Inhibition of the post-translational processing of microvillar hydrolases is associated with a specific decreased expression of sucrase-isomaltase and an increased turnover of glucose in Caco-2 cells treated with monensin

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The biosynthesis and post-translational processing of sucrase-isomaltase and dipeptidylpeptidase IV were studied by L-[^{35}S]methionine labeling, immunoisolation with monoclonal antibodies and SDS-PAGE in post-confluent Caco-2 cells treated with monensin (10 μM, 48 h). In addition to its classical effect on the post-translational processing of both hydrolases, i.e. an inhibition of the conversion of the high-mannose to the complex glycosylated form of the enzymes, monensin was found to have two other effects: a marked decrease of sucrase-isomaltase expression, but not of dipeptidylpeptidase IV; an increased turnover of glucose, as substantiated by increased rates of glucose consumption and lactic acid production and a decreased glycogen content. Whether these two effects are related to the particular differentiation and metabolic status of Caco-2 cells is discussed, as well as a possible role for the drug-induced modifications of glucose turnover on the decreased expression of sucrase-isomaltase.

Monensin *Microvillar hydrolase* *Protein glycosylation* *Glucose consumption* *Glycolysis* *Glycogen* *(Caco-2 cell)*

1. INTRODUCTION

Human colon cancer cell lines HT-29 and Caco-2 have been shown to undergo enterocytic differentiation in culture [1,2], the pattern of which is not of small-intestinal, but of the fetal colon type [3,4]. As in most malignant epithelial cells their turnover of glucose is different from that found in the normal, with the rates of glucose consumption and lactic acid production being much higher [5], and with glycogen being stored at high levels [6], a particularity also shared by human fetal colon cells [7] but absent from the normal post-natal colon [7]. It has been shown that the expression of microvillar hydrolases, and more particularly that of sucrase-isomaltase, could be modulated in these cells by imposing on them permanent modifications of their utilization of glucose, either by modifying the hexose composition of the culture medium, as in HT-29 cells [1,8,9], or in the case of Caco-2 cells by inducing permanent glycogenolysis [10]. These observations suggested that, at least in these cells, the regulation of the expression of sucrase-isomaltase was dependent, in part, on the turnover of glucose, the mechanism of this regulation still remaining unknown. Here, we compared the effect of monensin on the biosynthesis of sucrase-isomaltase and dipeptidylpeptidase IV in Caco-2 cells. Monensin, an ionophore which binds monovalent cations, has been shown in a variety of systems to block the processing of newly synthesized glycoproteins at the site of the Golgi complex [11–15]. In normal small intestinal [16] and kidney cells [17] it inhibits the conversion of the high-mannose to the complex form of microvillar

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hydrolases. We show here that treatment of Caco-2 cells with monensin results not only in modifications of the post-translational processing of the enzymes, as would be expected, but also, unexpectedly, in a specific decrease in expression of sucrase-isomaltase and in marked modifications of the turnover of glucose.

2. MATERIALS AND METHODS

Caco-2 cells, obtained from J. Fogh (Memorial Sloan Kettering Cancer Center, Rye, NY) were cultured in 25 cm^2 Corning plastic flasks in Dulbecco's modified Eagle's minimum essential medium (containing 25 mM glucose) supplemented with 20% inactivated (56°C, 30 min) fetal bovine serum and 1% non-essential amino acids as described in [10]. Monensin (Calbiochem Behring, La Jolla) was added to the culture medium of stationary cultures (i.e. when Caco-2 cells are fully differentiated [1,10]) for two consecutive periods of 24 h. For biosynthesis studies Caco-2 cells (passages 70–75) were harvested after 3 h exposure to 500 μCi L-[^{35}S]methionine (Amersham) in 2 ml methionine-free culture medium. Preparation of cell homogenates and brush border-enriched fractions (P2) was as in [10]. Immunoprecipitates, treatment with endo-β-acetylglucosaminidase H (Endo-H, Miles), SDS-PAGE and autoradiography were performed as in [18], using monoclonal antibodies HBB2/614/88 [18] for sucrase-isomaltase and HBB3/775/42 [18] for dipeptidylpeptidase IV (obtained from H.P. Hauri, Biocenter of the University of Basel, Basel). Glucose and lactic acid concentration in the medium and intracellular concentration of glycogen were measured as in [5]. The rates of glucose consumption and lactic acid production were calculated from the results obtained within the first 8 h after the medium change, i.e. when they are linear (see fig.3). Intracellular glycogen was measured after 48 h of treatment. Proteins were measured as in [19].

