•基础研究 BASIC RESEARCH•

家兔回肠淋巴管铸型的扫描电镜研究

滕诚毅,王晓平,魏双艳,王广友,汤凤彩

滕诚毅,牡丹江医学院解剖教研室 黑龙江省牡丹江市 157001 王晓平,艾默尔大学细胞生物学系 美国亚特兰大 30033 魏双艳,哈尔滨医科大学保健医院内科 黑龙江省哈尔滨市 150086 王广友,汤凤彩,哈尔滨医科大学神经生物学教研室 黑龙江省哈尔滨市 150086 滕诚毅,男,1961-02-14生,黑龙江省哈尔滨人,汉族,牡丹江医学院解剖教研室 副教授,主要从事淋巴系统的研究. 国家自然科学基金资助的科研项目, No.39070462 项目负责人:汤凤彩,150086,黑龙江省哈尔滨市南岗区保健路157号,哈尔滨医 科大学神经生物学教研室. tangfc@ems.hrbmu.edu.cn 电话:0451-6662943 传真:0451-6669576 收稿日期:2002-10-09 接受日期:2002-11-04

Lymphatic corrosion casts in rabbit ileum: scanning electronmicroscopic studies

Cheng-Yi Teng, Xiao-Ping Wang, Shuang-Yan Wei, Guang-You Wang, Feng-Cai Tang

Cheng-Yi Teng, Department of Anatomy, Mudanjiang Medical College, Mudanjiang 157011, Heilongjiang Province, China

Xiao-Ping Wang, Department of Cell Biology, Emory University, Atlanta 30033, USA

Shuang-Yan Wei, Department of Internal Medicine, Health Care Hospital of Harbin Medical University, Harbin 150086, Heilongjiang Province, China Guang-You Wang, Feng-Cai Tang, Department of Neurobiology, Harbin Medical University, Harbin 150086, Heilongjiang Province, China Supported by the National Science Foundation of China, No39070462 Correspondence to:Prof. Feng-Cai Tang, Department of Neurobiology, Harbin Medical University, Harbin 150086, Heilongjiang Province, China. tangfc@ems.hrbmu.edu.cn

Received:2002-10-09 Accepted:2002-11-04

Abstract

AIM:To investigate the three-dimensional organization and fine distribution of the lymphatics in rabbit ileum.

METHODS:Lymphatic corrosion cast with the Mercox were used for scanning electron microscopy (SEM), and semithin sections were used for light microscopy. The Mercox injected intraparenchymally into ileum wall were cut and put in a concentrated NaOH solution until the tissues were corroded away, and observed under SEM.

RESULTS: The central lacteals were found in the intestinal villi. The villi of the ileum contained two to three lacteals. The central lacteals were drained into the mucosal lymphatic capillary plexus. From the plexus, the lymphatic capillary descended into the lymphatics of submucosal layer and muscular layer. Then they were led into the serosal lymphatics and drained into the lymphatics of intestine mesentery. The cast of the lymphatics showed an appearance of a string of heads and the notch corresponding to the bicuspid valve of the lymphatics.

CONCLUSION: The three-dimensional organization of central lacteals and lymphatics in the rabbit ileum is demonstrated by lymphatic corrosion casts. Numerous impressions of the

endothelial nuclei, rich central lacteals and lymphatic capillary plexus in the ileum mucosa, lymphatic capillary and lymphatics in the submucosa and muscular layer are observed on the lymphatic corrosion cast.

Teng CY, Wang XP,Wei SY,Wang GY, Tang FC. Lymphatic corrosion casts in rabbit ileum: scanning electronmicroscopic studies. Shijie Huaren Xiaohua Zazhi 2003;11(4):446-448

摘要

目的:观察家兔回肠淋巴管的三维结构和微细分布.

方法:淋巴管铸型样品,通过扫描电子显微镜进行观察; 半薄切片样品,用光镜进行观察.淋巴管铸型剂是 Mercox, 采用回肠壁间接注射法.将注入铸型剂的回肠壁切下,置 入NaOH水溶液中腐蚀,直到肠壁组织完全脱离为止,然 后将淋巴管铸型样品置于扫描电子显微镜下观察.

结果:在小肠绒毛内清晰地显示出中央乳糜管,每个绒毛 中有2-3个中央乳糜管.中央乳糜管与黏膜层毛细淋巴管 丛相连通.黏膜层毛细淋巴管注入黏膜下层淋巴管,后者 与肌层淋巴管相吻合.肌层淋巴管连接浆膜层淋巴管,然 后汇入小肠系膜淋巴管.淋巴管呈串珠样外观,其表面存 有双凹切迹,该处相当于淋巴瓣的部位.铸型表面还可以 见到淋巴管内皮细胞核的压迹.

