学位論文

Changes in the mucus barrier during cisplatin-induced intestinal mucositis in rats

(ラットにおける Cisplatin 起因性消化管粘膜傷害に対するムチン量の変化に関する検討)

DM10032　山本　創

北里大学大学院医療系研究科医学専攻博士課程

臨床医科学群　消化器内科学

指導教授　小泉　和三郎

著者の宣言

本学位論文は、著者の責任において実験を遂行し、得られた真実の結果に基づいて正確に作成したものに相違ないことをここに宣言する。

論文要旨

【背景・目的】

抗癌剤起因性消化管粘膜傷害は臨床症状において、下痢、食欲不振としてあらわれ、時に治療継続が困難となる場合があるが、詳細は未だ解っていない。これまで我々は 5-FU をラットに投与すると胃から大腸に至るまでの消化管粘膜に様々な傷害が認められ、部位により傷害の程度に差があることを明らかにしてきた。

そこで今回は、消化器領域の癌に対する化学療法において5-FU同様に汎用される cisplatinを用いて、抗癌剤起因性消化管粘膜傷害に関し、検討を行った。また、抗癌剤起因性消化管粘膜傷害に対し、抗潰瘍薬として臨床で使用される各種 H2 受容体拮抗薬の効果を併せて検討した。

【方法】

Wistar 系雄性ラットに cisplatin を 6mg/kg 単回尾静脈投与し、1、3、7、11 日目に消化管(胃、空腸、回腸、大腸)を抗ムチンモノクローナル抗体使用による免疫組織学的検討及び生化学的粘液量測定を施行した。

(1)Cisplatin 投与におけるラット体重及び消化管粘膜の変化に対する検討

ラットを control 群、cisplatin 投与群の 2 群に設定。実験初日(day0)にのみ、cisplatin 群には生理食塩水にて希釈したcisplatinを6mg/kgの投与量で尾静脈より単回投与した。Control群に対してはcisplatin群で投与する体重あたりの投与量と同量の生理食塩水を尾静脈より単回投与した。投与後 1 日、3 日、7 日、11 日目に各群の体重測定及び消化管(胃、空腸、回腸、大腸)粘膜の傷害の程度、消化管粘液の測定を行なった。

次いで、H_2 受容体拮抗薬である famotidine(3mg/kg)、lafutidine(30mg/kg)の抗癌剤起因性小腸粘膜傷害に対する効果を検討するため、control 群、cisplatin 投与群、famotidine 投与群、lafutidine 投与群の 4 群を設定。実験初日(day0)にのみ、cisplatin 群にはゾンデにて経口的にcarboxymethylcellulose (CMC)を投与後、生理食塩水にて希釈したcisplatinを6mg/kgの投与量で尾静脈より単回投与し、control 群に対しては CMC 投与後、cisplatin 群で投与する体重あたりの投与量と同量の生理食塩水を尾静脈より単回投与した。両群ともに、3 日間(day0,1,2)1 日 2 回ゾンデにて CMC を投与した。Famotidine 群、lafutidine 群は各々famotidine(3mg/kg)、lafutidine(30mg/kg)をゾンデにて経口投与後、実験初日(day0)にのみ、生理食塩水にて希釈したcisplatinを6mg/kgの投与量で尾静脈より単回投与した。両群ともに、3 日間(day0,1,2)1 日 2 回ソンデにて各々famotidine、lafutidine を投与した。

(2)組織学的検討

得られた胃、空腸、回腸、大腸に対して hematoxylin & eosin (H-E)染色を施行し、各消化管粘膜の傷害の程度を評価した。次に、消化管粘液の主成分であるムチンにおいて、構造側鎖に、スルホ基を有する小腸のムチンを認識する抗ムチンモノクローナル抗体である PGM34 を使用して消化管粘膜の傷害の程度及び消化管粘液の分布を評価した。次いで、各細胞の細胞周期において、細胞周期合成期(G1、S、G2 期)の核を特異的に染色する抗体、ki-67 を使用して cisplatin 単独投与群及び famotidine、lafutidine 併用群の消化管粘膜増殖帯の細胞周期の過程を評価した。

(3)消化管粘液量の測定

各々の実験で得られた胃、空腸、回腸、大腸に対し組織の乾燥、粉砕、抽出を経て高分子画分(Fr-1)を作成し、hexose 量を測定。得られた hexose 量を粘液量とした。

【結果】

(1)Cisplatin 単独投与及び H_2 受容体拮抗薬併用に伴うラット体重の変化に関する検討

ラット体重においては、Cisplatin 投与群で、control 群と比し有意に体重の減少を認めた。

体重減少に関しては、cisplatin 投与群において著明に下痢を認めた事から、cisplatin 起因性の催吐作用による食欲減退と下痢による水分減少が考えられた。H_2 受容体拮抗薬併用では lafutidine 併用において、cisplatin 投与に伴う体重減少を抑制した。

(2)組織学的評価

Cisplatin 単独投与において H-E 染色にて消化管粘膜を評価すると、day3 の空腸、回腸に著明な傷害を認め、以降 day11 にかけて、傷害の自然回復を認めた。後述する消化管粘液量の測定結果と合わせ、空腸に比し回腸における粘膜傷害がより顕著であることが示された。次に、特に回腸における抗癌剤起因性粘膜傷害に注目し、各々Control 群、cisplatin 単独投与群及び famotidine 併用群、lafutidine 併用群において、抗ムチンモノクローナル抗体である PGM34 による回腸粘液量の分布を調べた。Cisplatin 投与群では、回腸に表層粘液の減少、粘液分泌細胞の減少及び消化管粘膜の傷害を認めた。これらは、lafutidine 併用下においては粘液量及び消化管粘膜の保持を認めた。Ki-67 による回腸粘膜の増殖帯の評価に関しては、famotidine、lafutidine 併用群の両者において、cisplatin 単独投与群と同様に cisplatin 投与下において、増殖帯は control 群に比し抑制されていた。以上より、cisplatin 起因性の小腸粘膜傷害は空腸に比し、回腸がより傷害される事が示されたが、lafutidine 併用下では、傷害は抑制された。また、lafutidine は増殖帯の細胞周期に直接関与しないことから、cisplatin の細胞傷害作用に影響を与えず、粘液量を保持していることが示唆された。

