TOXICITY OF METHYLGlyOXAL TOWARDS RAT ENTEROCYTES AND COLONOCYTES

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SUMMARY: Effect of methylglyoxal, a bacterial metabolic product, on protein, DNA, and RNA synthesis in rat enterocytes and colonocytes was investigated. Results showed that 1 mM methylglyoxal inhibited protein, DNA, and RNA synthesis to the extent of 65-85, 65-80, and 10-20 per cent, respectively, in villus and crypt cells and colonocytes. The inhibitory pattern was similar in these various cell types. The inhibitory effect on protein and DNA synthesis was more marked than that on RNA synthesis. Inclusion of thiol compounds up to 4 mM concentration did not protect the cells from the inhibitory effect of methylglyoxal. No alteration in the level of cellular reduced glutathione and glyoxalase enzyme activity was observed when cells were incubated with 2 mM methylglyoxal. These results suggest that the antiproliferative action of methylglyoxal on eukaryotic cells may be through the inhibition of macromolecular synthesis.

INTRODUCTION:

Enteric bacteria normally present in the gastrointestinal tract of mammals produce methylglyoxal by the action of the enzyme methylglyoxal synthase [1]. We earlier showed the production of methylglyoxal by different aerobic and anaerobic bacteria isolated from faeces of normal South Indians [2]. Methylglyoxal is a cytotoxic compound that inhibits proliferation of both normal and cancerous cells [3]. The important sources suggested for methylglyoxal are dihydroxyacetonephosphate, an intermediate of glycolysis [4], and aminoaacetone, an intermediate of glycine and threonine metabolism [5-9]. Formation of methylglyoxal from dihydroxyacetonephosphate is catalysed by methylglyoxal synthase [1,10]; and that from aminoaacetone, by aminoxidase enzyme [5,11].

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Methylglyoxal is detoxified by the glyoxalase system, which catalyses conversion of methylglyoxal to D-lactic acid via the intermediate S-D-lactoylglutathione [12]. This glyoxalase system comprises two enzymes, glyoxalase-I and -II and glyoxalase-I requires reduced glutathione as cofactor [13]. Aronsson and Mannervik [14] suggested that glyoxalase enzyme present in mammalian tissues may have a protective function against methylglyoxal produced by enterobacteria. We earlier reported the presence of glyoxalase-I in mammalian intestine; the enzyme was purified from monkey intestinal mucosa [15].

Toxic effect of methylglyoxal on E. coli and malignant ascites cells has been reported [16-18]. If the suggested source of methylglyoxal in mammals is enteric bacteria, then the cells lining the intestinal tract are the ones immediately affected by this toxic compound. Moreover, methylglyoxal is antimitotic, and the epithelial cells lining the intestinal tract have a high mitotic activity. Hence we studied the effect of methylglyoxal on synthesis of macromolecules and on levels of reduced and oxidised glutathione in isolated villus and crypt cells and colonocytes of the rat. Possible protection by thiol compounds from methylglyoxal was also investigated and is reported in this communication.

MATERIALS AND METHODS:
Acetaldehyde and orcinol were purchased from BDH Chemicals Ltd, England. Diphenylamine from Fluka, West Germany, BSA, DNA sodium salt, DTT, EDTA-tetrasodium salt, GSH, GSSG, methylglyoxal (40% w/v), N-ethylmaleimide, o-phenalddehyde, p-hydroxydiphenyl, p-nitrophenylphosphate-disodium, RNA from calf liver and crypan blue were purchased from Sigma Chemical Co, USA. L-[U-14C]leucine from Amersham International plc, UK. [Me-3H]thymidine and [5-3H]uridine were from Bhabha Atomic Research Centre, India.

Separation of villus and crypt cells from rat small intestine: Villus and crypt cells were isolated according to Weiser's method [19]. The first three, middle three, and last three fractions were pooled to obtain villus cells, the mixed cell fraction (containing both villus and crypt cells), and crypt cells, respectively. Purity of the separated villus and crypt cells were determined by the marker enzyme alkaline phosphatase, which was assayed as described [20].
The separated enterocytes were suspended in oxygen-saturated, calcium-containing Krebs-Hensleit saline [21] with 0.25% (w/v) BSA and used for the isotope uptake study.

Separation of colonocytes: Colonocytes were isolated from rat colon by the method described by Roediger and Truelove [22]. Isolated colonocytes were suspended as described above for enterocytes.

Cell viability: Viability of the isolated cells was checked by dye-exclusion method using trypan blue and succinate tetrazolium salt mixture and kininase of vitamin production. Measurement of lactic acid was done as described [23].

