Eur J Nutr (2009) 48:499–503
DOI 10.1007/s00394-009-0035-9

SHORT CONTRIBUTION

Food derived carbonyl compounds affect basal and stimulated secretion of interleukin-6 and -8 in Caco-2 cells

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Received: 4 March 2008 / Accepted: 4 June 2009 / Published online: 21 June 2009

Abstract
Background The carbonyl compounds methylglyoxal (MG) and glyoxal (G) are reactive intermediates generated in a variety of foods and beverages during processing and prolonged storage.
Aim and methods We investigated direct effects of these compounds on intestinal cells determining the basal and stimulated secretion of IL-8 and IL-6 in vitro.
Results MG or G induced a concentration dependent enhancement of IL-8 and IL-6 secretion compared to baseline levels. A co-incubation with pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) or lipopolysaccharides (LPS) and increasing MG concentrations further enhanced IL-8 and IL-6 secretion. For G, however, this additive effect was only observed in TNF-α and IL-1β treated cells, but not after co-incubation with LPS.
Conclusion These results suggest a pro-inflammatory effect of G and MG at high concentrations in human intestinal cells by stimulating IL-8 and IL-6 cytokine levels. Effects of G and MG in combination with other cytokines may negatively affect inflammatory processes.

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Keywords Caco-2 cells · Glyoxal · Methylglyoxal · Interleukin-8 · Interleukin-6

Introduction

Most publications on methylglyoxal (MG) and glyoxal (G) have focused on their endogenous generation from cellular glycolytic intermediates mainly investigating the functional significance in diseases such as diabetes mellitus. There are several reports showing that plasma MG concentrations in poorly controlled diabetic patients are in the range of about 400 μM and that local tissue MG concentration may even be much higher than plasma levels [11, 21]. High concentrations of these carbonyl compounds were associated with vascular endothelial cells inflammatory injury representing an important early pathogenic feature of atherosclerosis and other pathophysiological processes [4, 13, 15]. Besides, they have also been reported to directly cause inflammatory processes. Carbonyl compounds and dietary glycation end products have been shown to affect the secretion of cytokines such as IL-8 and IL-6 which have the potential to induce inflammation [20, 23, 25]. Besides this endogenous generation, MG and G are by-products mainly originating from processing of foods such as coffee, wine and milk products [6–8, 17–19]. One could speculate that their oral intake over a prolonged period of time may exert degenerative alterations in different tissues. Futhermore, mutagenic and carcinogenic effects on the intestine have already been demonstrated in vitro and in vivo [2, 3, 14]. However, there are only few publications on direct local effects of MG and G within the intestine. Here, most studies concentrate on their cytotoxic and cancerogenic effect using concentrations ranging from μM to mM [2, 3]. Based upon those data on cell toxic

effects we used concentrations below the cytotoxic level in order to see whether such concentrations affect intestinal cells, i.e. influence IL-8 and IL-6 secretion with and without concomitant stimulation by pro-inflammatory modulators.

Materials and methods

Cell culture

The intestinal cell line Caco-2 was obtained from LGC Promochem GmbH (Wesel, Germany) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), penicillin/streptomycin (100 U/mL and 0.1 mg/mL), and 2 mM L-glutamine (all materials were supplied by Invitrogen, Karlsruhe, Germany). For intervention experiments, Caco-2 cells were seeded on 6-well plates (1×10^5 cells/well) and cultured for 14 days after having attained confluence.

Chemicals

Lipopolysaccharides from *E. coli* (055:B5) and MG were purchased from Sigma Chemicals (Deisenhofen, Germany), G from Fluka (Frankfurt, Germany); recombinant human TNF-α and IL-1β were obtained from R&D Systems (Wiesbaden, Germany). Methylthiazoletetrazolium bromide (MTT) dye was purchased from Calbiochem (Karlsruhe, Germany).

Cell proliferation and cytotoxicity

Cell proliferation and cytotoxicity were determined using the MTT assay as described elsewhere [10].