(3) 消化管粘液量の測定

消化管粘液量において、cisplatin を投与すると、胃、回腸、大腸においてday3 より顕著に粘液量が減少し、day11 にかけて回復する傾向を認めた。組織学的な評価と合わせ、回腸における cisplatin 起因性粘膜傷害とH_2受容体拮抗薬併用による効果を評価すると、lafutidine 併用下において、著明に粘液量減少は抑制された。

【考察】

(1)Cisplatin 単独投与及びH_2受容体拮抗薬併用に伴うラット体重の変化に関する検討

Cisplatin 投与に伴う体重減少に関しては、cisplatin 起因性の催吐作用による食欲減退と吸収の場である空腸、回腸粘膜上皮が傷害される事に起因する栄養、水分の吸収障害及び炎症に起因する血管透過性亢進による水分漏出が併発し、その結果として発症する下痢による水分減少、栄養不良が主たる要因と考えられた。Lafutidine 併用における体重減少の抑制に関しては、lafutidine 投与により、空腸、回腸粘膜上皮の傷害が抑制され、これにより上述の吸収障害、漏出が抑制される事に起因するラット体水分量、筋肉や体脂肪量の保持が主たる要因と考えられた。

(2)組織学的評価及び消化管粘液量の測定

Cisplatin 投与群において空腸、回腸とも投与 3 日目に粘膜上皮の最大の傷害を認め、7 日目以降自然回復を認めた。これは cisplatin 単回投与での血中濃度の減少が二相性を示し、第二相の半減期がおよそ 100 時間程度である事、また、ラットの小腸粘膜上皮の turn over はおよそ 48~72 時間である事に起因する両者の時相が主たる要因として示唆された。空腸と比し、回腸において粘膜上皮に有意に傷害を認めた事に関しては、両者の血流量の差異や、元来の粘膜上皮の脆弱性の差異などの因子が考えられるが、これらは今後も検討が必要である。回腸における lafutidine 併用下での粘液量減少の抑制及び消化管粘膜の保持に関しては、Ki-67 を使用した免疫染色の結果より、増殖帯からの粘膜新生ではなく、lafutidine 独自の作用であるカプサイシン感受性知覚神経を介する粘液産生機構によって粘液産生が保たれ、これにより相対的な管腔側からの細菌、消化酵素などの刺激が緩和された事が粘膜傷害抑制をもたらしたのではないかと考えている。

【結論】

Cisplatin 投与は、ラットにおいて消化管の特に回腸を中心に傷害を生じることが確認でき、傷害抑制には第二世代のH_2受容体拮抗薬併用投与が効果的であることが示唆された。

Contents

1.Introduction

Gastrointestinal (GI) mucositis, a frequent complication of antineoplastic chemotherapy, can reduce treatment effectiveness because it leads to dose reductions, increased healthcare costs, and an impaired quality of life [1, 2]. Clinical practice guidelines recommend antiacid secretory drugs such as H_2-receptor antagonists for the prevention and treatment of GI mucositis [3–6]. Recently, some of the newer H_2-receptor antagonists (so-called second-generation H_2-receptor antagonists) have been frequently used in Japan. These agents have a unique component structurally differing from conventional H_2-receptor antagonists and promote gastric mucosal defense mechanisms, including mucus secretion [7]. Although medical therapy has improved the management of symptoms in patients with chemotherapy-induced mucositis [3–6], their effects on mucosal defense mechanisms remain poorly understood.

At present, 5-fluorouracil (5-FU) and cisplatin-based chemotherapy is most widely used to treat advanced GI cancer [8, 9]. Several experimental studies have investigated the mechanisms of small-intestinal-mucosal injury induced by 5-FU [10, 11], and we have recently reported significant changes in the mucus barrier of the rat during 5-FU-induced GI mucositis [12]. On the other hand, cisplatin is well known to be associated with renal toxicity [13, 14], but there is a dearth of information about its effects on the GI mucosa.

Mucin, a major component of mucus, is considered to play an important role in the physiological defense of the GI mucosa. Our previous studies showed quantitative and qualitative changes in GI mucin in normal and diseased animals, as well as in humans, and demonstrated the importance of mucin in the GI mucosal barrier [15–18]. We have also established several monoclonal antibodies (mAbs) that react with mucin synthesized and secreted by specific mucus-producing cells of the rat GI mucosa [18–20].

The first objective of this study was to sequentially compare the effects of cisplatin on mucus in different portions of the rat GI tract. Next, we evaluated the efficacy of two different types of H_2-receptor antagonists, famotidine, and lafutidine, against cisplatin-induced intestinal-mucosal injury in rats. We also assessed the effects of these drugs on ileal mucin accumulation. Famotidine is a well-known conventional H_2-receptor antagonist, and lafutidine is a second-generation H_2-receptor antagonist group, characterized by possessing a six-membered aromatic ring [7].

2.Materials and methods

2.1.Animals and Drug Treatment

Seven- or eight-week-old male Wistar rats (CLEA-Japan, Tokyo, Japan) were used in this study. The animals were housed in our animal care facility for 1 to 2 weeks to allow body weight to stabilize. At the beginning of the experiment, the animals were weighed. During treatment, food and water were provided ad libitum. The animals were weighed again and sacrificed on the assigned day of each experiment. The stomach, proximal and distal small intestine (corresponding to the jejunum and ileum, resp.), and colon were removed. This study was conducted in accordance with the guidelines of the Animal Laboratory Center of Kitasato University School of Medicine.

