EC-18 suppressed tumor growth and alleviated side effects caused by cisplatin in the HNSCC implantation model

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ABSTRACT

Introduction: Head and neck squamous cell carcinomas (HNSCC) are very unfavorable carcinoma that lowers the quality of life. In addition, side effects associated with treatment such as oral mucositis also deteriorate enough to be classified as a disease. This study verified the synergistic antitumor effect of EC-18 (PLAG, 1-Palmitoyl-2-linoleoyl-3-acetyl-roc-glycerol) with or without cisplatin as a chemotherapy and side effects alleviation effects in the metastatic mouse oral squamous carcinoma (MOSCC) orthotopic model.

Method: After inserting mouse-derived squamous oral carcinoma into the right side of the tongue (n=12), it was treated with EC-18 alone or with cisplatin for three weeks. The changes in feed rate and body weight were quantitatively verified on a 2-day interval. Changes in tumor size and oral mucositis symptoms (toluidine-blue positive) were analyzed on the sacrifice day. In addition, changes in the amount of damage-associated molecular pattern (DAMP) increased by tumor and cisplatin, and changes in related active factors were quantitatively analyzed.

Result: Compared to the positive control group, the tumor size was reduced by 42% in the group treated with EC-18 alone (P<0.05). In addition, the tumor size was further reduced by 25% in the group co-treated with EC-18 and cisplatin compared to the cisplatin alone (P<0.05), and it was observed that the tumor was disappeared in 3 mice in the co-treated group. In the positive control group, feed rate and body weight gradually decreased after about 12 days. Also, in the cisplatin-treated group, feed rate and body weight decreased rapidly after six days. On the other hand, in the group treated with EC-18 alone, there was no decrease in body weight and diet, and in the treatment with cisplatin, the diet and body weight gradually recovered after 14 days. Unlike the occurrence of oral mucositis symptoms independent of tumor growth inhibition by cisplatin, simultaneous treatment with EC-18 recovered oral mucositis symptoms along with the reduction of tumor growth (P<0.05). This effect could be explained by the fact that the expression of inflammation-related factors (TNFalpha, IL-6) and DAMP (HMGB1, Adenosine) were effectively controlled by EC-18 (P<0.05).

