following the approval of the design by the Animal Ethics Committee. Sixteen male Sprague-Dawley rats (ages: between 11 and 13 weeks) were randomly assigned to one of two groups: control group (C, n: 6) and tube gastrostomy group (TG, n: 6). Rats were kept in a room at a constant temperature (22 ± 2°C) with 12-h light and dark cycles, in individual cages and were fed a standard rat chow. The animals were allowed free access to water before the experiment. The feeding, body weight, and general clinical conditions of the animals were monitored daily. On the first day and the 10th day of surgery rats were anesthetized and blood samples were collected for the assessment of biochemical analysis (aspartate transaminase, alanine transaminase, albumin, total protein, urea, creatinine, glucose, and electrolytes). During the entire experiment period the rats were allowed a same amount of rat chow and water.

Gastrostomy Apparatus

A novel and simple gastrostomy tube was derivated from a silicone foley catheter. A silicone foley catheter (6 French) was shortened to 4 cm. Catheter balloon valve was cut off and the orifice was closed. The main lumen of the catheter was plugged with a stopper and the tube gastrostomy apparatus (TGA) was constituted (Figure 1).

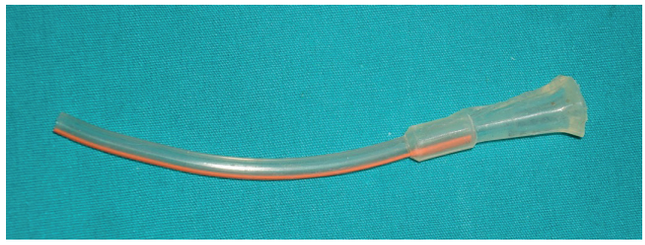


Figure 1. Tube gastrostomy apparatus

The Experimental Design

After an overnight fasting period, control group (group C) was anesthetized using 5 mg/kg of xylazine and 30 mg/kg of ketamine hydrochloride intramuscularly prior to the experiment. A midline incision was performed, and the stomach was visualized. The midline incision and the skin were closed with 3/0 prolene sutures.

The group tube gastrostomy (group TG) was anesthetized using 5 mg/kg of xylazine and 30 mg/kg of ketamine hydrochloride intramuscularly. An incision was performed, and the stomach was visualized. A 1 cm incision was made in the midline and opening of the peritoneum. Anchoring sutures were placed and anterior gastric wall was lifted. The gastric wall is then opened. The TGA was placed into the stomach, 4/0 prolene purse string suture was performed and then fixed with absorbable seromuscular stitches (3/0 prolene) to the anterior parietal peritoneum. The TGA was pulled through from a tunnel under the skin and fixed to the lateral abdominal wall with a 2/0 silk suture. The midline incision and the skin were closed with 3/0 prolene sutures (Figure 2).

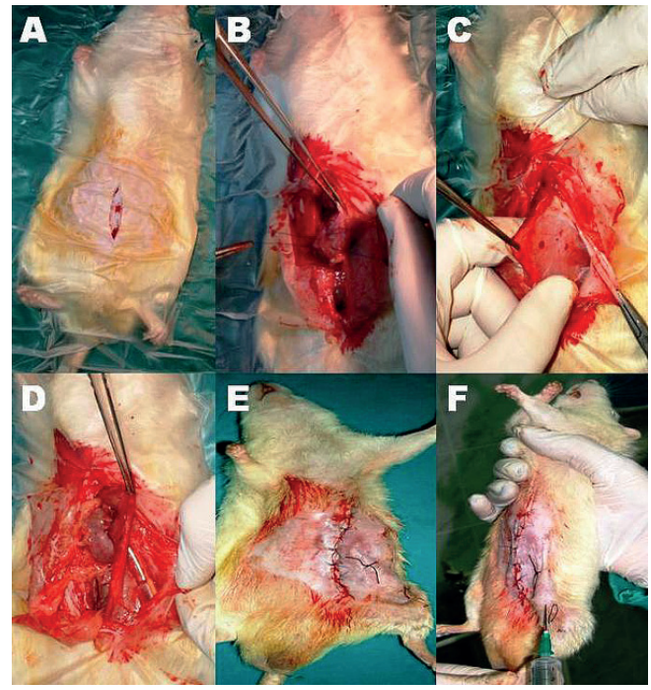


Figure 2. Closure of the wound (A, B, C, D, E, F)

Statistical analysis

The numeric results were expressed as mean±sd, and median (min-max). Differences between preoperative and postoperative values in each group were assessed by Wilcoxon sign rank test. Changes from preoperative to postoperative between gastrostomy and laparotomy groups were assessed by Covariance analysis (ANCOVA). A P-value <0.05 was considered as statistically significant. Statistica 7.0 (StatSoft Inc. Tulsa, OK, USA) statistical software was used for statistical analyses.