结论:淋巴管铸型清晰地显示家兔回肠壁黏膜层丰富的中 央乳糜管和毛细淋巴管丛以及黏膜下层、肌层毛细淋巴管 和大量的淋巴管的三维结构.

滕诚毅,王晓平,魏双艳,王广友,汤凤彩. 家兔回肠淋巴管铸型的扫描电镜研究. 世界华人消化杂志 2003;11(4):446 - 448

http://www.wjgnet.com/1009-3079/11/446.htm

0 引言

小肠淋巴管的形态研究,已有不少报道^[1-19],但对回 肠壁各层毛细淋巴管、淋巴管的微细分布和各层淋巴 管道的流注关系,仍有不同观点^[1-8].应用合成树脂 (Mercox)制备回肠淋巴管铸型,研究回肠壁内各层淋巴 管道的立体形态和微细分布,国内尚无报道.我们采用 Mercox淋巴管间接注射法^[20,21],进行淋巴管铸型,在 扫描电镜下,观察了家兔回肠淋巴管道的立体结构.

1 材料和方法

1.1 材料 成年健康家兔 16 只,雌雄不拘. 其中 2 只制 备回肠的半薄切片,两只做回肠的石蜡组织切片,其 余 12 只制备回肠的淋巴管铸型样品. 铸型剂是 Mercox,为日本东京生产的一种颗粒微细而均匀的合成树脂 (Velenehospital, Tokyo, Japan).

1.2 方法 在戊巴比妥麻醉下, 打开家兔腹腔. 用1 ml 或2 ml 注射器, 连接特制的淋巴管注射针头, 吸入 混有硬化剂 MA 的 Mercox 溶液,在解剖显微镜下,用 手推法将 Mercox 溶液注入回肠黏膜层和黏膜下层[5,12]. 当观察到蓝色的铸型剂已到达回肠系膜时,切下该段 回肠.在对系膜缘打开肠腔,进行流水冲洗,而后黏膜 面向上固定于塑料板上,浸于生理盐水中,置60 温箱内 2 h. 取出样品,依次浸入 200 g/L NaOH 水溶 液和 150 g/L NaOH 水溶液中各 2 h,当肠壁组织完全 被腐蚀后,样品经流水冲洗,用眼科器械清除表面渗 出物.样品浸于蒸馏水中冰冻,并切成4×4mm的小 温箱干燥,固定于扫描电镜样品台上, 块,经37 进行导电处理后,于离子喷涂器内喷金,取加速电压 10-15KV,于S-520扫描电镜下观察和摄片[12,13].家兔 回肠材料经透射电镜样品系列处理后,包埋于 Epn 812中,做半薄切片,次甲基蓝一天青 染色,在光镜 下进行观察.

2 结果

2.1 黏膜层的淋巴管道 在小肠绒毛黏膜上皮的深侧有 中央乳糜管.中央乳糜管起于盲端,盲端对向回肠的 腔面.其纵轴与回肠的横径平行.中央乳糜管呈顶端膨 大(直径 70-120 μm)的杵状,基部变细(直径 20-40 μm) 后,直接注入黏膜层毛细淋巴管丛(图1,2).中央乳 糜管长度150-280 μm,密度6-9个/mm².在一个小肠 绒毛内可见到2-3个中央乳糜管.黏膜层的毛细淋巴管 特别丰富,其走行弯曲,互相连续成丛(图1),其直径 15-20 μm.根据半薄切片光镜的观察,黏膜层毛细淋 巴管丛位于黏膜固有层的深侧,黏膜肌的浅方(图3).从 黏膜层毛细淋巴管丛向深方发出许多吻合支,穿过黏 膜肌注入黏膜下层淋巴管丛(图1).



图 1 家兔回肠黏膜层的淋巴管铸型样品.SEM × 70, 标尺 =50 μm L: 中央乳糜管;C:毛细淋巴管丛;S:黏膜下淋巴管;M:肌层淋巴管.

2.2 黏膜下层、肌层和浆膜层的淋巴管道 家兔回肠黏 膜下层有丰富的淋巴管,并彼此吻合形成黏膜下淋巴 管丛,从该丛发出侧支进入肌层,与肌层淋巴管相 交通(图 1). 肌层淋巴管注入浆膜层淋巴管,最后汇入 小肠系膜集合淋巴管.在黏膜下层、肌层和浆膜层也 见到带盲端的毛细淋巴管,但数量较少.在半薄切片上 可以见到淋巴瓣(图 3),在淋巴管铸型样品上,也可见 到淋巴瓣的双凹压迹及圆形或卵圆形的淋巴管内皮细 胞核的压迹(图 4).



