Geranylgeranylanacetone Suppresses Spontaneous Apoptotic DNA Fragmentation in Cultured Guinea Pig Gastric Pit Cells

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We recently found that guinea pig gastric pit cells in culture undergo spontaneous and rapid apoptotic DNA fragmentation, which may represent the rapid death of gastric pit cells in vivo. In this study, we observed that pretreatment of cells with geranylgeranylanacetone, an antiulcer drug with heat-shock protein-inducing activity, suppressed the spontaneous apoptotic DNA fragmentation in a dose-dependent manner. Pretreatment of cells with low concentrations of ethanol or heat-shock also prevented the spontaneous apoptotic DNA fragmentation. These observations indicate that the suppression of the apoptotic DNA fragmentation by geranylgeranylanacetone involve the induction of heat-shock proteins.

Key words: geranylgeranylanacetone; apoptosis; gastric pit cell

Gastric pit cells (surface mucous cells) have a rapid cell turnover rate in vivo. The short turnover cycle is the result of not only rapid proliferation of progenitor cells but also rapid cell death at the gastric surface. In the normal stomach, there is a balance between the proliferation of the immature cells and the death of senescent cells at the top of the surface layer. Alteration in the rate of gastric pit cell death may cause various gastric diseases. For example, alteration in the rate of the gastric pit cell death by Helicobacter pylori was suggested to be involved in the development of atrophic gastritis and gastric carcinogenesis associated with H. pylori infection. Recently, we found that guinea pig gastric pit cells undergo spontaneous and rapid apoptotic DNA fragmentation in vitro, which may represent the rapid cell death of gastric pit cells in vivo (Tsutsumi et al., unpublished observations).

Geranylgeranylanacetone (GGA) is a unique antiulcer drug that protects the gastric mucosa from various stresses. The action of GGA on the gastric mucosa does not depend on endogenous prostaglandins (PGs). Recently, GGA was shown to induce heat-shock proteins (HSPs; HSP90, 70, and 60) directly in cultured gastric pit cells and in the gastric mucosa and protect cells from stress conditions. Based on our results, we proposed that induction of HSPs by GGA protects cells from various stresses through suppression of stress-dependent apoptosis. We showed that apoptosis of gastric pit cells caused by high concentrations of ethanol is suppressed by GGA and suggested that this suppression is mediated by induction of HSPs by GGA. In this study, we show that the spontaneous and rapid apoptotic DNA fragmentation in cultured guinea pig gastric pit cells is inhibited by pretreatment of cells with GGA. We also report that pre-exposure of gastric pit cells to low concentrations of ethanol or heat-shock, well-known HSP-inducing conditions, also suppresses the spontaneous apoptotic DNA fragmentation.

Gastric mucosal cells were isolated from guinea pig fundic glands, as described previously. Cells were cultured in the presence of 10% FCS with and without GGA 10^{-6} M. Unattached cells were removed, and the attached cells were cultured in medium without GGA. Apoptotic DNA fragmentation was monitored, as described. As shown in Fig. 1A, rapid and spontaneous apoptotic DNA fragmentation was observed in cells that were not treated with GGA. When cells were pretreated with GGA, the onset of spontaneous DNA fragmentation was delayed (Fig. 1A).

The dose dependency of the effect of GGA on spontaneous apoptotic DNA fragmentation is shown in Fig. 1B. GGA at 10^{-6} M or higher concentrations was necessary for the suppression of the apoptotic DNA fragmentation (Fig. 1B). The concentration of GGA (10^{-6} M) was similar to that needed for the induction of HSPs. We also examined the incubation period dependency of the effect of GGA on apoptotic DNA fragmentation and found that incubation with GGA 10^{-6} M for longer than 2 h was required for this action of GGA (data not shown). These results support the idea that the GGA-dependent suppression of apoptotic DNA fragmentation is mediated at least in part by the induction of HSPs.

The results described above suggest that the suppression of spontaneous apoptotic DNA fragmentation by GGA is due to the induction of HSPs. Thus induction of HSPs by other

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Fig. 1. Effect of GGA on Spontaneous Apoptotic DNA Fragmentation

Gastric mucosal cells prepared from guinea pigs were cultured in RPMI 1640 medium containing 10% FCS with 10^{-6} M (A) or indicated concentrations (B) of GGA and further incubated in the absence of GGA for the indicated periods (A) or 4 h. Apoptotic DNA fragmentation was monitored as described previously.

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means was predicted to inhibit the DNA fragmentation. We therefore examined the effect of pretreatment of cells with 1—3% ethanol on spontaneous DNA fragmentation. At these concentrations, ethanol induces HSP in cultured gastric mucosal cells without affecting cell viability.\textsuperscript{11} Preexposure to 1—3% ethanol appeared to suppress DNA fragmentation (Fig. 2). Pretreatment of cells with low concentrations of ethanol also prevented apoptosis by high concentrations of ethanol.\textsuperscript{10} Thus low concentrations of ethanol may prevent the apoptosis in gastric mucosal cells in general.

We examined whether heat-shock also blocked spontaneous apoptosis. Cells were incubated at 43°C for various periods, and spontaneous DNA fragmentation was monitored. Heat treatments for longer than 15 min inhibited apoptotic DNA fragmentation (Fig. 3). The appearance of the effect of heat-shock coincided with the timing of HSP induction in cultured gastric mucosal cells.\textsuperscript{12} These ethanol and heat treatments did not affect the viability and attachment of cells as in the case of GGA treatment (data not shown).

It is not known whether the maturation-dependent death of gastric mucosal cells should be enhanced or attenuated to protect the gastric mucosa against various metabolic insults. In this study, we showed that a cytoprotective compound, GGA, inhibited spontaneous cell death by suppressing apoptosis, suggesting that antiapoptotic agents may be beneficial for increasing gastric mucosal defense under conditions of stress. In conditions of stress, such as exposure to toxic chemicals, the gastric mucosal cells are damaged, and the number of cells may decrease, which unfavorably alters the balance between aggressive and defensive factors. From this point of view, inhibition of maturation-dependent rapid cell death would appear to be beneficial for the protection of the gastric mucosa against various metabolic insults, as it would increase the number of gastric mucosal cells. It has been suggested that the enhanced apoptosis of surface mucous cells caused by \textit{H. pylori} infection plays an important role in development of atrophic gastritis.\textsuperscript{2,3}

REFERENCES