2.2.Effects of Cisplatin on Body Weight and GI Tract

Rats were divided into a cisplatin group and control group (n=4-5 per group). In the cisplatin group, cisplatin (Sigma-Aldrich Inc., St. Louis, MO, USA) was suspended in saline solution and injected into a tail vein at a dose of 6 mg/kg on day 0. In the control group, rats were similarly given a single dose of saline solution on day 0. Body weight, histological changes of the stomach, jejunum, ileum, and colon, and the mucin content of these organs were assessed on days 1, 3, 7, and 11 days after injection as described below.

2.3.Effects of H_2-Receptor Antagonists on Cisplatin-Induced Mucosal Damage

Rats were divided into the following 4 groups (n=4-5): a control group, cisplatin group, cisplatin plus famotidine group, and a cisplatin plus lafutidine group. Cisplatin was suspended in saline solution and injected into a tail vein at a dose of 6 mg/kg on day 0 in the cisplatin group, cisplatin plus famotidine group, and the cisplatin plus lafutidine group. The control group similarly received saline solution on day 0. In the cisplatin plus famotidine group and the cisplatin plus lafutidine group, the respective antiulcer drugs were suspended in 0.5% carboxymethyl cellulose (CMC) (Kanto Chemical Co. Inc., Tokyo, Japan) solution immediately before use. The first dose of each antiulcer drug (famotidine 3 mg/kg; lafutidine 30 mg/kg) was given by oral gavage 30 minutes before the injection of cisplatin on day 0. Additional doses of famotidine or lafutidine were similarly given once daily on days 1 and 2. Control animals received 0.5% CMC instead of the antiulcer drugs. Rats in all groups were fasted from day 2 onward and were sacrificed on day 3.

2.4.Histological Examination

Specimens of each tissue were immediately fixed for 3 h in Carnoy's solution, freshly prepared as described elsewhere [21]. After fixation, the tissues were dehydrated in ethanol, cleared in xylene, embedded in paraffin, and sliced into 3 mm thick paraffin sections, which were then prepared for immunostaining with antimucin monoclonal antibodies (mAb). Immunohistochemical staining was done using the avidin-biotin-peroxidase method and an LSAB2 Kit (Dako, Carpinteria, CA, USA). Briefly, endogenous peroxidase activity was blocked with 0.3% H_2O_2, and the tissue was then sequentially incubated with 10% (v/v) normal swine serum, an anti-mucin mAb (PGM34), biotinylated anti-mouse immunoglobulins, streptavidin horseradish peroxidase (HRP), and 0.02% 3,3-diaminobenzidine in 50 mM Tris-HCl, pH 7.6, containing 0.005% H_2O_2. Counterstaining was done with hematoxylin and eosin (H-E). The immunohistochemical reactivity of the mAb was assessed with the use of an optical microscope. Villus height in the epithelium of the jejunum and ileum was measured in 5 rats per group. The villus height was measured at 3 sites of 3 high-power fields (total, 9 sites) in each rat and the mean value and standard deviation were calculated.

The epitope of the mAb PGM34 was recently shown to be a specific sulfated oligosaccharide of the mucin molecule. This mAb stains all goblet cells of rat small intestine [20]. The Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2, and mitosis) but is absent in resting cells (G_0), making it an excellent marker for determining the so-called "growth fraction" of a given cell population [22–24]. Paraffin sections of the small intestinal mucosa, 3 μm thick, were used for immunostaining with Ki-67, performed by the same method as outlined above. A primary mAb against Ki-67 (MIB-5; Dako, Glostrup, Denmark) was used instead of the antimucin mAb. The number of Ki-67 positive cells was measured for each sample.

2.5.Biochemical Examination

Specimens from each tissue were lyophilized and powdered for extraction of mucin as described previously [15]. Each sample was suspended in 50 mM Tris-HCl, pH 7.2, containing 2% Triton X-100 (Triton-Tris buffer), homogenized, and then incubated at 37°C for 1 h. After centrifugation at 8000 g for 30 min at 4°C, the supernatant was collected, and an aliquot was applied to a Bio-Gel A-1.5 m column and eluted with the Triton-Tris buffer. The void volume fraction (Fr-1) monitored by hexose measurement was collected as mucin. The hexose content of this fraction was measured by the phenol-sulfuric acid method using galactose as the standard. The mucin content (Fr-1 hexose value) was expressed as micrograms of Fr-1 hexose per gram of dry tissue weight.

2.6.Statistical Analysis

Differences in mean values among groups were analyzed by one-way analysis of variance with Scheffe's test; values of less than 0.05 were considered to indicate statistical significance.

3.Results

3.1. Body Weight Change

During the 11-day study period, body weight increased in a stepwise fashion in the control rats, but body weight gain significantly decreased after the injection of cisplatin (Figure 1). During the first 3 days after treatment, body weight decreased in the rats given cisplatin (6 mg/kg i.v.). As shown in Table 1, there was virtually no change in the body weight of rats given cisplatin plus famotidine as compared with those given cisplatin alone. In contrast, lafutidine inhibited cisplatin-induced body weight loss.

3.2.Changes in Morphology and Mucin Content of GI Mucosa after Cisplatin Treatment

Mucosal damage characterized by epithelial sloughing and mucosal ulceration of villous tips was detected in the GI tract mucosa of each rat after injection of cisplatin. On day 3 after treatment with cisplatin, severely injured epithelial mucosa was seen in the small intestine, especially the ileum, whereas evidence of GI mucosal injury was minimal on day 1. As shown in Figures 2(b) and 2(c), cisplatin treatment markedly decreased the villus height in the intestine. The villus area fully recovered by day 11 after cisplatin challenge. The simultaneously measured mucin contents of the rat GI mucosa are shown in Figure 3. The content was most markedly reduced in the ileum on day 3 and increased thereafter. On day 11 after cisplatin challenge, the ileal mucin content had returned to the baseline level.