图2 放大的中央乳糜管(L)和黏膜层毛细淋巴管(C),SEM × 250,标尺=50 µm.



图 3 半薄切片样品.显示黏膜层毛细淋巴管(C)、毛细血管(<),黏膜 下层淋巴管(S)、淋巴瓣()和静脉(V)、动脉(A) × 200,标尺 =20 μm.



图 4 淋巴管铸型样品.显示黏膜层毛细淋巴管(C)和较大淋巴管上淋巴 瓣的双凹压迹(),右下角的图显示淋巴管内皮细胞核的压迹() SEM × 400(右下角的图 × 800,标尺 =10 μm)标尺 =50 μm.

3 讨论

以 Mercox 做铸型剂,采用淋巴管间接注射法,可以 清晰地显示小肠器官内淋巴管的三维结构及微细分 布,这是以往其他研究方法无可比拟的^[1,20,21],但也 必须注意在注入铸型剂时,针尖切勿刺入大血管.在 观察和分析结果时也要与血管铸型相鉴别^[5].通过淋巴 管铸型扫描技术及半薄切片的双重观察,进一步证实 家兔回肠具有丰富的淋巴管道^[20,21].本研究在家兔回肠黏 膜层观察到大量的中央乳糜管和毛细淋巴管丛,这与徐 玉东 et al^[1]、郑国宝 et al^[2]的结果一致.Fukushima et al^[9] 在大鼠小肠内观察到,小肠绒毛内相邻的几个中央乳 糜管的基部相互连接成窦;本研究在家兔回肠未见到 此种情况.我们观察到家兔回肠内的中央乳糜管没有相 互融合,而是直接注入黏膜层毛细淋巴管网.在黏膜层 未有见到较粗的淋巴管,这与有些学者^[5]的报道不同, 回肠大量的中央乳糜管、毛细淋巴管和淋巴管与小肠 的吸收功能,肠道免疫相适应^[22-31].

4 参考文献

- 1 Xu YD, Tang FC, Zhong SQ, Hai LS, Zhang YF, Xu QJ, Wang YX . Scanning electron microscopy of jejunum lymphatic casts in rabbit. *Zhong guo Jiepou Xuebao* 1996;27:349-351.
- 2 Zheng GB, Wang YX. Fine distribution of intramural lymphatics of small intestine. *Zhongguo Jiepouxue Zazhi* 1997;20:490-493
- 3 Shimoda H, Kato S, Kudo T, Usui T. Lymphatic network and nerve plexus in the myenteric layer of the monkey jejunum: a topographic study using an enzyme-histochemical mehtod. Arch Histol Cytol 1998;61:65-73
- 4 Azzali G.Ultrastructure of small intestine submucosal and serosal-muscular lymphatic vessels. *Lymphology* 1982;15:106-111
- 5 Ohtani O,Ohtsuka A. Three-dimensional organization of lymphatics and their relationship to blood vessels in rabbit small intestine. A scanning electron microscopic study of corrosion casts. *Arch Histol Jap* 1985;48:255-268
- 6 Yamanaka Y, Araki K, Ogata T. Three-dimensional organization of lymphatics in the dog small intestine :a scanning electron microscopic study on corrosion casts. *Arch Histol Cytol* 1995; 58:465-474
- 7 Khoo SM, Edwards GA, Porter CJ, Charman WN. A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. *J Pharm Sci* 2001;90:1599-1607
- 8 Hokari R, Miura S, Nagata H, Fujimori H, Koseki S, Kato S, Kurose I, Sekizuka E, Granger DN, Ishii H.Intercellular cell adhesion molecule-1 regulates lymphocyte movement into intestinal microlymphatics of rat Peyer's patches. *J Leukoc Biol* 2001; 70:896-902
- 9 Fukushima K, Sasaki I, Masuda T, Nagura H, Naito H, Funayama Y, Matsuno S. Three-dimensional structure of mucosal and submucosal lymphatics in rat small intestine. *Tohoku J Exp Med* 1998;185:9-14
- 10 Caliph SM, Charman WN, Porter CJ. Effect of short-, medium-, and long-chain fatty acid-based vehicles on the absolute oral bioavailability and intestinal lymphatic transport of halofantrine and assessment of mass balance in lymph-cannulated and noncannulated rats. *J Pharm Sci* 2000;89:1073-1084
- 11 Ichikawa S, Shiozawa M, Iwanaga T, Uchino S. Immunohistochemical demonstration of peptidergic nerve fibers associated with the central lacteal lymphatics in the duodenal villi of dogs.

