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Aminoglutethimide-induced lysosomal changes in adrenal gland in mice

Mayu Mutsuga, Yoshiji Asaoka, Naoko Imura, Tomoya Miyoshi, Yuko Togashi*

Toxicology and Pharmacokinetics Laboratories, Pharmaceutical Research Laboratories, Toray Industries, Inc., 6-10-1, Tebiro, Kamakura, Kanagawa 248-8555, Japan

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ABSTRACT

Aminoglutethimide is a steroidogenesis inhibitor and inhibits a cholesterol side-chain cleavage enzyme (CYP11A1) that converts cholesterol to pregnenolone in mitochondria. We investigated histopathological changes induced by 5-day administration of AG in mice. Cytoplasmic vacuoles of various sizes and single cell necrosis were found in zona fasciculata cells in AG-treated mice. Some vacuoles were positive for adipophilin, whereas others were positive for lysosome-associated membrane protein-2 on immunohistochemical staining, indicating they were enlarged lipid droplets and lysosomes, respectively. Electron microscopy revealed enlarged lysosomes containing damaged mitochondria and lamellar bodies in zona fasciculata cells, and they were considered to reflect the intracellular protein degradation processes, mitophagy and lipophagy. From these results, we showed that AG induces excessive lipid accumulation and mitochondrial damage in zona fasciculata cells, which leads to an accelerated lysosomal degradation in mice.

1. Introduction

Aminoglutethimide (AG), which inhibits the activity of a cholesterol side-chain cleavage enzyme (CYP11A1), is used in the treatment of Cushing's syndrome characterized by excessive synthesis of glucocorticoid in the adrenal cortex (Santen and Misbin, 1981; Fleseriu and Petersenn, 2012; Pozza et al., 2012), prostate cancer (Lam et al., 2006; Kruit et al., 2004), and breast cancer (Ceci et al., 1985; Brufman and Biran, 1990). One of the major adverse effects of AG is adrenal dysfunction (Santen and Misbin, 1981) due to excessive suppression of steroid biosynthesis. Histopathological changes, such as cytoplasmic vacuolation, mitochondrial damage, and hypertrophy and/or hyperplasia in the adrenocortical cells, have been reported in patients with Cushing's syndrome treated with AG (Motlík et al., 1973; Marek and Motlik, 1975). However, the relationship between these changes and AG is unclear because Cushing's syndrome itself causes morphological changes in the adrenal gland (Imaki et al., 2004). In experimental animals, histopathological changes similar to those in patients with Cushing's syndrome treated with AG have been reported: cytoplasmic vacuolation in rats, hamsters, and Mongolian gerbils; mitochondrial injury in rats; and hypertrophy and/or hyperplasia in rats and hamsters (Akana et al., 1983; Ito, 1971; Robba et al., 1987; Malendowicz, 1992; Kadioglu and Harrison, 1975).

Blood cholesterol is taken up into cells in the adrenal cortex and then synthesized to various steroid hormones by steroidogenic enzymes in the mitochondria and endoplasmic reticulum (Kraemer, 2007; Miller and Auchus, 2011; Xie et al., 2006). The pathway of steroidogenesis differs among animal species. In humans, cortisol is produced as the principal glucocorticoid by the action of CYP17 (Harvey, 2016), whereas corticosterone is secreted in mice and rats because they have negligible expression of CYP17 (Harvey, 2016; Keeney et al., 1995; Le Goascogne et al., 1991). However, CYP11A1 is involved in processes upstream of the steroid cascades in both human and rodents (Harvey, 2016; Shih et al., 2008), suggesting a common target of AG.

We examined histopathological changes in the adrenal glands in mice orally treated with AG for 5 days, and revealed that AG induced excessive lipid accumulation and mitochondrial damage in zona fasciculata cells, which leads to an accelerated lysosomal degradation in mice.

2. Materials and methods

2.1. Animal experiments

Male Crlj:CD1(ICR) mice (Charles River Laboratories Japan, Inc., Yokohama, Japan) were housed in a plastic cage with softwood chip bedding under controlled conditions (12 h light/dark cycle), and fed a standard diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water ad libitum. AG (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was orally administrated to 7-week-old mice at 0, 125, 250, 500 mg/kg/day in 0.5% methylcellulose (Junsei Chemical Co., Ltd., Tokyo, Japan) solution once daily for 5 days (4/dose group). The highest dose level was set below the LD_{50} of mice intraperitoneally treated with AG (Aboul-Enein et al., 1975), and lower levels were set with a common

* Corresponding author.
E-mail address: Yuko_Togashi2@nts.toray.co.jp (Y. Togashi).

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Table 1
Body weight and adrenal weight in mice treated with AG.

Dose (mg/kg/day)	0	125	250	500
Number of animals	4	4	4	4
Body weight (g)	34.3 ± 0.6	34.5 ± 1.5	33.7 ± 1.7	31.2 ± 2.4
Adrenal weight (mg)	6.6 ± 1.0	8.0 ± 1.1	9.5 ± 3.4	8.6 ± 0.6
Adrenal weight (mg/g body weight)	0.19 ± 0.03	0.23 ± 0.04	0.28 ± 0.09	0.28 ± 0.04

Data represent as the means ± S.D.
Not significantly different from the vehicle-treated mice (Dunnett's multiple comparison or Steel's test).
The body weight and adrenal weight were measured before the necropsy; three of the 4 animals treated with 500 mg/kg/day were necropsied on Day 3 due to a moribund state.

ratio of 2. The day after the final administration, mice were euthanized under anesthesia, and the adrenal grand was removed and weighted. During the experimental period, the mice were not fasted. This study was reviewed by the Animal Care and Use Committee, approved by the head of the test facility, and performed in accordance with Guidelines for the Animal Experiments, Research & Development Division, Toray Industries, Inc.