3.3. Effects of H_2-Receptor Antagonists on Cisplatin-Induced Mucosal Damage

The effects of two types of H_2-receptor antagonists on cisplatin-induced damage of the ileal mucosa were compared on day 3 after cisplatin injection. As shown in Figure 4, in the control rats, immunohistochemical reactivity for PGM34 was detected in goblet cells, as well as in the surface mucus gel layer of the ileum. Cisplatin treatment markedly reduced the villus height and decreased the number of PGM34-positive goblet cells.

In the rats treated with cisplatin plus lafutidine, appreciable damage was rarely found in sections of the ileal mucosa, whereas famotidine did not prevent cisplatin-induced ileal mucosal damage. Likewise, Table 2 compares the effects of the H_2-receptor antagonists on the mucin content of the ileal mucosa on day 3 after the induction of mucosal damage by cisplatin. The mucin content of the ileum decreased after treatment with cisplatin to 67.4% of the control value. Lafutidine significantly inhibited the cisplatin-induced decrease in the ileal mucin content to 86.9% of the control value. In contrast, concurrent treatment with famotidine had no discernible effect on the mucin content as compared with treatment with cisplatin alone. Figure 5 shows the morphologic changes of ileal Ki-67-positive cells after treatment. In the control rats, immunohistochemical reactivity for Ki-67-positive cells was detected in the proliferative zone. Cisplatin treatment remarkably reduced the number of Ki-67-positive cells. In the animals treated with cisplatin plus lafutidine, the expression of Ki-67-positive cells decreased. Nonetheless, the ileal mucosa was maintained in the cisplatin plus lafutidine group.

4.Discussion

We found that intravenous injection of cisplatin in a single dose of 6 mg/kg caused GI mucosal damage altered the GI mucin content and inhibited body weight gain of rats. Our results are consistent with those of prior studies showing that mucosal damage characterized by epithelial sloughing of villous tips occurs 3 to 7 days after treatment with cisplatin [25]. In our preliminary study, 10 mg/kg of cisplatin was also found to induce GI mucosal injury in rats, similar to 6 mg/kg, but a considerable number of animals died after treatment with the higher dose. Previous works showed that 3 mg/kg of cisplatin-induced nephrotoxicity, but not GI mucosal injury in rodents [26]. Taken together, these findings indicate that the dose of cisplatin used in the present study was appropriate for evaluating effects on the GI mucosa. The present data demonstrate that cisplatin, at clinically appropriate doses, not only inhibits renal function but also influences mucin metabolism.

Accumulation of mucin in the GI mucosa is closely related to mucosal protective capability, acting as a mucus barrier [15-17]. In the stomach, mucin is a key element in protecting the gastric epithelium against various irritants [15, 17]. The present study showed that a decreased mucin content in all parts of the GI tract is a cause of mucositis after treatment with cisplatin. Our most notable finding was a remarkable cisplatin-induced reduction in the mucin content of the ileum. Although the protective property of intestinal mucin has received limited attention as compared with gastric mucin, our results suggest that the ileal mucosa is especially vulnerable to the adverse effects of cisplatin.

A specific type of mucin is expressed in distinct mucus-producing cells of the mammalian GI tract [21, 27]. Using the original antimucin mAb PGM34, we studied the preventive effect of lafutidine on cisplatin-induced alterations in rat ileal mucus. This mAb recognizes the sulfuric acid residue structure attached to mucin molecules [20]. Our results showed that lafutidine prevented cisplatin-induced small intestinal mucosal damage in rats. Lafutidine, a second-generation H_2-receptor antagonist, has been reported to stimulate mucin accumulation independently of its H_2-receptor antagonistic properties and to protect against necrotizing-agent-induced mucosal damage in the rat [7]. Moreover, at clinical dose levels, lafutidine not only inhibits acid secretion but also strengthens the mucus barrier of the human gastric mucosa [28].

Our finding that lafutidine prevented mucosal damage indicates that changes in the mucus barrier are the "causes" of cisplatin-induced mucositis. Although further studies are needed to clarify the functions of specific types of mucin, a reduction in PGM34-positive mucin may contribute to the initiation or progression (or both) of chemotherapy-induced mucosal injury in the rat small intestine.

The results of Ki-67 immunohistochemical staining proved that cisplatin alters cell proliferation in the rat intestinal epithelium. Consequently, attenuation of cisplatin-induced mucositis might be attributed to a reduction in its growth-inhibitory activity. Recently, supplementation of nutrients such as glutamine and vitamins was shown to attenuate cisplatin-induced mucosal damage by increasing intestinal-cell turnover [25, 29]. Our present study showed that lafutidine prevented cisplatin-induced alterations in rat intestinal mucus, without affecting cell turnover. Our previous study showed that lafutidine directly stimulated mucin production by rat mucus cells [7]. Thus, the preventive effect of lafutidine against cisplatin-induced intestinal damage may be attributed to the increased production of mucin by goblet cells that remained viable after cisplatin treatment.

In this study, famotidine did not attenuate the morphologic alterations or the changes in the mucin content of the intestinal mucosa in rats treated with cisplatin. First-generation H_2-receptor antagonists such as cimetidine and famotidine have been reported to reduce the production and secretion of rat GI mucin [7]. Our findings suggest that famotidine did not promote the function of goblet mucus cells in this study. Although further investigations are needed to clarify the detailed mechanism of cisplatin-induced intestinal injury, the activation of the goblet cells, if appropriately manipulated, might lead to more effective prevention of cisplatin-induced GI mucositis.

