Letter

Gastric mucosal changes induced by polyethylene glycol 400 administered by gavage in rats

Yoshihide Ueda*, Masaru Tsuboi*, Yasufumi Ota, Maki Makita, Takuya Aoshima, Madoka Nakajima and Isao Narama

BioSafety Research Center, Foods, Drugs and Pesticides (BSRC), 582-2, Shioshinden, Iwata, Shizuoka 437-1213, Japan

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ABSTRACT — Polyethylene glycol 400 (PEG 400) is widely used with a variety of pharmaceutical formulations, and is often added to dosing formulations in preclinical toxicity studies. The aim of the present study was to characterize the effects of PEG 400 on the rat gastrointestinal tract. Three dosage levels (5, 50 or 100 v/v%) of PEG 400 were administered at a volume of 5 ml/kg/day by gavage for 15 days to the rats (5 males and 5 females in each group). At the end of the treatment, the whole lengths of gastrointestinal tracts were examined pathologically. Although there were no gross abnormalities at necropsy, the histopathological examination revealed several changes localized to the stomach mucosa, but not in the intestine. The changes consisted of infiltration of eosinophils and globule leukocytes, increased in the height of the entire mucosal layer, elongation of the surface mucous epithelial and mucous neck cell layers with increased intracellular mucous in the glandular stomach, and the spongiosis (intercellular edema) of the squamous epithelium in the forestomach. These changes near the limiting ridge tended to increase in severity and extent in a dose-dependent manner. These results suggest that repeated oral administration of concentrated PEG 400 can easily induce the mucosal changes in the stomach of the rats.

Key words: PEG 400, Repeated oral dosing, Gastric mucosa, Eosinophil, Globule leukocyte

INTRODUCTION

Polyethylene glycols (PEGs) are commercially available polyethers of ethylene glycol, and are widely used as thickeners, ointment bases, emulsifiers, or ophthalmic solutions (Hermansky et al., 1995; Sheftel, 1990, 2000). The physical nature of PEGs vary according to their mean molecular weights and determine their applications. PEG 400 is a viscous liquid at room temperature and is widely used for various purposes. It has been supposed to have only minimal toxicity in general use (Bartsch et al., 1976; Sheftel, 2000; Smyth et al., 1955). Taking advantage of these characteristics, PEG 400 is occasionally applied as a vehicle of dosing formulations in preclinical toxicity studies. However, some researchers have reported more dynamic actions of this chemical in animals especially in the gastrointestinal tract (Hermansky et al., 1995; Cho et al., 1992). We have also encountered the effect of loose stools in PEG 400-dosed rats, suggesting that the substance may produce functional changes in the large intestine.

This study was conducted to characterize the effects of PEG 400 on the rat gastrointestinal tract following administration of dosing 0, 5, 50 or 100 v/v% of PEG 400 by gavage daily for 15 days.

MATERIALS AND METHODS

Preparation of PEG 400 and dosing solutions

Polyethylene glycol 400 (PEG 400), purchased from Sanyo Chemical Industries, Ltd. (Kyoto, Japan), was dissolved in distilled water (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) to prepare each of the dosing solutions (5, 50 or 100 v/v%) before every treatment.

Animals and housing conditions

Both male and female 6-week-old Crl:CD(SD) rats (SPF) were purchased from Charles River Laboratories

Correspondence: Masaru Tsuboi (E-mail: m-tsuboi@anpyo.or.jp)
*These authors equally contributed to this work.
Japan, Inc. (Kanagawa, Japan). Following their receipt, the animals were quarantined and acclimated to the study environment for a week, and then subjected to the PEG 400 treatments. The animals were individually housed in stainless/steel mesh cages, and maintained in a barrier-sustained facility under the following conditions: (Temperature: 23 ± 3°C; Relative humidity: 55 ± 20%; Lighting: 12-hr light/dark cycle). All animals were allowed free access to a sterilized pellet diet, CRF-1 (Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water.