Cytokine-assay

To determine IL-8 and IL-6 secretion, cells were incubated either with or without carbonyl compounds, cytokines (TNF-α, IL-1β) or LPS. After incubation, the supernatant was removed and subjected to a ProteoPlex® Human Cytokine Array (Merck, Frankfurt, Germany) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism software 4.03 (San Diego, CA, USA). All results are given as mean ± SD of duplicate determinations representing three independent experiments. Statistical significance of the differences between various groups was evaluated by one-way analysis of variance (ANOVA). Differences were considered statistically significant at $^*P < 0.05$.

Results

Growth inhibition and cytotoxicity of MG and G in Caco-2 cells

As shown in Fig. 1, MG and G induced a concentration-dependent growth inhibition associated with an increasing cytotoxic effect. A significant inhibition of cellular growth was induced by concentrations above 10 mM MG or G. For further experiments, only concentrations below the cytotoxicity level were used.

Influence of MG and G on constitutive IL-8 and IL-6 secretion

Caco-2 cells constitutively secreted cytokines with the highest basal level for IL-8 of 178 ± 23 pg/mL and for IL-6 of 27 ± 6 pg/mL (Fig. 2). After 24 h incubation, the secretion of IL-8 and IL-6 was affected in a dose-dependent manner. Exposure to MG leads to maximum IL-8 and IL-6 concentrations of 488 ± 14 and 67 ± 9 pg/mL, respectively. Similar to MG, G enhanced IL-8 and IL-6 secretion to peak levels of 364 ± 18 and 47 ± 9 pg/mL, respectively.

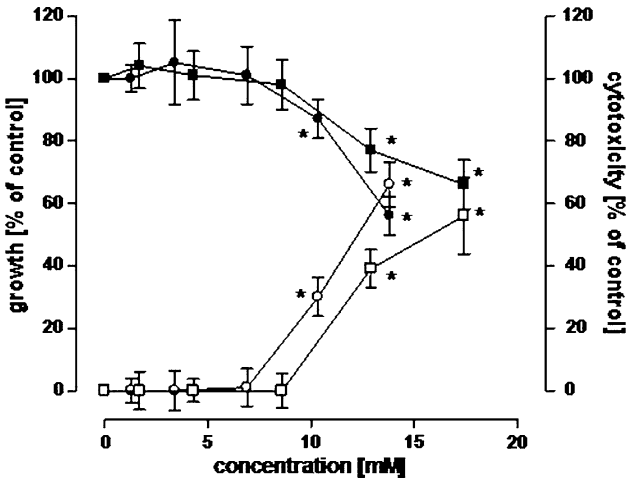

Fig. 1 Effects of MG and G on cell growth and cytotoxicity. Caco-2 cells were cultured for 24 h with increasing concentrations of MG (*circle*) and G (*squares*). Growth (*filled symbols*) and cell toxicity (*open symbols*) were determined by using the MTT assay. Values represent three independent experiments in duplicates. The results are expressed as % of controls ($^*P < 0.05$ vs. untreaed control)

