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大鼠小肠甘丙素在肠型放射病时的变化

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提 要 本文用免疫细胞化学（ICC）、H.E染色及显微分光光度计测定等技术，对20Gy γ线肠型放射病时大鼠小肠GAL神经的免疫反应性结构做了定性和定量分析。结果如下（1）照后小肠粘膜层损伤随时间延长而加重。（2）照后各组小肠GAL-LI神经元胞体无明显改变，而GAL纤维出现排列紊乱。（3）照后大鼠小肠内GAL的含量有下降趋势，且照后48h和72h组与非照射组之间存在显著差别（$P<0.01$）。作者结合GAL在肠道的生理功能，将照射后小肠GAL的变化规律与肠型放射病的病理特征及过程相联系，推测照后GAL的变化可能与受照后小肠运动机能、供血障碍以及粘膜损伤等病理改变有关。

关键词 小肠；辐射损伤，实验性；甘丙素；免疫细胞化学

自Tatemoto发现[1]甘丙素（Ga lanin,GAL）以来，在该肽分布、性质及功面能诸方已有许多报道。但做为新的神经肽—GAL在肠型放射病（肠型病）中的变化和作用尚未见国内、外报告。因此，本文采用免疫细胞化学（ICC）和显微分光光度计测定技术，对肠型病时大鼠小肠GAL做了定性及定量分析，并辅以病理组织学观察，以期对GAL在肠型病中的变化及可能的机制进行初步的探讨。

1 材料和方法

1.1 实验动物

取52只雄性Wistar纯系大鼠，体重200g～250g，分组见表1。根据本校防原医学教研室大鼠肠型放射病模型，即用^{60}Co-γ射线20Gy，对大鼠行一次性全身照射，照射率为0.6997Gy/min(150cm×28'32")。照后分组取材，均以空肠段为研究材料。

表1 实验分组

Tab.1 Group of Experiment (N)

Techniques	Control	AIRS		
		24h	48h	72h
HE Stain	3	3	3	3
ICC*	10	10	10	10

* Two immunostained whole mount stretch preparation of every sectional group of each animal were choosed and measure by microspebctrophotometer.

1.2 H.E染色

动物经腹腔注射麻醉后，剖腹取空肠数节入Zomboni's液固定[2]，按H.E染色法制片，光镜观察。

1.3 ICC染色

动物麻醉后，经Zomboni's液灌注固定，取空肠段分别进行冰冻切片和分层铺片处理，标本按Sternberger[3]的PAP法进行免疫细胞化学染色，并设立各种替代对照。ICC主要试剂见表2。

表2 ICC主要试剂及稀释度

Tab.2 ICC Main Agents and Dilution

Main Agents	Dilution	Productive Origin
Rabbit Antigalanin	1:10 000	INC/USA
Goat anti-rabbit IgG	1:20	The Second Military Medical College
Rabbit PAP	1:80	The Second Military Medical College

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1.4 显微分光光度计测定ICC染色

应用西德Leitz-MPV-3型显微分光光度计配用PDP11/03型电子计算机定量测定。扫描范围10×10μm（此范围能容纳铺片神经网格的交叉点，每次随机选取一单独交叉点置于此范围中进行测量），每组10只动物，每只动物选取两张铺片，每张铺片测10个点，4个时相组共计800个点，对所测各个吸光度值进行统计学处理。

2 结 果

2.1 H.E染色观察结果

在照后24h小肠粘膜层呈现损伤变化，且随照后时间的延长而逐渐加重，至照后72h时小肠绒毛变短，粘膜上皮大部分脱落。〔图1〕

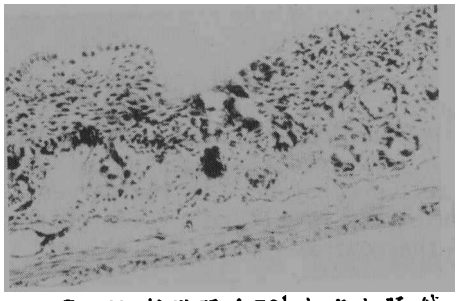


图1 20Gy γ-射线照后72h大鼠小肠绒毛变短，粘膜脱落。×1 200

Fig.1 The mucosa of small intestine in rats after exposing to 20 GY γ-ray, the vili became short and the mucosa has peeled off. ×1 200

2.2 ICC观察结果

照后各时相组小肠肌间神经丛GAL-I神经元结构无破坏。GAL-I神经纤维密度仅在照后72h小肠粘膜层略有增加，且肌间神经丛纤维网与非照射组相比〔图2〕出现排列紊乱，显色不均。〔图3〕