Conclusion: EC-18 can effectively inhibit the growth of HNSCC and alleviate the side effects caused by existing anticancer drugs at the same time. Therefore, it can be a desirable therapy for HNSCC patients.
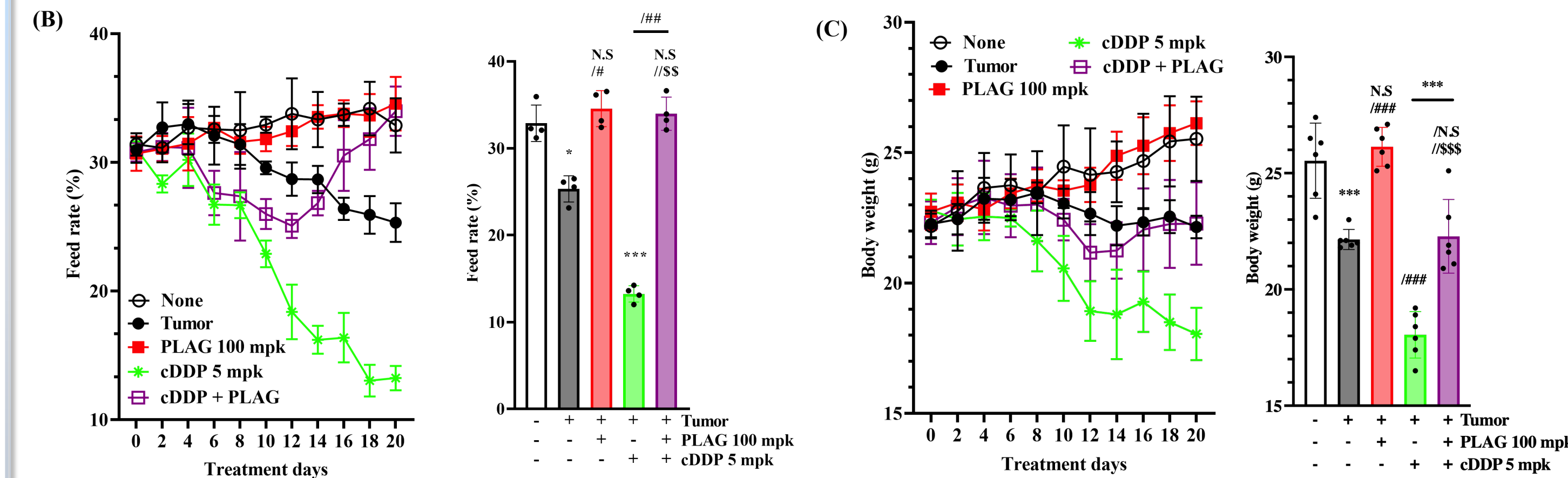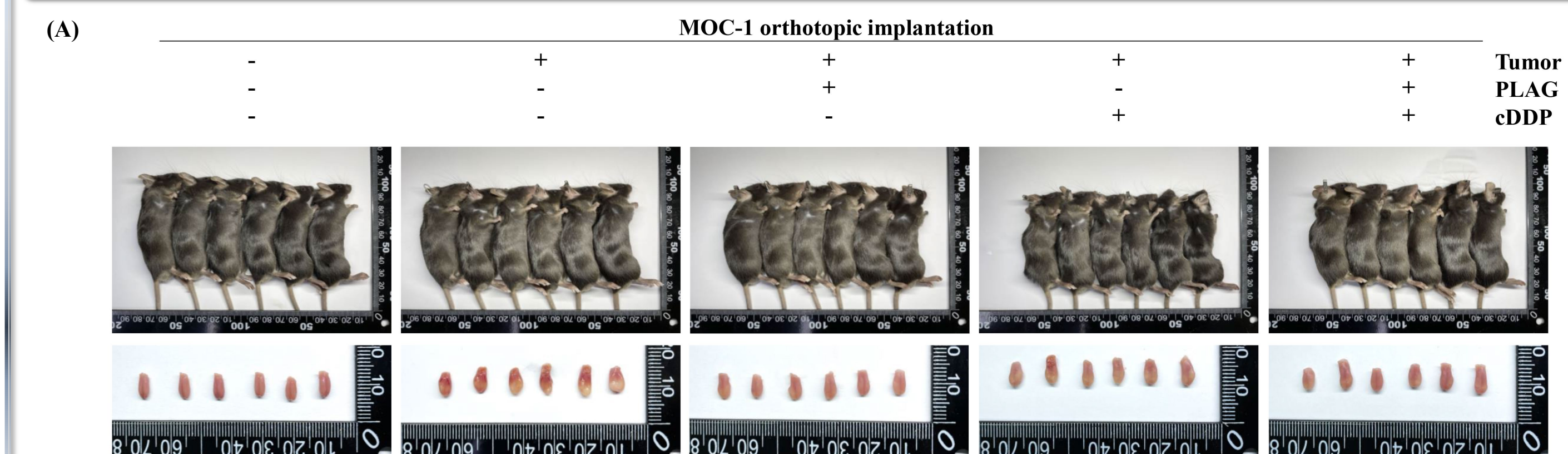
EXPERIMENTAL DESIGN
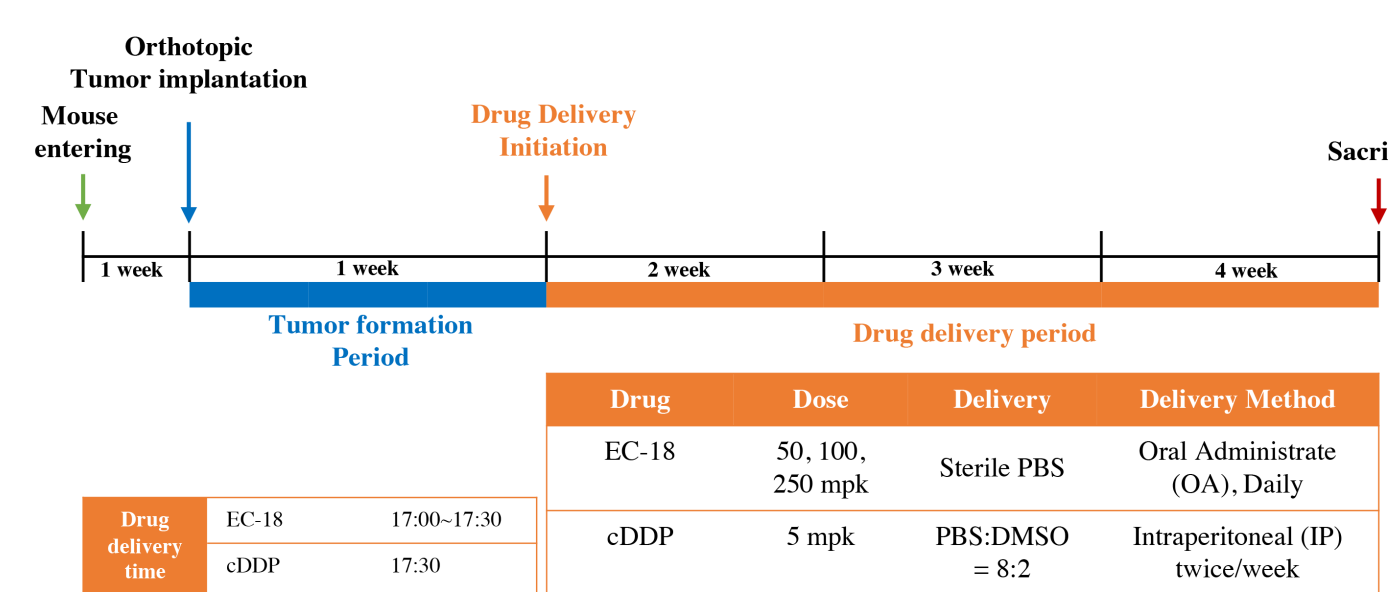
1. Compound concentration
  - PLAG : 100 mpk
  - Cisplatin (cDDP) : 5 mpk