In conclusion, our study had two major findings. First, alteration of the mucus barrier function is a cause of cisplatin-induced mucositis. Second, lafutidine might effectively prevent chemotherapy-induced mucositis by activating intestinal mucus cells.

Acknowledgments

Takafumi Ichikawa is currently receiving grants from the Japanese Ministry of Education, Science and Culture, the Integrative Research Program of the Graduate School of Medical Sciences, Kitasato University, and Taiho Pharm. Co. Ltd., Tokyo, Japan. For the remaining authors none were declared.

Figures and Legends

Figure 1.

Time-course of body weight of rats on 1, 3, 7, and 11 days after treatment with cisplatin. The body weight of each rat was measured immediately before sacrifice. Data are presented as means ± SE (n=4-5). *P<0.05 and **P<0.01

Figure 2.

Microscopical findings of the gastrointestinal mucosa stained with hematoxylin and eosin. The animals were given cisplatin 6 mg/kg i.v. and were sacrificed at various time points (1, 3, 7, and 11 days) after treatment.

Figure 3.

Time-course of the mucin content of the gastrointestinal mucosa. The animals were given cisplatin 6 mg/kg i.v. and were sacrificed at various time points (1, 3, 7, and 11 days) after treatment. Mucin content is expressed as micrograms of Fr-1 hexose per gram of dry tissue weight for each type of mucosa. Data are presented as means ± SE (n=4-5). *P<0.05 and **P<0.01 .

Figure 4.

Comparison of the effects of H_2-receptor antagonists on cisplatin-induced ileal mucosal damage as evaluated by immunostaining with PGM34. The animals were given cisplatin 6 mg/kg i.v. and were sacrificed 3 days after treatment. The villus height of the ileum significantly decreased in the cisplatin group as compared with the control group and significantly increased in the cisplatin + Laf group as compared with the cisplatin group. Means (±SE) n=5 (each group); Fam: famotidine; Laf: lafutidine; * P<0.05

Figure 5.

Changes in the immunoreactivity of Ki-67 of the ileal mucosa after treatment. The number of Ki-67-positive cells in three pits is shown. The arrows indicate Ki-67-positive cells. Means (±SE) n=5 (each group); Fam: famotidine; Laf: lafutidine; *P<0.05

Figure 1

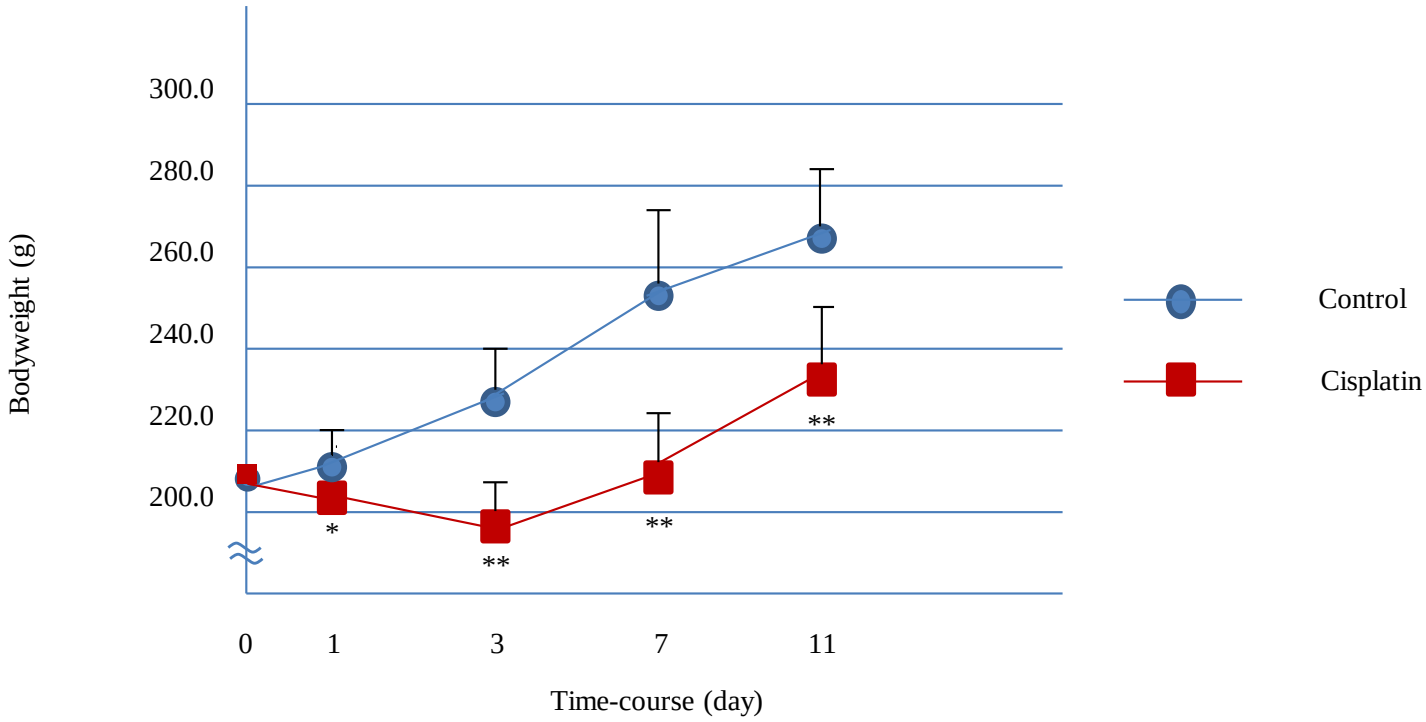
Bodyweight (g)
300.0
280.0
260.0
240.0
220.0
200.0
*
**
**
**
0
1
3
7
11
Time-course (day)
Control
Cisplatin