Arch Histol Cytol 1991;54:241-248

- 12 Drake RE, Abbott RD. Effect of increased neck vein pressure on intestinal lymphatic pressure in awake sheep. *Am J Physiol* 1992; 262:892-894
- 13 Kellersman R, Zhong R, Kiyochi H, Garcia B, Grant DR. Reconstruction of the intestinal lymphatic drainage after small bowel transplantation. *Transplantation* 2000;69:10-16
- 14 Thielke KH, Pabst R, Rothkotter HJ. Quantification of proliferating lymphocyte subsets appearing in the intestinal lymph and the blood. *Clin Exp Immunol* 1999;117:277-284
- 15 Lowden S,Heath T. Lymphatic drainage from the distal small intestine in sheep. *J Anat* 1993;183:13-20
- 16 Yasunaga A,Kato S, Uchida Y,Miyauchi R. Enzyme-histochemical study on the fine distribution of the intramural lymphatics at the ileocecal junction of the monkey intestine. *Okajimas Folia Anat Jpn* 1991;68:259-269
- 17 Benoit JN, Zawieja DC. Effects of f-Met-Leu-Phe-induced inflammation on intestinal lymph flow and lymphatic pump behavior. *Am J Physiol* 1992;262:199-202
- 18 Ichikawa S,Kyoda K, Iwanaga T, Fujita T, Uchino S. Nerve terminals associated with the central lacteal lymphatics in the duodenal and ileal villi of the monkey. *Acta Anat (Basel)* 1993; 146:14-21
- 19 Ichikawa S, Sreedharan SP, Goetzl EJ, Owen RL. Immunohistochemical localization of peptidergic nerve fibers and neuropeptide receptors in Peyer's patches of the cat ileum. *Regul Pept* 1994;54:385-395
- 20 Tang FC, Wang YX, Han MD, Li YL, Hai LS. Observation of the corrosion casts of stomach lymphatics in the rabbit. *Acta Anatomy Sinica* 1992;23:333-336
- 21 Tang FC, Zhang YF, Xu YD,Zhong SQ, Wang XP, Wang YX. Scanning electron microscopic studies of lymphatic corrosion casts in the rabbit appendix. *China Natl J New Gastroenterol* 1996;2:238-240
- 22 Tsilibary EC, Wissig SL. Lymphatic absorption from the peritoneal cavity: regulation of patency of mesothelial stomata. *Microvase Res* 1983;25:22-39
- 23 Leak LV, Burke JF. Fine structure of the lymphatic capillary and the adjoining connective tissue area. *Am J Anat* 1966;118:785-809
- 24 Dobbins WO, Rollins EL. Intestinal mucosal lymphatic permeability: An electron microscopic study of endothelial vesicles and cell junctions. *J Ultrastruc Res* 1970;33:29-59
- 25 Sacchi G, Weber E, Agliano M, Comparini L. Subendothelial nerve fibers in bovine mesenteric lymphatics:An ultrastructural and immunohistochemical study. *Lymphology* 1994;27:90-96
- 26 Guarna M,Pucci AM, Alessandrini C,Volpi N, Fruschelli M, D'Antona D, Fruschelli C. Peptidergic innervation of mesenteric lymphatics in guinea pigs:An immunocytochemical and pharmacological study. *Lymphology* 1991;24:161-164
- 27 McHale NG, Roddie IC. The effect of transmural pressure on pumping activity in isolated bovine lymphatic vessels. *J Physiol* 1976;261:255-269
- 28 Alessandrini C, Gerli R, Sacchi G, Ibba L, Pucci AM, Fruschelli C. Cholinergic and adrenergic innervation of mesenterial lymph vessels in guinea pig. *Lymphology* 1981;14:1-6
- 29 Nikles SA, Heath TJ. Pathways of lymph flow through intestinal lymph nodes in the horse.*Anat Rec* 1992;232:126-132
- 30 Lowden S,Heath T. Ileal Peyer's patches in pigs: intercellular and lymphatic pathways. *Anat Rec* 1994;239:297-305
- 31 Serizawa H, Miura S, Tashiro H, Imaeda H, Shiozaki H, Ohkubo N, Kimura H, Tanaka S, Tsuchiya M. Alteration of mucosal immunity after long-term ingestion of an elemental diet in rats. J Parenter Enteral Nutr 1994;18:141-147