2. Compound delivery
  - O.A : PLAG (Daily)
 - I.P : cDDP (5 mpk, 1 injection/week)

RESULT

1. PLAG alleviates severe weight loss caused by cDDP in the MOC-1 HNSCC implantation model

Analysis of the alleviates severe weight loss by tumor and cDDP following PLAG treatment in MOC-1 HNSCC implantation model.
(A) Confirmation of change in shape of test mice and tumor size in tongue tissue based on the date of sacrifice. (B) Analysis of feed-rate change according to PLAG and cDDP treatment. (C) Analysis of whole-body weight change according to PLAG and cDDP treatment
Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the cDDP only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

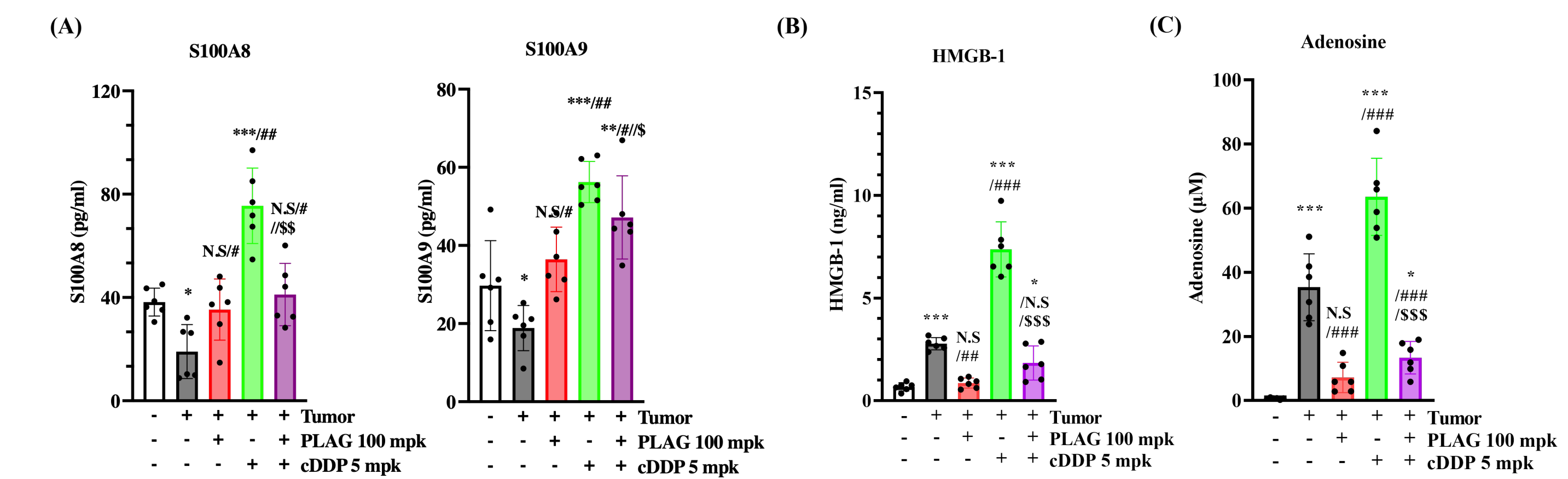### 2. PLAG alleviates oral mucositis (OM) caused by cDDP
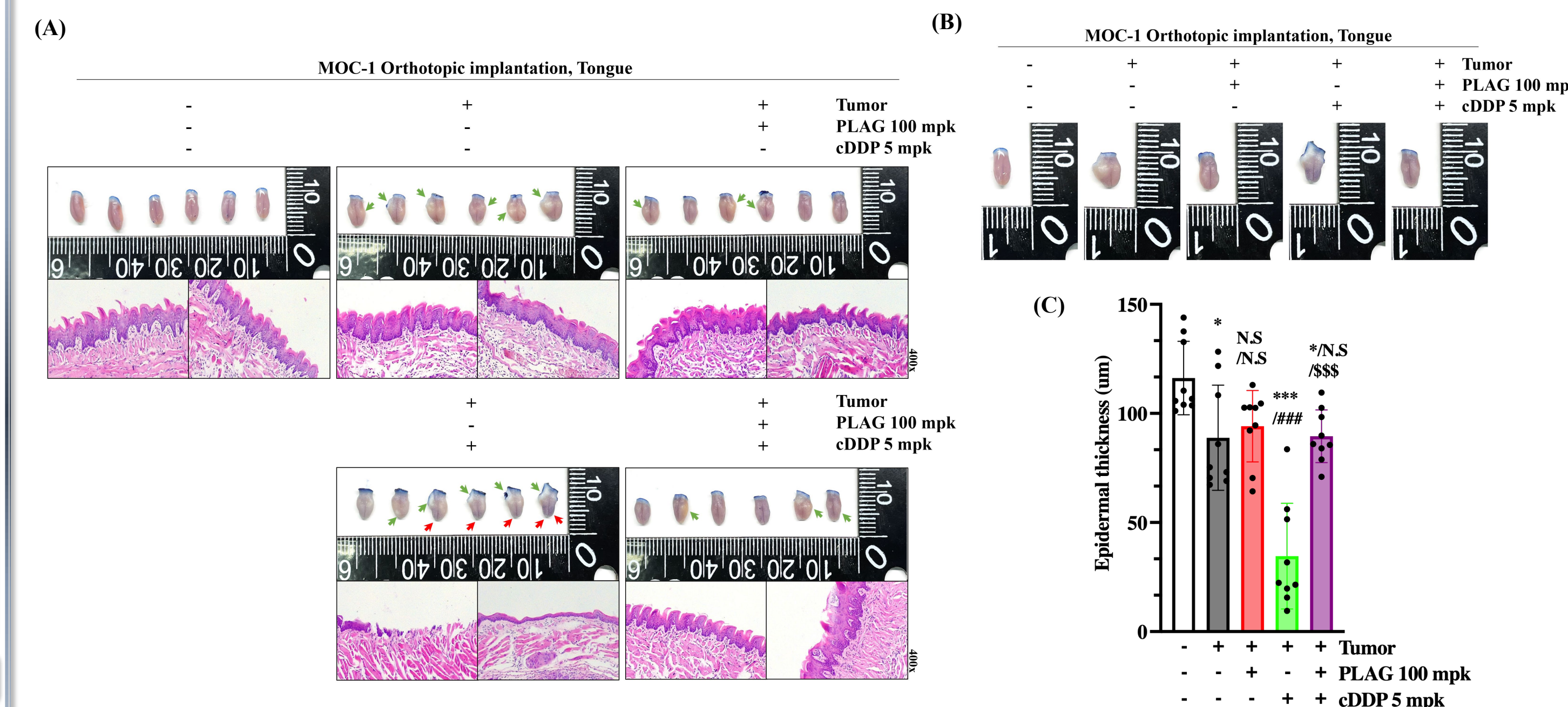
Analysis of the alleviates mild-OM by tumor and cDDP following PLAG treatment in MOC-1 HNSCC implantation model.
(A) Verification of mild-oral mucositis symptom relief effect according to PLAG treatment. Red arrow; tongue damage area, Green arrow; tumor region. (B) Analysis of the effect of relieving tongue damage caused by cDDP using TB (toluidine-blue) staining. (C) Analysis of changes in the thickness of the tongue epidermal layer.
Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the cDDP only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