2.2. Histopathology

Tissue samples were fixed in a 10% neutral-buffered formalin solution, embedded in paraffin, and cut into 2–4 μm thick sections. Subsequently, hematoxylin and eosin (HE) staining of paraffin sections and Sudan black staining of 2% osmium tetroxide-fixed paraffin sections were performed.

2.3. Immunohistochemistry for LAMP-2 and adipophilin

Immunohisotchemical stainings for lysosome associated membrane protein-2 (LAMP-2) and adipophilin were performed as previously described (Asaoka et al., 2013). Sections were treated with epitope retrieval solution (ImmunoSaver, Nissin EM Co., Ltd., Tokyo, Japan) for 5 min at 98 °C, and incubated with primary antibody; rat monoclonal IgG antibody against mouse LAMP-2 (sc-19991, Santa Cruz Biotechnology, Inc., Dallas, Texas, USA) or a guinea pig polyclonal antibody against adipophilin (GP40, Progen Biotechnik GmbH, Heidelberg, German), at 4 °C overnight. The sections were then incubated with secondary antibodies and a VECTASTAIN Elite ABC kit (Vector Laboratories, Inc., Burlingame, CA, USA).

2.4. Electron microscopy

Right adrenal glands were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin. Then, ultrathin sections were double-stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope (HT-7700; Hitachi High-Technologies Co., Ltd., Tokyo, Japan).

2.5. Statistical analysis

The differences in body weight and adrenal weight between the vehicle- and AG-treated mice were analyzed with Dunnett's multiple comparison or Steel's test (SAS Institute Japan Ltd., Tokyo, Japan). The level of significance was set at 5%.

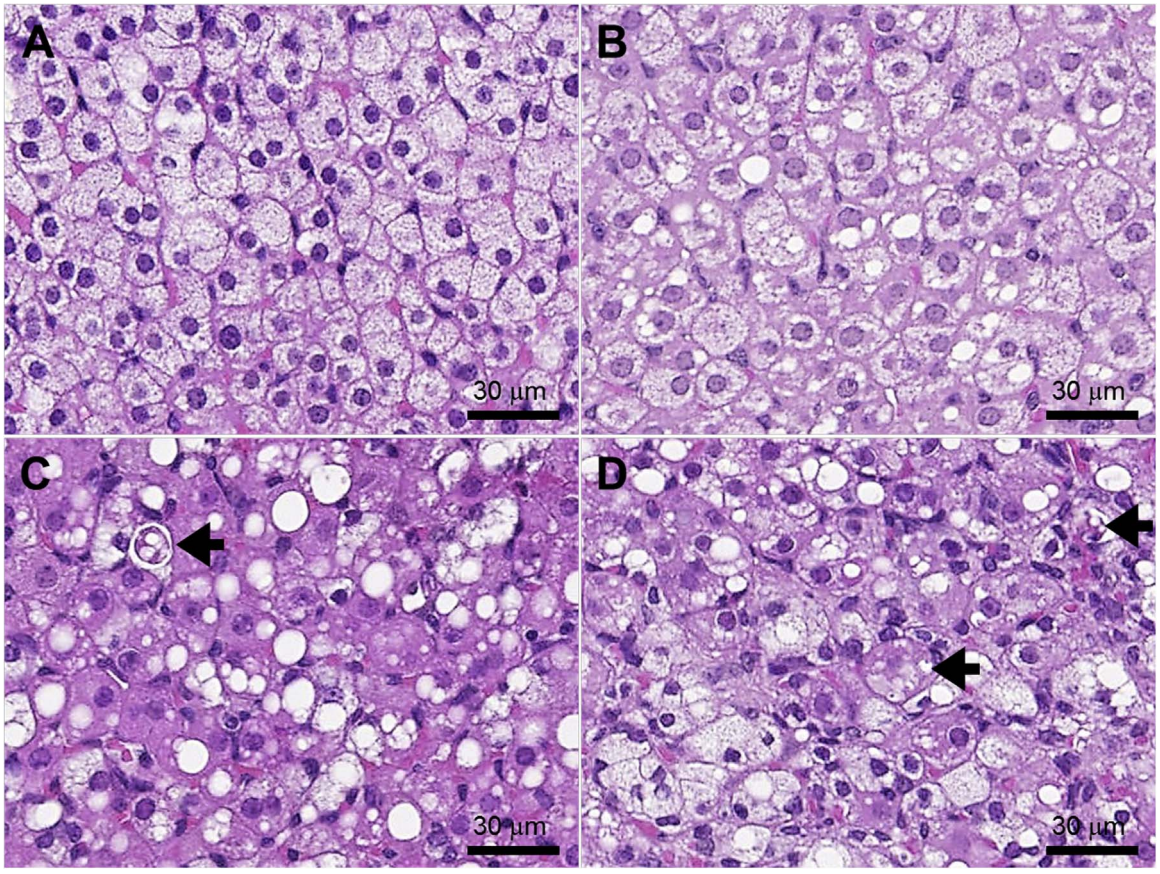

Fig. 1. Histopathological changes in the zona fasciculata of the adrenal gland in mice treated with AG for 5 days.Cytoplasmic vacuolation and single cell necrosis were found in zona fasciculata cells of the adrenal gland in AG-treated mice.(A) Vehicle-treated mice. (B) AG-treated mice, 125 mg/kg/day. (C) AG-treated mice, 250 mg/kg/day. (D) AG-treated mice, 500 mg/kg/day. Black arrow, single cell necrosis.