2.3 显微分光光度计测量结果

发现小肠GAL含量随照后时间的延长而呈下降趋势〔表3〕，且照后48h及72h组与非照射组总吸光度值存在显著性差别（$P<0.01$）。

3 讨 论

3.1 大鼠小肠GAL-I神经的主要生理效应及在肠型病时的变化特征

目前认为GAL是胃肠功能的调节物，其作用在大鼠小肠主要表现对平滑肌运动、粘膜细胞分泌及小肠血流等方面起调节作用[1][4]。在肠型放射病时，大鼠小肠粘膜层损伤明显，GAL-I神经胞体无结构破坏，各层GAL-I纤维分布随照后时间延长出现排列紊乱，染色不均，且小肠GAL含量呈下降趋势。

表3 显微分光光度计测定结果

Tab.3 The Results of Microspectrophotometer Measuring ($\bar{x}\pm s$)

Groups	Control	AIRS 24h	AIRS 48h	AIRS 72h
Numeration	63.37±4.73	61.60±5.31	50.71±4.77	42.88±4.69

3.2 照后大鼠小肠GAL含量变化的可能意义

根据GAL在大鼠小肠的主要生理学效应，将照后GAL的变化与肠型放射病的病理过程及特征相联系，推测在照射后该肽对肠型放射病的某些病理过程可能起到一定作用。如GAL具有促平滑肌收缩、血管收缩、升高血压等作用，可能加重肠型放射病时的小肠运动功能及机体血循环功能障碍。至于照射后GAL含量的减低是肽能神经元合成GAL的能力减低或是GAL的消耗过多，以及GAL在肠型放射病时具体的病理生理作用机制都还有待于进一步的实验研究。

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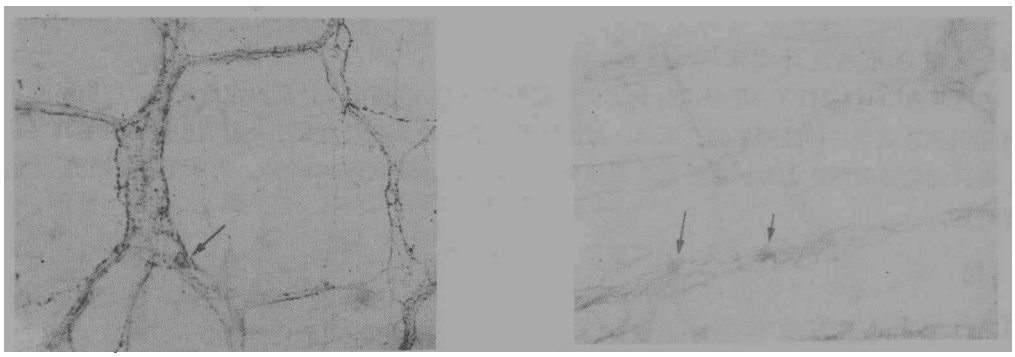


图 2　正常大鼠空肠肌间神经丛铺片，示GAL-I纤维网及GAL-I神经胞体（箭头）。×1 200

Fig.2 Whole mount preparation of rat jejunum (control gronp) showing the myenteric plexus. Note the GAL-I nerve network and neuronal bodies(arrow). ×1 200

图 3　20Gy γ-射线照后72h空肠肌间神经丛铺片，示GAL-I纤维和胞体（箭头），神经纤维杂乱，染色强度弱于对照组，且显色不均。×1 200

Fig.3 Whole mount preparation of rat jejunum after exposing to 20GY γ-ray. Note the distribution of the GAL-I were in a mass and the intensity of immunoreactive substances in the nerves and neurons is lower and uneven than that of the control group (Fig.2) . ×1 200

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The Changes of Intestinal Galanin in Rats with Acute Intestinal Radiation Sickness

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Abstract The changes of galanin in the small intestines of the rats which were inflicted with acute intestinal radiation sickness (AIRS) by exposing to 20 Gy gamma rays were studied with immunocytochemistry (ICC), HE staining of tissue sections, and microspectrophotometer. It was found that the damage of intestinal mucosa was being intensified as AIRS progressed; there no marked change of GAL-LI neurons but the arrangement of GAL-LI fibers was disordered in the irradiated rats as compared with those of the control; the galanin content of the small intestines was significantly lower in the irradiated rats than in the control especially in the 48th and 72nd hour after irradiation ($P<0.01$).

Considering the physiological function of galanin in the small intestines and the characteristics of the pathological changes of AIRS, the authors suggest that the changes of galanin in the irradiated small intestines are closely related to the disturbance of intestinal motility, interference of blood circulation, and mucosa damage resulting from AIRS.

Key words intestine small; radiation injuries; experimental/PA; galanin; immuncytochemistry

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