3. PLAG treatment effectively restores the abnormally increased concentration of DAMP

Verification of restored abnormally increased DAMP concentration by tumor and cDDP to normal following PLAG treatment.
(A) Quantitative analysis of changes in the concentration of S100A8/A9 (alarmin) in blood. (B) Quantitative analysis of changes in the concentration of HMGB-1 ,which was associated with tissue damage in blood. (C) Quantitative analysis of changes in excessively increased blood adenosine concentration.
Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the cDDP only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

4. PLAG normalizes cDDP-induced expression of inflammatory cytokines

Quantitative analysis of release changes of inflammation-related factors according to PLAG treatment.
The massively increased IL-6 and TNFalpha by cDDP were restored to normal following the PLAG treatment.
Compared with the negative control: *P<0.033, **P<0.002, ***P<0.001; Compared with the tumor only: #P<0.033, ##P<0.002, ###P<0.001; Compared with the cDDP only: $P<0.033, $$P<0.002, $$$P<0.001 (each experiment n=6). N.S, Not significant. Mean ± SD.

5. PLAG effectively increases the antitumor efficacy of cDDP

PLAG alone effectively inhibits tumor growth. In combination with cDDP, the tumor growth inhibitory effect was further increased.
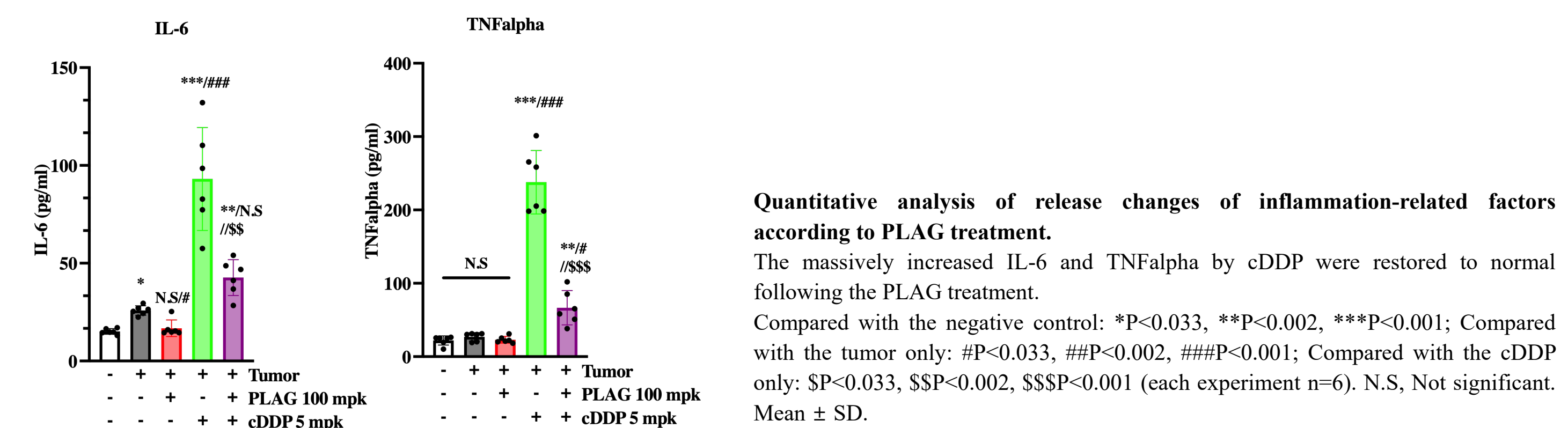
CONCLUSION

- PLAG (EC-18) effectively restores the severe weight loss caused by HNSCC and cDDP.

- In particular, it effectively relieves the symptoms of oral mucositis (tongue tissue damage and disruption of the epidermal layer) in the tongue tissue caused by cDDP.

- PLAG effectively restores the excessive increase of DAMP and inflammation related factors in blood accompanying tissue damage caused by cDDP to normal levels.

- Most importantly, treatment of PLAG not only can effectively control tumor growth in HNSCC but also has the potential to eliminate tumors following treatment with cDDP completely..