3. RESULTS

To determine the maximum concentration of the drug compatible with an absence of effect on cell viability, exponentially growing (day 4–5) and stationary cultures (day 14–15) were treated for 48 h with various concentrations of monensin. The optimal concentration was found to be 10 μM. In exponential cultures this concentration did not modify the doubling time of the cells; in stationary cultures it had no effect on cell viability as assessed by trypan blue exclusion, total cell number and total protein content per flask. The phase-contrast morphology of the cells was not modified, except for a marked augmentation of the size of domes (which are a typical feature of post-confluent Caco-2 cells [2] and are indicative of active transepithelial ion transport properties [20]).

The effect of monensin on the biosynthesis and post-translational processing of sucrase-isomaltase and dipeptidylpeptidase IV was studied on day 16, i.e. when the rate of synthesis of the enzymes is maximum. Identification of the high-mannose

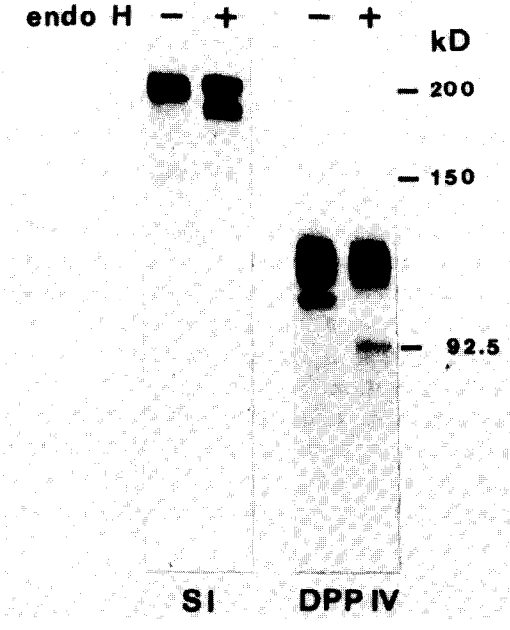

Fig.1. Fluorograms of SDS-PAGE of sucrase-isomaltase (SI) and dipeptidylpeptidase IV (DPP IV) immunoisolated with the corresponding monoclonal antibodies from the cell homogenates of untreated Caco-2 cells (day 16 in culture) labeled for 3 h with L-[^{35}S]methionine. Immunoprecipitates were run on SDS-PAGE in the absence of (−) or after a treatment with Endo-H (+). The upper band of the enzymes (insensitive to Endo-H) corresponds to the complex glycosylated form and the lower band (sensitive to Endo-H) to the high-mannose form.

(sensitive to Endo-H treatment) and complex form of the enzymes (unsensitive to Endo-H) is as indicated in fig.1. Treatment with monensin results in a double effect: (i) It inhibits the conversion from the high-mannose to the complex form of both sucrase-isomaltase and dipeptidylpeptidase IV; as shown in fig.2 only the high-mannose form of the enzymes could be detected in the cell homogenate, in contrast to control cells where both the high-mannose and complex forms of the enzymes are present. In the brush border-enriched fraction (P2) only the complex form of the enzymes is present in control cells, whereas in monensin-treated cells only a faint band is detectable; this band corresponds to the high-mannose form and the reason for its presence is most likely that the P2 fraction is not pure brush border membrane. (ii) The second effect is a marked decrease of the labeling of sucrase-isomaltase. As shown in fig.2 the intensity of labeling of sucrase-isomaltase in the cell homogenate from monensin-treated cells is much lower than the total labeling of the enzyme in control cells, in contrast to dipeptidylpeptidase IV which, although in different molecular forms, has the same intensity of labeling under both conditions.

In the course of these studies we observed that treatment with monensin resulted in more rapid acidification of the culture medium as compared to control cells, suggesting a higher rate of production of lactic acid. This prompted us to investigate further a possible effect of monensin on glucose utilization. The results reported in table 1 show that treatment with monensin indeed results in a more than 2-fold increase of the rates of glucose consumption and lactic acid production and a

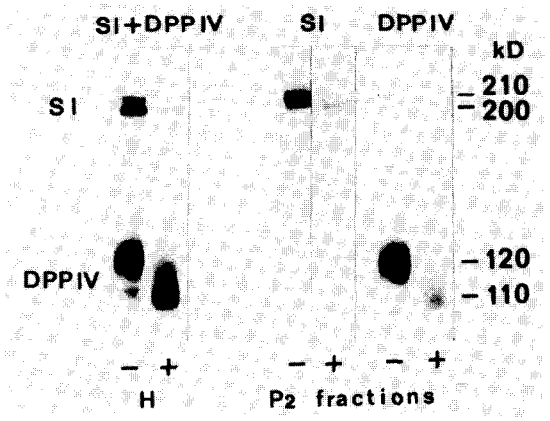

Fig.2. Fluorograms of SDS-PAGE of immunoprecipitates of L-[^{35}S]methionine-labeled sucrase-isomaltase (SI) and dipeptidylpeptidase IV (DPP IV) from the cell homogenate (H) and a brush border-enriched fraction (P2) of Caco-2 cells treated (+) or not treated (−) with monensin (10 μM, 48 h). For immunoprecipitation of the enzymes the same amount of cell proteins was used in both control and treated cells. Immunoprecipitates from the cell homogenates were done with the concomitant use of both anti SI and anti DPP IV monoclonal antibodies; single antibodies were used for the P2 fraction. The two bands of SI and DPP IV in the cell homogenate from control cells correspond for each enzyme to the high-mannose (lower bands) and complex form (upper band) of the enzymes (see fig.1); in the P2 fraction from control cells only the complex form of the enzymes is present. Note the absence of complex form of the enzymes in monensin-treated cells. Also note the very low intensity of labeling of SI in monensin-treated cells as compared to the control.