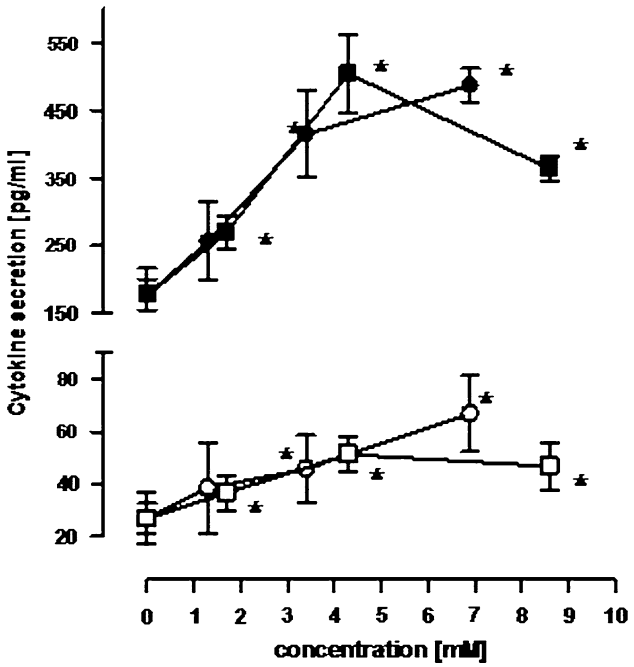

Fig. 2 Cytokine secretion of Caco-2 cells by MG and G. Caco-2 cells were stimulated with increasing concentrations of MG (*circles*) and G (*squares*) for 24 h prior to the determination of cytokine IL-8 (*filled symbols*) and IL-6 (*open symbols*) concentrations in the supernatant. Each value represents three independent experiments in duplicates given as mean ± SD (*$P < 0.05$ vs. unstimulated cells)

Effect of MG and G on TNF-α-stimulated secretion of IL-8 and IL-6

Incubation with TNF-α significantly increased IL-6 and IL-8 secretion. In addition, co-incubation with MG and G further up-regulated IL-8 and IL-6 secretion as shown in Table 1.

Effect of MG and G on IL-1β-stimulated secretion of IL-8 and IL-6

IL-1β itself significantly increased IL-8 and IL-6 release from Caco-2 cells compared to their basal secretion. This IL-1β-stimulated IL-8 and IL-6 secretion was further enhanced by MG and G (Table 1).

Effect of MG and G on LPS-stimulated secretion of IL-8 and IL-6

LPS increased IL-8 and IL-6 secretion in intestinal cells. A further enhancement of IL-8 and IL-6 secretion was achieved by simultaneous incubation with MG and LPS, but not with G and LPS (Table 1).

Discussion

The intestinal mucosa is an important site for the absorption of nutrients and has an important role as a barrier against potentially injurious agents in the intestinal lumen. However, it has been reported that intestinal epithelial cells produce various inflammatory mediators, such as IL-8 and participate in the development of intestinal inflammation. In this study we investigated the effect of carbonyl compounds such as G and MG on the IL-6 and IL-8 secretion of Caco-2 cells under basal und stimulated conditions. Caco-2 cells have been previously used in studies of intestinal inflammation, because they represent the first cell linage of the gastrointestinal tract interacting with luminal factors. So far, the natural amounts of G and MG in food seem to be rather low [5–7]. However, it is known that the amount may markedly increase due to food processing and storage. It is difficult to estimate the concentrations of carbonyl compounds to which intestinal cells are exposed. One of the major reasons for the scarcity of data is that until recently appropriate quantitative methods for screening purposes in food and beverages were not available. It can be expected that new mass spectrometric methods may reveal more accurate quantitative data for carbonyl compounds in processed food in the near future [18, 24]. Recently, Tan et al. (2008) measured MG levels in beverages and showed that regular carbonated beverages

Table 1 Secretion of IL-6 and IL-8 (in pg/mL) after co-stimulation with the inflammatory mediators TNF-α, IL-1β and LPS in the presence or absence (PC, positive control) of MG and G

	IL-6			IL-8		
	TNF-α (10 ng/mL)	IL-1β (10 ng/mL)	LPS (1 μg/mL)	TNF-α (10 ng/mL)	IL-1β (10 ng/mL)	LPS (1 μg/mL)
PC	47 ± 5	47 ± 5	68 ± 7	1158 ± 63	1110 ± 38	341 ± 14
MG (3.4 mM)	61 ± 6*	82 ± 6*	89 ± 7*	1585 ± 14*	1524 ± 16*	452 ± 26*
MG (6.9 mM)	64 ± 6*	80 ± 7*	96 ± 10*	1826 ± 18*	1625 ± 27*	525 ± 103*
G (4.3 mM)	61 ± 7*	66 ± 6*	64 ± 15	1742 ± 127*	1321 ± 58*	403 ± 106
G (8.6 mM)	62 ± 6*	73 ± 10*	70 ± 10	1606 ± 189*	1528 ± 47*	363 ± 106

Each value represents three independent experiments in duplicates given as mean ± SD (*$P < 0.05$ vs. positive control)

can contain levels of MG up to 1.4 mg/L (20 μM) [24]. Furthermore, a systematic evaluation of the MG and G content in food is still missing; only a limited number of foods and beverages have been investigated so far.