Figure 2

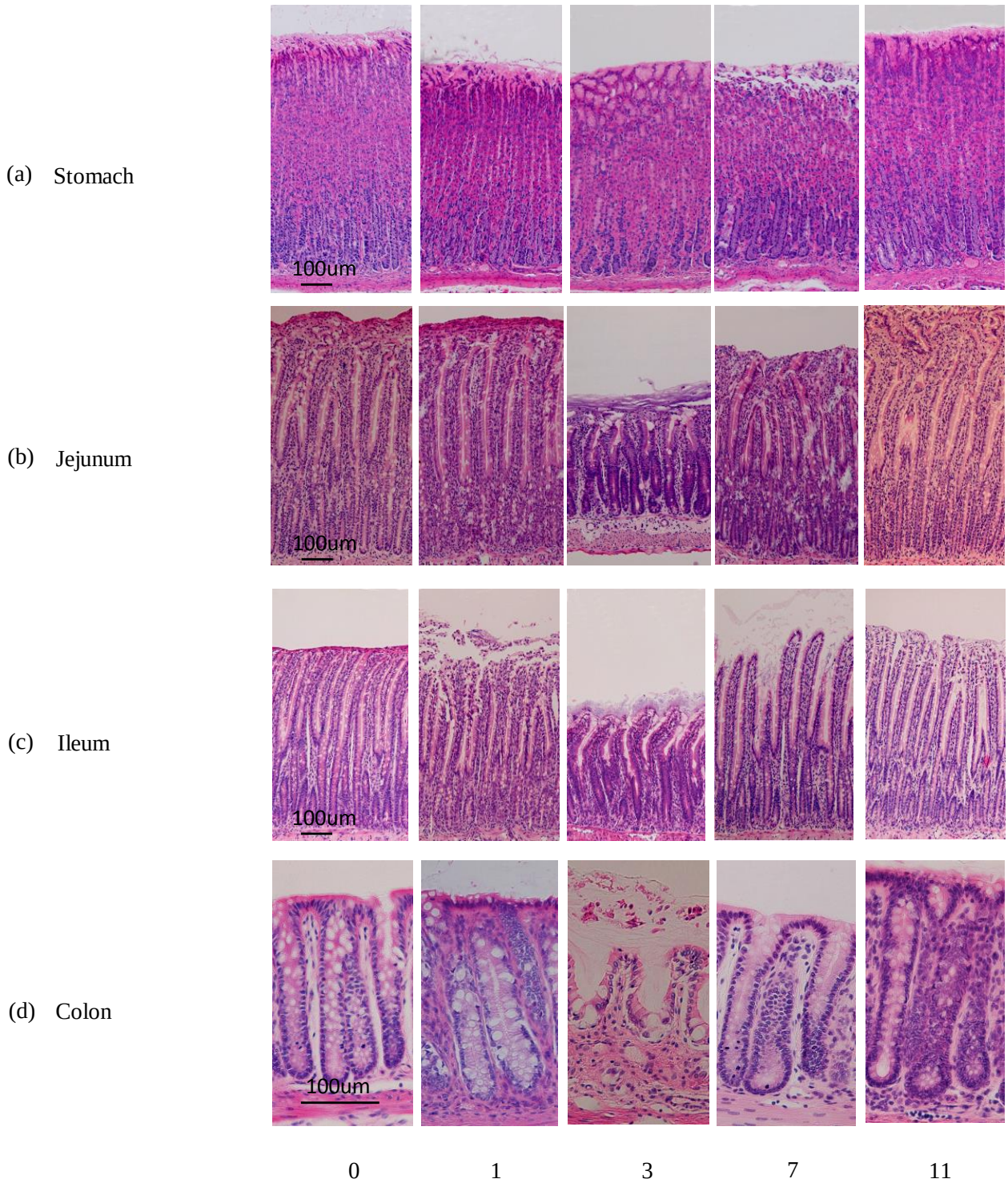
(a) Stomach
(b) Jejunum
(c) Ileum
(d) Colon
100um
0
1
3
7
11
Time-course (day)