Result

The procedure was ended in the 10th day of experiment. No mortality was observed in group C. One rat died within 2 days after the procedure in Group TG and autopsy was performed to clarify the death. The main cause of the death was peritonitis due to inappropriate placement of the tube gastrostomy apparatus and the leakage of gastric contents from the gastrostomy orifice. One rat had incision site detachment in group TG. Rat's wound was revised. The rats were monitored daily and no abnormal behavior consists of self harming incision site, resistance to oral intake or attending to displace TG were observed among rats in both groups.

The values of aspartate transaminase, alanine transaminase, albumin, total protein, urea, creatinine, glucose, and electrolytes

in groups C and TG in preoperative period and at the end of the experiment were shown in Table 1. There was statistically significant difference in increasing alanine transaminase level ($p<0.05$) and decrease in the total protein and body weight ($p<0.05$) at the group TG at the end of experiment. There was significant increase in urea levels in Group C ($p<0.05$) at the end of experiment. The statistically significant decrease was observed in the same period in group C between aspartate transaminase, albumin, total protein, and body weight ($p<0.05$). Glucose ($p=0.047$) and aspartate transaminase ($p=0.050$) level decrease changes and weight loose ($p=0.034$) from preoperative period to the end of the experiment between gastrostomy and laparotomy groups were statistically significantly.

Table 1. Biochemical parameters of the groups.

	Group TG		Group C		p
	Preoperative Period	**End of the experiment**	**Preoperative Period**	**End of the experiment**	
Ur	30.5±5.9 29 (25-42)	26.17±5.11 25.5 (18-31)	30 (28-32) 30.17±1.60	26.17±3.37* 26 (21-31)	0.620
Cr	0,34±0,16 0,29 (0,24-0,67)	0.34±0.07 0.35 (0.2-0.42)	0.23±0.03 0.23 (0.2-0.29)	0.32±0.02* 0.33 (0.30-0.37)	0.581
Gl	245.67±48.1 237 (196-316)	220.5±31.94 213 (188-278)	246.83±27.44 253 (196-276)	251±26.82 259.5 (217-279)	0.047
Na	138±5.5 136 (134-149)	141.17±2.63 140.5 (139-146)	136±1.26 136.5 (134-137)	135.67±3.44 135.5 (131-140)	0.014
K	5.38±1.6 4.75 (4.3-8.7)	5.25±1.02 5.15 (3.9-6.7)	5.1±0.55 4.9 (4.6-6)	5.76±1 5.45 (4.7-7)	0.439
Cl	100±1.26 99.5 (99-102)	100.33±3.07 99.5 (97-105)	99.17±2.04 99 (97-102)	101.5±5.4 101 (95-111)	0.623
ALT	47,33±4,8 47,00(42-55)	47.67±10.5 43 (38-65)	50.5±7.06 51 (41-60)	67±15.07* 68 (46-88)	0.050
AST	129±6.63 126.5 (124-141)	149.5±10.13* 152 (135-160)	137.83±45.83 118 (100-221)	161.83±21.64 161.5 (137-195)	0.245
Prt	6.3±0.27 6.25 (5.9-6.7)	5.71±0.37* 5.9 (5.1-6)	5.9±0.16 5.9(5.7-6.1)	5.31±0.24* 5.3 (5-5.6)	0.744
Alb	1.43±0.13 1.45 (1.2-1.6)	1.66±0.58 1.75 (0.8-2.4)	1.3±0.06 1.3 (1.2-1.4)	1.03±0.13* 1 (0.9-1.2)	0.203
Wg	365±17 364.5 (345-388)	343.17±18.06* 340.5 (323-364)	350.33±41.09 346 (302-408)	339.67±39.67* 334.5 (293-397)	0.034