Study design and treatment

The acclimated animals were randomly assigned at 7-week-old to 4 groups (5 males and 5 females per group) after being stratified based on the body weights. Thereafter, the animals received 0, 5, 50 or 100 v/v% of PEG 400 once a day for 15 days at a volume of 5 ml/kg, while their general conditions, body weights and food consumptions were monitored. After the end of the treatment, all the animals were examined hematologically and pathologically.

Hematology and pathology

After the PEG 400 treatments, blood samples for the hematological examination were collected from the abdominal aorta of all rats under the deep ether anesthesia (euthanized by exsanguination) to determine the hematological parameters (hematocrit, hemoglobin content, red blood cell count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte, platelet, white blood cell, neutrophil, lymphocyte, monocyte, eosinophil and basophil count) using ADVIA 120 (Bayer Corp., Tarrytown, NY, USA).

At necropsy, the overall gross changes of the systemic organs including their external surface and orifices were examined. Subsequently, the gastrointestinal (GI) tract (esophagus to rectum) was extracted and immersed in 10% neutralized formalin solution followed by the injection of the fixative from the esophagus. After fixation for 2 days, the stomach (at the greater curvature), small intestine, colon and rectum were trimmed longitudinally, while the cecum was trimmed transversely. The paraffin-embedded specimens of the GI tract were sectioned at 3 μm thickness and stained routinely with hematoxylin and eosin (H&E). To characterize the globule leukocytes, the stomach sections were additionally stained with Giemsa, toluidine blue (pH 2.5) and AB-PAS, in addition to immunohistochemical staining using antibodies for CD8 (Serotec Ltd., Oxford, UK, dilution 1:100), ED-1 (Serotec Ltd., dilution 1:50), and rat mast cell protease 2 (RMCP 2, MS-RM4, Moredun Scientific Ltd., Midlothian, Scotland, dilution 1:50) by the labeled streptavidin-biotin method (DAKO LSAB2 System-HRP, Carpinteria, CA, USA).

Statistical analyses

The incidences of the histological findings in the stomach were analyzed by Fisher’s exact test (Hollander and Wolfe, 1999). Statistical significance was set at the two-sided 5% level of probability level.

Animal ethics

This study was conducted in compliance with the “Law Concerning the Protection and Control of Animals”, “Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain” and “Guidelines for Animal Experimentation (BSRC Experimental Animal Ethics Committee Authorization)”.

RESULTS AND DISCUSSION

General condition, body weight, food consumption, hematology and necropsy

Most of both male and female rats treated with 50 or 100 v/v% of PEG 400 passed loose stools intermittently during the treatment period (data not shown). Although this clinical sign was subtle even in the 100 v/v% group, the number of animals passing loose stools increased in a dose-dependent manner. No other significant changes were observed in the general condition, body weight, food consumption, hematologic indices (data not shown).

Histopathological changes in the stomach

Several histological changes were observed in the stomach, but not in the intestine, of the PEG 400-treated rats (Table 1). In the glandular stomach, the mucous neck cells increased in number as well as in size because of the larger mucous content in all the PEG 400-treated males and females treated with 50 or 100 v/v% of PEG 400 (Figs. 1A and 1B). The surface mucous cells also proliferated and contained a larger amount of mucous in their cytoplasm, resulting in thickening of the gastric mucosa and elongation of the gastric glands, especially in the surface area. The affected regions frequently showed mild infiltrations by globule leukocytes (GLs) and eosinophils (Figs. 1C and 1D). The GLs exclusively infiltrat-
Fig. 1. Changes in the glandular stomach and forestomach of the rats treated with PEG 400. Glandular stomach of the control male (A) and the 100 v/v% PEG 400-treated male (B): Thickening of the gastric mucosa caused by elongation of the surface and neck area of the gastric glands. Enlarged mucous neck cells and surface mucous cells filled with cytoplasmic mucous were more prominent in the PEG 400-treated animals than in the controls. (C): The submucosa and lamina propria in the 100 v/v% PEG 400-treated male; eosinophil infiltration is prominent. (D): Glandular gastric mucosa in the 100 v/v% PEG 400-treated male; GLs (arrows) bearing eosinophilic globules in the cytoplasm infiltrating the inter-epithelial cell spaces. (E): Epithelial spongiosis at the limiting ridge in the 100 v/v% PEG 400-treated male. (F): GLs (arrows) infiltrating the inter-epithelial cell spaces at the limiting ridge. (G): Giemsa staining reveals weak metachromasia of the GLs. (H): Immunohistochemistry reveals GLs showing positive staining (brown) for a marker of the mucosal mast cells (RMCP 2). Scale bar in A and B: 150 μm; in C-D, E, G and H: 50 μm; in F: 3 μm.
ed the epithelial layer, while the eosinophils were also noted in the submucosal layer and lamina propria. These changes were constant near the limiting ridge, and tended to increase in severity and extent in a dose-dependent manner. Therefore, the changes were considered to be induced by the PEG 400 dosing. In fact, similar changes were also observed in the rats in our additional studies conducted using PEG 400 purchased from 2 other companies (data not shown).