Incorporation of radiolabeled isotopes: The effect of methylglyoxal on incorporation of labeled compounds into protein, DNA, and RNA of the cells was assayed in 2.0-ml cell suspensions (1.5 to 2.0 x 10^6 cells) containing different concentrations of methylglyoxal ranging from 0.1 to 3.0 mM. Uptake of radiolabeled compounds in the absence of methylglyoxal (control) was considered as 100 percent (incorporation) synthesis, and the percent inhibition at different concentrations of methylglyoxal was calculated based on that value. A control for non-specific absorption of radiolabelled isotopes and a zero-time control with methylglyoxal were carried out, and we found that the 'counts' for both of these controls were the same.

Uptake of [14C]-leucine into protein: To a 2.0-ml cell suspension, 0.025 μCi [14C]-leucine and the required volume of 100 mM stock methylglyoxal were added, and the total volume of the assay mixture was made up to 2.5 ml with oxygen-saturated, calcium-containing Krebs-Hensleit saline with 0.25% (w/v) BSA. In the control, methylglyoxal was replaced by Krebs-Hensleit saline. Each cell suspension was incubated at 25°C for 2 h, and 1.25 ml of 20% TCA and 1.25 ml of 2% phosphotungstic acid were added. The protein precipitate was centrifuged, washed twice with 5% TCA, and dissolved in one ml of 0.5N NaOH. This solution was transferred to a scintillation vial containing 10 ml of scintillation fluid and counted [24]. Leucine incorporation was expressed as DPM per mg protein.

Uptake of [3H]-thymidine into DNA: The cell suspension containing 0.25 μCi [3H]-thymidine was incubated at 25°C for 2 h. At the end of incubation, 100% TCA was added to a final concentration of 20%, and the mixture was kept at 4°C for 10 min and then centrifuged. The precipitate was suspended in 2.0 ml of 10% TCA, kept at 4°C for 10 min, and centrifuged. To this TCA precipitate was added 2.0 ml of 5% TCA, after which the tube was kept in a boiling water bath for 30 min, and centrifuged. The supernatant was counted, and DNA synthesis was expressed as DPM per mg DNA.

Uptake of [3H]-uridine into RNA: To the cell suspension, 0.25 μCi [3H]-uridine was added, and incubation was carried.
out at 25°C for 1 h. After incubation, 1.25 ml of 10% perchloric acid was added, and the precipitate was centrifuged, washed twice with 0.2N perchloric acid, and dissolved in 2.0 ml of 0.1N KOH. The solubilized RNA was counted, and RNA synthesis was expressed as DPM per mg RNA.

Other assay procedures: Glyoxalase-I was assayed as described [12]. Protein concentration was determined by the method of Lowry et al. [25] with bovine serum albumin as standard. RNA was extracted by the method of Schmidt and Thannhauser [26] and estimated by use of orcinol reagent [27]. DNA was extracted from the cells as described by Burton [28] and estimated with diphenylamine reagent [29]. Reduced glutathione content of the control and 2 mM methylglyoxal-treated cells was assayed as described by Hissin and Hilf [30].

RESULTS:

Purity of the isolated cells was judged by the marker enzyme alkaline phosphatase. When incubated, more than 85% of the cells were viable up to 2 h as judged by lactate production and dye exclusion (Fig.1). Treatment with various concentrations of methylglyoxal did not alter the viability up to 2 hours, and the viability was similar for villus cells, crypt cells, and colonocytes. Isotope uptake with [14C]-leucine, [3H]-thymidine, and [3H]-uridine at different time intervals were studied separately in enterocytes and colonocytes. Optimum uptake of [14C]-leucine and [3H]-thymidine was observed at 2 h; and that of [3H]-uridine, at 1 h (Fig. 1). This optimum uptake pattern was similar in villus and crypt cells and colonocytes. Thus, for the study of the effect of methylglyoxal on protein and DNA synthesis, cells were incubated for 2 h; and for RNA synthesis, the duration of incubation was 1 h. Figure 2a shows the effect of methylglyoxal on the inhibitory pattern of protein synthesis in villus, and crypt cells and colonocytes. At 1 mM methylglyoxal more than 70% inhibition was observed and nearly complete inhibition was achieved at a 2 mM methylglyoxal concentration. Figure 2b shows the inhibition of DNA synthesis by methylglyoxal. 1 mM Methylglyoxal inhibited DNA synthesis 67, 65, 79, and 33 percent in villus, crypt, and mixed cells and colonocytes, respectively. The percent inhibition was greater at higher concentrations of
methylglyoxal. The inhibitory pattern of RNA synthesis is shown in Figure 2c. The percent inhibition of RNA synthesis at 1 mM methylglyoxal was 9, 16, 20, and 18 in villus, crypt, and mixed cells and colonocytes, respectively. The maximum inhibition achieved in villus and crypt cells at 3 mM methylglyoxal was 30 and 21 percent, respectively, whereas in colonocytes it was 70 percent.