Table 1

Effect of monensin on the rates of glucose consumption and lactic acid production and on the intracellular glycogen content in post-confluent Caco-2 cells

	Glucose consumption (nmol/h per mg protein)	Lactic acid production (nmol/h per mg protein)	Intracellular glycogen (μg/mg protein)	Proteins per flask (mg)
Control cells	393 ± 19	668 ± 32	265 ± 15	5.46 ± 0.22
Monensin (10 μM)	842 ± 12	1768 ± 28	147 ± 7	5.45 ± 0.21

For details, see section 2. Caco-2 cells (passage 72) were treated with monensin between days 14 and 16 in culture; each value represents the mean and SE of 3–5 different flasks

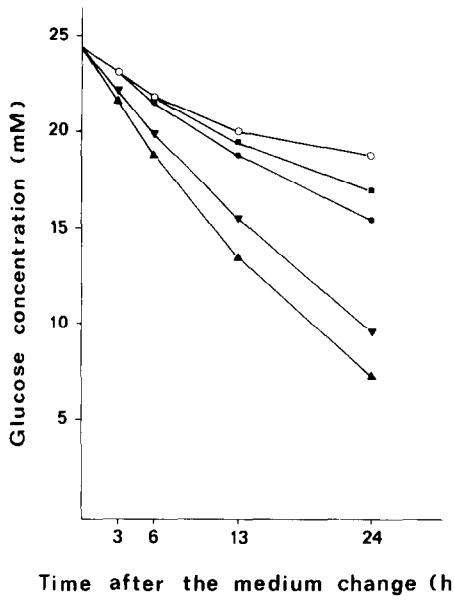

Fig.3. Evolution of the concentration of glucose in the culture medium of Caco-2 cells (day 15) after a medium change. (○) Control cells; cells treated with monensin: (■) 0.5 μM, (●) 1 μM, (▼) 5 μM, (▲) 10 μM. Cells were cultured in 25 cm^2 plastic flasks in the presence of 5 ml culture medium.

nearly 50% decrease of glycogen accumulation, without modification of the cell protein content. The effect on the rate of glucose consumption is dose-dependent (fig.3) and is reproducible at each medium change (not shown).

4. DISCUSSION

Although Caco-2 cells are malignant colonic cells and therefore should not be considered a totally relevant 'in vitro' model for normal small intestinal cells, they have been shown to be an appropriate model for the study of the biosynthesis and intracellular transport of microvillar hydrolases [18]. The present results confirm the relevance of this model for such studies as far as treatment of the cells with monensin results in the same inhibition of the conversion of the enzymes from their high-mannose to their complex form as demonstrated for sucrase-isomaltase and dipeptidylpeptidase IV in small intestinal cells [16] or for dipeptidylpeptidase IV in kidney cells [17].

In addition to this classical effect monensin, however, has two other effects in Caco-2 cells: one is a marked and specific decrease of the expression of sucrase-isomaltase which appears to be particular to Caco-2 cells, as it has not been observed in normal intestinal cells [16]; whether this modification of sucrase-isomaltase expression results from decreased biosynthesis and/or increased degradation of the enzyme has to be documented further. The second effect is a marked increase in glucose turnover. This latter effect is not restricted to Caco-2 cells and is independent of the differentiation state of the cells as it has also been observed in a number of undifferentiated human colon carcinoma cell lines (unpublished), suggesting that it could be related to the particular metabolic status of malignant epithelial cells.

The mechanism by which monensin interferes with the processing of membrane glycoproteins is far from being clear [11], as well as that responsible for its particular additional effects in Caco-2 cells. Whether there is a relationship in these cells between the monensin-dependent increase in glucose turnover and the specific inhibiting effect of the drug on sucrase-isomaltase expression is however questionable in regard to a number of observations. Indeed, previous studies have shown that increased glucose consumption, or decreased glycogen accumulation, or the association of both, are concomitant with decreased expression of sucrase-isomaltase but not, or to a much lesser extent, of other hydrolases in situations such as Caco-2 cells treated with forskolin [10], undifferentiated HT-29 cells grown in the presence of glucose [1,8,9] or the human fetal colon in the late stages of gestation [7,21].

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