Along with the changes in the glandular stomach, spongiosis (intercellular edema) of the squamous epithelium with infiltration by GLs was observed in the forestomach of predominantly male rats in all of the PEG 400-treated groups (Figs. 1E and 1F). These changes were also localized near the limiting ridge, even in the 100 v/v% group. GL has been defined as a mononuclear cell containing eosinophilic globules in the cytoplasm and infiltrating some mucous membranes (Akpavie and Pirie, 1989; Kent, 1966). However, the precise GL lineage remains controversial: some GLs have been thought to belong to the large granular lymphocyte lineage (Baert and Frederix, 1985; Konno et al., 1994), and other GLs have been assumed to belong to the mast cell lineage (Ikeda and Yamashina, 1993; Narama et al., 1999). Although some GLs in the rat GI tract have often been reported to appear in response to infections by parasitic nematodes (Whur, 1966; Taliaferro and Sarles, 1939), it was confirmed by routine examination of the environmental condition that there were no infections by any such nematodes in the present study performed in a barrier-sustained animal room using SPF animals. According to the reports of Shaffer et al. (1950) and Cho et al. (1992), PEG 400 would be, at least partially, absorbed into the circulation, and may affect the secretory environment of the stomach. Thus, the mucosal changes described in this study might reflect the altered endogastric secretion resulting from the administration of PEG 400.

The actual mechanism remains unclear at present and further investigation is required. In the past, such chemical-induced GL and eosinophil infiltrations of the rat glandular stomach have been reported following administrations of 2-amino-2-ethyl-1,3-propanediol (Ishida et al., 2004 JECDB), 3,3′-thiobispropionic acid (Sudo et

Table 1. Histopathological findings of the stomach in the rats treated with PEG 400 for 15 days

<table>
<thead>
<tr>
<th>Organ</th>
<th>Finding</th>
<th>Male (v/v%)</th>
<th>Female (v/v%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Forestomach</td>
<td>Mucosal spongiosis</td>
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</tr>
<tr>
<td></td>
<td>Infiltration, Globule leukocyte</td>
<td>0/5</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>Infiltration, Eosinophil</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Glandular</td>
<td>Edema</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>stomach</td>
<td>Swelling and Proliferation,</td>
<td>0/5</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>Mucous neck cell</td>
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<td>0/5</td>
</tr>
<tr>
<td></td>
<td>Swelling and Proliferation,</td>
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<tr>
<td></td>
<td>Surface mucous cell</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>Infiltration, Globule leukocyte</td>
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<td>1/5</td>
</tr>
<tr>
<td></td>
<td>Infiltration, Eosinophil</td>
<td>0/5</td>
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</tr>
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Significant difference (*: p < 0.05, **: p < 0.01) vs control.

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al., 2003 JECDB) and iron lactate (Narama et al., 1999). Therefore, these reports, together with our findings in the PEG 400-treated rats, may lead to a better understanding of the pathogenesis and significance of these gastric cellular changes.

In conclusions, repeated oral administration of concentrated PEG 400 causes mucosal changes in the rat stomach. Therefore, special attention must be paid while administering PEG 400 by gavage to rats in preclinical toxicity studies.

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REFERENCES