Protective effect of thiol compounds on methylglyoxal toxicity was checked. When cysteine and GSH at different concentrations up to 4 mM were included separately in the incubation system, we found that the inhibitory effect of methylglyoxal was not abolished. Cellular glutathione level was measured in control and methylglyoxal-treated cells, and it was not affected by 2 mM methylglyoxal treatment. GSH levels in villus, crypt, and mixed cells and colonocytes were 2.60, 2.64, 2.77, and 2.31 nmol per mg protein, respectively. Glyoxalase-I activity in the cells was also not affected when the cells were treated with 2 mM methylglyoxal.

**Fig. 1** Uptake of radiolabeled isotopes by colonocytes and percent viability at different time intervals. Colonocytes (1.5 - 2.0 x 10⁶) were incubated at 25°C for different time intervals separately with 0.025 μCi [¹⁴C]-leucine (○), 0.25 μCi [³H]-thymidine (■), or 0.25 μCi [³H]-uridine (▲). Percent viability of cells (○) was determined by trypan blue exclusion. Experiments were performed as described in the text. Points represent the average of duplicate experiments.
Fig. 2  Percent inhibition of (a) protein synthesis, (b) DNA synthesis, (c) RNA synthesis in villus, crypt, and mixed cells, and colonocytes in the presence of different concentrations of methylglyoxal. The percent inhibition values are calculated based on 100 percent synthesis in the control.

DISCUSSION

Methylglyoxal is an antimitotic compound that inhibits the growth of bacteria [16], viruses [31], and different neoplastic cell lines. Methylglyoxal also has a carcinostatic effect and inhibits the growth of ascites carcinoma, mammary carcinoma, leukaemia, adenocarcinoma, lymphocarcinoma, and sarcoma [32,17,18]. Impairment of several biochemical mechanisms such as mitochondrial respiration, protein synthesis, and secretion by methylglyoxal toxicity has also been reported [33].

The cells lining the gastrointestinal tract are in immediate contact with the luminal contents, which may contain bacterial metabolites including methylglyoxal. Our
earlier work showed that bacteria isolated from faeces are capable of producing methylglyoxal [2]. Cells lining the gut have high mitotic activity and methylglyoxal has an antimitotic effect. The present study shows that methylglyoxal can inhibit protein, DNA, and RNA synthesis when incubated in vitro with various cell types isolated from the rat gastrointestinal tract (Fig.2). Inhibition of protein synthesis by methylglyoxal in E. coli and in neoplastic cells has been reported by others [16,17]. In E. coli 1 mM methylglyoxal inhibited the uptake of labeled leucine, thymidine, and uracil to the extent of 93.9, 50.5, and 25.3%, respectively. Similarly in malignant ascites cells 1.5 mM methylglyoxal inhibited protein, DNA, and RNA synthesis to the extent of 86, 38, and 21%, respectively. The inhibitory pattern presented in this work is similar to those reported values. The inhibitory effect on protein and DNA synthesis was much more pronounced than that on RNA synthesis (Fig.2). It was suggested that the antiproliferative effect of methylglyoxal may be mediated through one of its metabolites, S-D-lactoylglutathione [34]. It has been suggested that methylglyoxal may inhibit protein synthesis by binding reversibly to exposed guanine residues of tRNA, thus preventing their participation in protein synthesis [35]. It has also been suggested that the inhibition of DNA and RNA synthesis could be due to the reaction of methylglyoxal with guanine residues of DNA and its precursors [31,36].

Methylglyoxal, being a keto aldehyde, may react with sulphydryl groups of thiol compounds. It was reported that in E. coli, cysteine can protect the cells from the inhibitory action of methylglyoxal. In the present study, however, the thiol compounds used could not protect the cells from the inhibition of macromolecular synthesis by methylglyoxal. Glutathione is one of the important nonprotein thiol compounds in the cell. The level of GSH was not altered by methylglyoxal treatment. However, since our method of estimation was not very sensitive, it is possible
that changes in its level might not have been detected. Glyoxalase-I activity also remained unaltered in the cells treated with methylglyoxal. We were unable to determine the level of methylglyoxal in the cytosol of the isolated cells after treating the cells with methylglyoxal. This may reflect either rapid metabolism of methylglyoxal inside the cells by the glyoxalase enzyme or low sensitivity of the colorimetric method used for its estimation.

Methylglyoxal may kill the cells by more than one mechanism. Although it affects synthesis of protein, DNA and RNA, the target of its lethal effect may be protein synthesis, as suggested by various authors [16,37]. Another possible mechanism for inhibition of cell division is its interaction with sulfhydryl groups. It is known that sulfhydryl groups are important in a great number of enzymes involved in cell division [38]. Methylglyoxal and other α-keto aldehydes readily interact with sulfhydryl groups forming addition products [3,39], and this interaction may account for the inhibitory effect of methylglyoxal on cell division [16,40]. Earlier we showed that methylglyoxal can alter the protein thiol and free amino groups of isolated intestinal cells [41]. The presence of glyoxalase enzyme in mammalian intestine may be a protective mechanism for highly vulnerable mucosal cells of the gastrointestinal tract against this toxic compound.

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REFERENCES


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