* $p<0.05$ compared with preop, Ur: urea, Cr: creatinine, Gl: glucose, Na: sodium, K: potassium, Cl: chloride, ALT: alanine transaminase, AST: aspartate transaminase, Prt: total protein, Alb: albumin , Wg: weight

Discussion

In this experimental study a novel and simple tube gastrostomy model was developed which allows experiment animal unrestrained movements. The results of the current study indicate that this novel tube gastrostomy model is useful in short period of experimental animal model in which orogastric route is not suitable during experiment. Researches on diseases associated with gastrointestinal system, studies on the effects of nutrition solutions and toxin ingestion experiments are commonly used and accepted as a pioneer or extrapolation to the human condition. Administration of an experiment material, drug, toxin, or nutrition solution through the gastrointestinal system may be challenging due to the used route, operation techniques, time, and sedation requirement. The simplest method of drug administration is adding research material to the food or water of the experiment animal. This method was applied by many authors in animal experiment models. Höfer et al. [3] investigated the dose and route dependent effects of cadmium in the rat uterus. The method of the experiments was constructed on the oral intake of cadmium in drinking water of the rats for four weeks [3]. On another experimental study, Pal et al. [4] examined the effect of garlic on isoniazid and rifampicin-induced hepatic injury in rats by adding the investigated drugs in the water of rats for 28 day through the experiment period. Like as in many experimental methods although the effects of investigated material access is through the gastrointestinal tract, the expected or investigated effect of drug on end or examined organ is extra gastrointestinal. We support that the medications in which the experiment procedure requires strict dose adjustment, an ostomy procedure may be added to the experiment method to arrange the appropriate doses applied. The orogastric route is the other alternative method for application the investigated material. Stainless steel rods or silastic tubing with a feeding tube is generally used in vast majority of experiments. Buts et al. [5] examined the effects of Saccharomyces boulardii on ileal adaptation after proximal enterectomy in rats. The authors applied Saccharomyces boulardii and saline solution by nasogastric intubation from day 1 to day 8 after surgery by a rod though the esophagus. They mention that at the end of experiment the death rate was less that 5% but they did not clarify the causes of the deaths. Sezer et al. [6] investigated the effect of Saccharomyces boulardii on reducing irinotecan-induced intestinal mucositis and diarrhea. During the experiment period three rats died within 2 h after the procedure. The investigators performed an autopsy to clarify the deaths. The main causes of the deaths were iatrogenic tracheal intubations and respiratory insufficiency due to aspiration of S. boulardii on its administration via the feeding tube. In conclusion, the main problems in the usage of the orogastric route can be enumerated as time-consuming processes, sedation requirements in many experiment protocols, two personnels for the handling and the manipulation, need for experience, and the mortality due to tracheal intubation [1, 2, 7-10]. Tube ostomies are frequently used but anesthesia requirement, operative mortality, wound infections, damage to the ostomies of the animals between themselves, limitation in the movements of the animals, and high cost are the disadvantages of these routes. Emre et al. [1] published a novel model for tube gastrostomy in 2007. A modified Janeway tube gastrostomy model was performed by a tube constructed from stomach wall. The orifice of the tube was sutured to the abdominal wall and a catheter was inserted in the stomach from this orifice for medication or other applications. Also this and other ostomy models are simple to perform the limitations of these procedures are still not solved. We support that the most important limitation of the ostomy models are the leakage of applied material larger than 1 cc from ostomy and self

harming or injuring the ostomy orifice by other experiment animals. In current study the significant statistically difference was observed in the weight loose between the experiment group and control group. This paradox seems to be a limitation but the weight loose was less than 10% in both groups.

The hypothesis which we support is this novel gastrostomy model is a simple, maintaining unrestrained movements, and useful model with a similar mechanism of chemotherapy ports and peritoneal dialysis catheters with an easy application of experiment material. We believe that this new constructed tube gastrostomy model may be an alternative route for the experimental models in which orogastric route is unavailable to use.

Authors' contributions

AS was involved in the study design, data collection, and writing and editing all aspects of this manuscript. MAY and TS were involved in the study design and editing this manuscript. MAY and TS were also involved in the study design, data analysis, and editing this manuscript. NS was involved in the study design, data analysis, and statistical analysis. All of the authors have read and approved the final version of this manuscript.

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