Figure 3

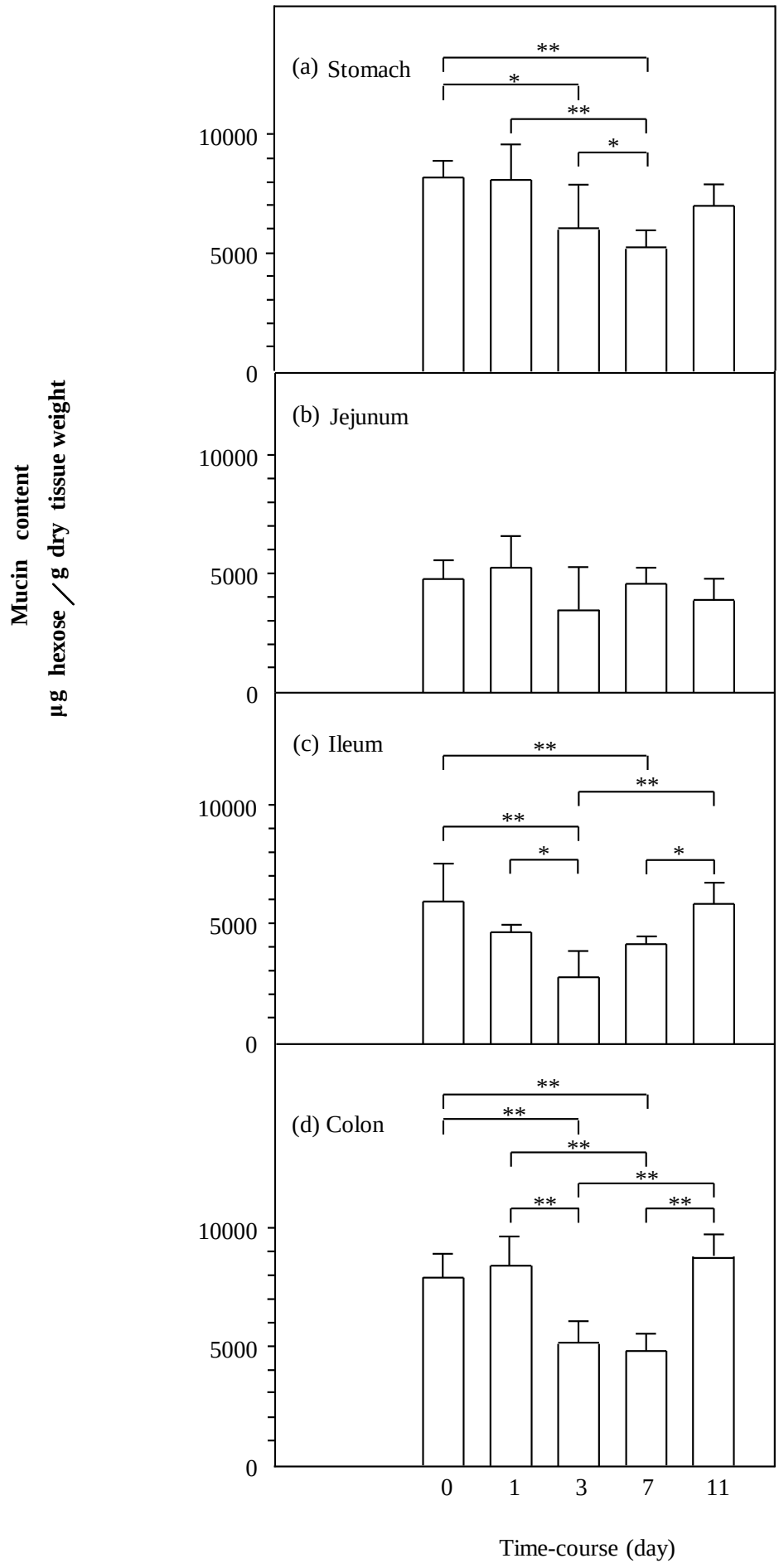
(a) Stomach
(b) Jejunum
(c) Ileum
(d) Colon
**
*
**
*
10000
5000
0
0
1
3
7
11
Time-course (day)
Mucin content
μg hexose／g dry tissue weight

Figure 4

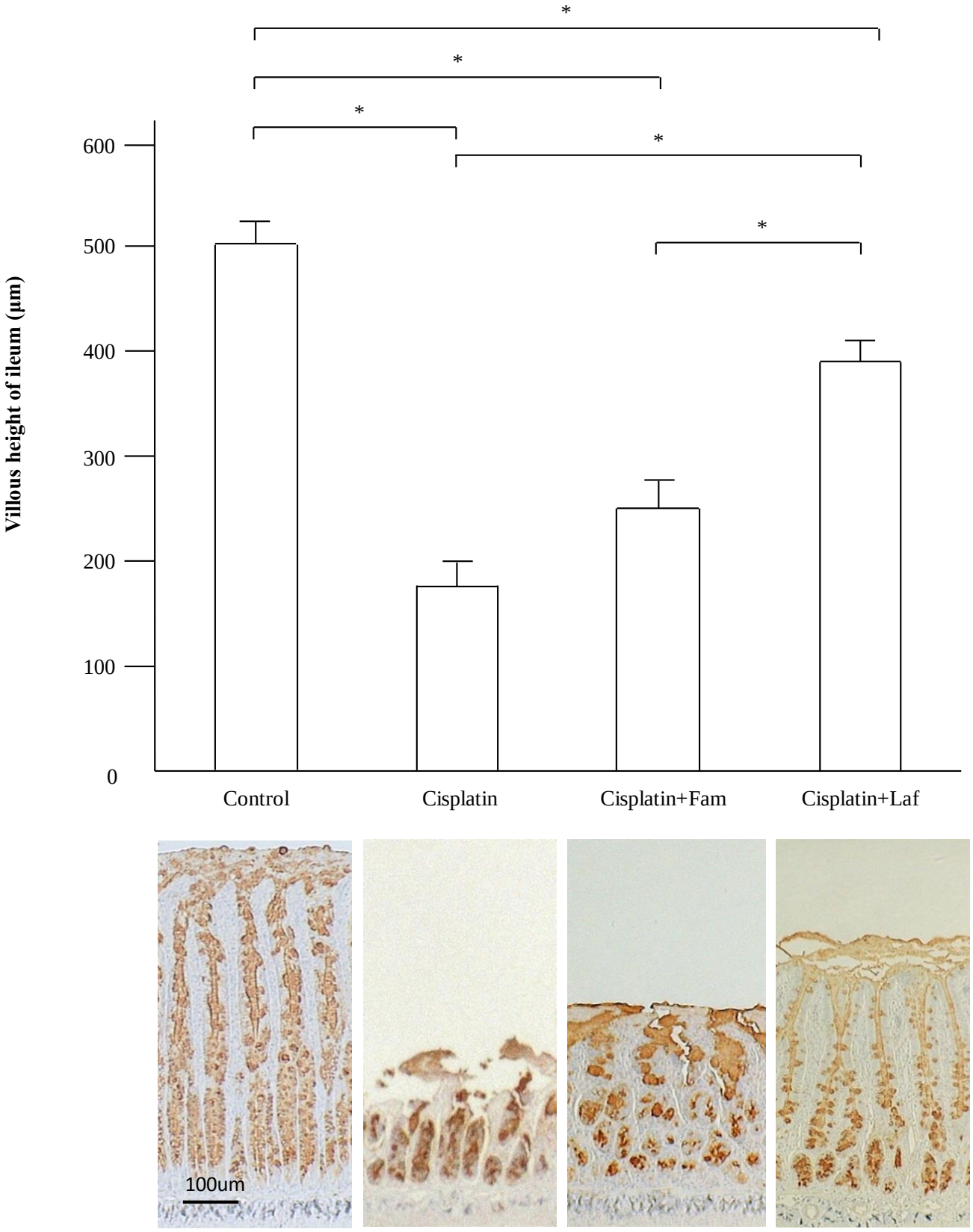
*
*
*
*
*
600
500
400
300
200
100
0
Villous height of ileum (μm)
Control
Cisplatin
Cisplatin+Fam
Cisplatin+Laf
100um