Thus, for screening purposes we used MG and G in concentrations which did not inhibit cell growth by cytotoxic effects (Fig. 1). Lower mM concentrations, however, were found to be enhancers of IL-8 and IL-6 secretion in intestinal epithelial cells (Fig. 2). Up-regulated IL-6 and -8 secretion by MG and G have also been observed in other in vitro experiments using non-intestinal epithelial cells [1, 12, 26]. A possible explanation is that carbonyl compounds function as oxidative stressors. Impairment of different signaling pathways caused by a G- and MG-induced production of reactive oxygen species (ROS) may lead to the activation of different kinase cascades stimulating the synthesis of IL-6 and IL-8. This influence of G or MG on MAPK-pathways has already been reported [1, 12]. Because of the multiple signal cascades influencing the IL-6 and -8 transcriptional and translational expression levels it is conceivable that G and MG induced different cascades by generating different oxygen species [1, 9].

Besides the action of G and MG on the basal secretion of IL-6 and IL-8 we also observed an increased IL-8 and IL-6 secretion after stimulation with TNF-α and IL-1β (Table 1). Under physiological conditions, epithelial cells secrete low levels of such cytokines, but their production could be rapidly induced by a wide range of stimuli such as pro-inflammatory cytokines like TNF-α or IL-1β. Both are known to trigger colonic inflammation [22]. The increasing effect of MG and G on this cytokine induced IL-6 and IL-8 secretion could be a result of activating different signal pathways at the transcription and translation level [9]. The effects of MG and G on TNF-α and IL-1β stimulated cytokine secretion differed from those when LPS was used as stimulus. Whereas MG increased the LPS effect, G failed to enhance LPS stimulated IL-6 and IL-8 secretion. Ndelene et al. [16] hypothesized that the involvement of the MAPK pathway in regulating LPS-mediated IL-8 secretion is also redox dependent. In our experiments, the failure of G to further enhance LPS-induced IL-6 and IL-8 secretion could be a consequence of a lower generation of ROS and thus less participation in the signal cascade activation. The ability of MG and G to up-regulate the TNF-α- and IL-1β-, and in part LPS-induced secretion of IL-6 and IL-8 in intestinal epithelial cells may suggest an active role of carbonyl compounds in initiating or enhancing inflammatory processes.

In that context, it is noteworthy that we investigated the direct effect of MG and G on intestinal epithelial Caco-2 cells by measuring their cytokine secretion. To gain insight into the possible effects of luminal MG and G exposure to the first mucosal cell linage we used Caco-2 cells as a rather simple model. The complex situation in the gut, however, is difficult to mimic in vitro. E.g. exposure of intestinal cells to luminal factors results in secretion of cytokines which can contribute to the acute inflammatory response (TNF-α, IL-1β and IL-8) and the activation of T-lymphocytes such as IL-12 and INF-γ (Th1 cells) or IL-4 and IL-5 (Th2 cells) and GM-CSF (macrophage). The secretion of these target specific cytokines in the basal compartment of the GALT results in further immune modulation. The application of co-culture experiments which allows the cross-talk between epithelial cells and leukocytes/macrophages could reveal more details in further experiments.

Finally, despite the above mentioned problems in evaluating the food-derived G and MG content, Caco-2 cells seemed to tolerate high concentrations of G and MG. It is well known, that dependent on the cell line, the responsiveness to stimuli is different and non-transformed intestinal cells may respond to lower levels of G and MG.

It remains to be shown in further investigations whether MG and G induce inflammatory processes in vivo as well, since the concentrations used in these experiments were quite high although not in a cytotoxic range.

Acknowledgments The authors would like to thank Nadine Metz for her excellent technical assistance. This work was supported by the Hessian Ministry of Higher Education, Research, and the Arts (HMWK).

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