Figure 5

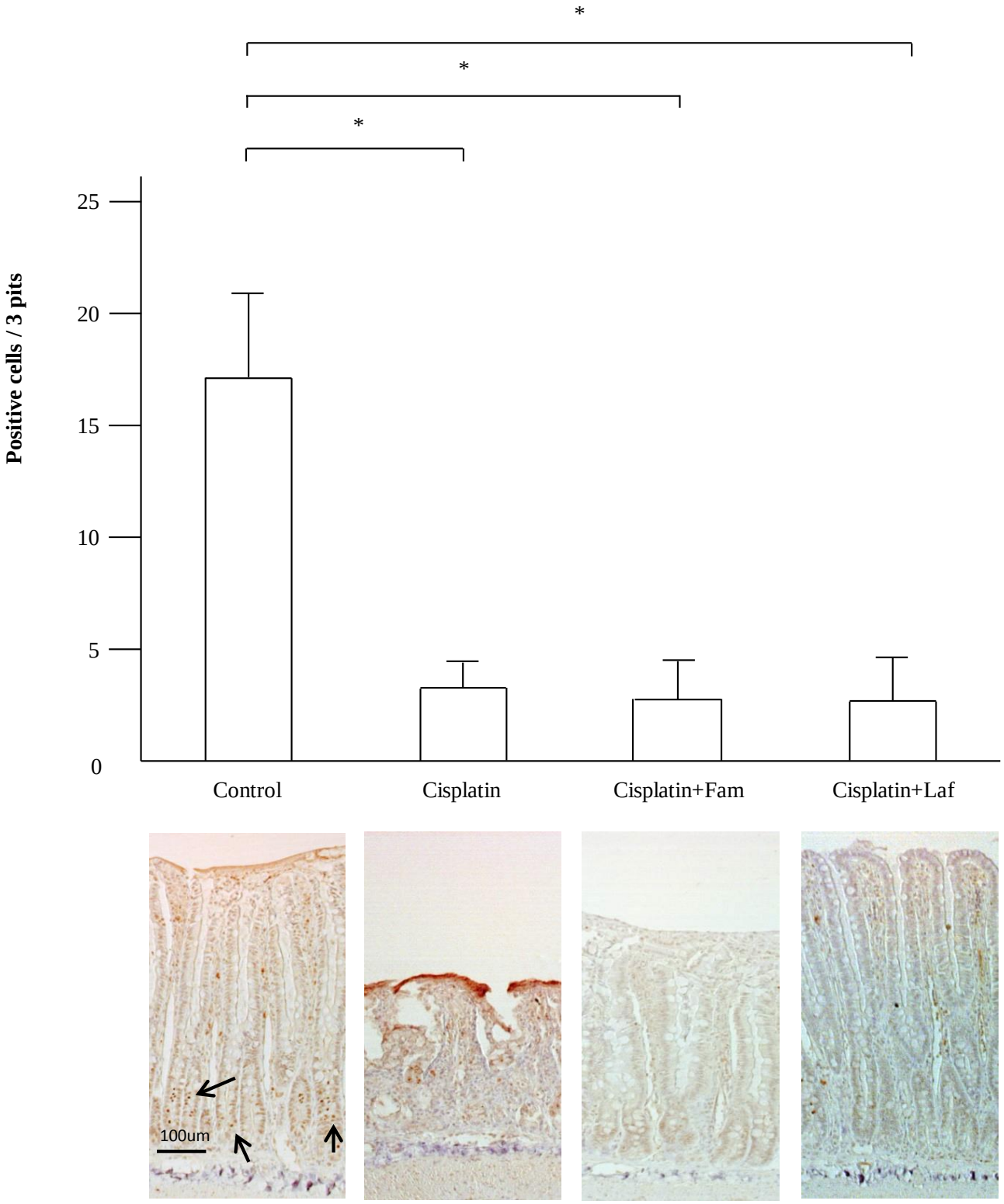
*
*
*
Positive cells / 3 pits
25
20
15
10
5
0
Control
Cisplatin
Cisplatin+Fam
Cisplatin+Laf
100um

Table 1 **Bodyweight of the rats before and 3 days after treatment in each experimental group**

	n	Body Weight (g) Before	After	
Control	4	231.7 (±4.9)	237.5 (±4.3)	*
Cisplatin	4	233.2 (±14.3)	220.8 (±11.0)	*
Cisplatin+Fam	5	234.8 (±2.1)	225.6 (±2.0)	
Cisplatin+Laf	5	235.8 (±5.1)	231.4 (±1.9)	

Fam:Famotidine Laf:Lafutidine
Means(±S.E) *:P<0.05

Table 2

Changes in the mucin content of the ileal mucosa

	n	Hexose value	
Control	4	2575.2 (±433.5)	**
Cisplatin	4	1736.7 (±502.6)	**
Cisplatin+Fam	5	1779.6 (±262.4)	*
Cisplatin+Laf	5	2239.0 (±438.8)	*

The animals were given cisplatin 6mg/kg i.v. and were sacrificed 3 days after treatment.
Mucin content is expressed as micrograms of Fr-1 hexose per gram of dry tissue weight for each mucosa.
Fam:Famotidine Laf:Lafutidine
Means(±S.E) *:P<0.05 and **:P<0.01