SHORT COMMUNICATION

INTESTINAL ABSORPTION OF CYSTINE AND CYSTEINE IN NORMAL HUMAN SUBJECTS AND PATIENTS WITH CYSTINURIA

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SUMMARY

1. An intestinal perfusion technique has been used to study absorption \textit{in vivo} of \textit{L}-cystine and \textit{L}-cysteine in six normal human subjects and three patients with homozygous cystinuria.

2. No significant absorption of \textit{L}-cystine was detected during perfusion of the cystinuric patients, whereas \textit{L}-cysteine absorption was normal. These results imply that \textit{L}-cystine and \textit{L}-cysteine are normally absorbed by different transport processes.

3. No significant reduction of \textit{L}-cystine to \textit{L}-cysteine occurred in the gut lumen during the perfusion experiments. No more oxidation of \textit{L}-cysteine to \textit{L}-cystine occurred in the gut lumen during the perfusion experiments than in the test solutions which were simultaneously incubated \textit{in vitro}.

Key words: cystine absorption, cysteine absorption, intestinal perfusion, cystinuria.

The results of oral tolerance tests (Dent, Senior & Walshe, 1954; London & Foley, 1965; Foley & London, 1965) and studies \textit{in vitro} on jejunal biopsy material (McCarthy, Borland, Lynch, Owen & Tyor, 1964; Thier, Segal, Fox, Blair & Rosenberg, 1965; Rosenberg, Crawhall & Segal, 1967) suggest that there is an intestinal transport defect for cystine in patients with homozygous cystinuria. Two of the studies (Foley & London, 1965; Rosenberg et al., 1967) indicate that cysteine (the reduced form of cystine) may be absorbed normally in cystinuria.

The aim of the present study has been to investigate absorption \textit{in vivo} of cystine and cysteine in normal human subjects and patients with homozygous cystinuria. An intestinal perfusion technique has been used so that absorption from the jejunum \textit{in vivo} could be accurately measured. Intestinal contents are continuously aspirated during the perfusion procedure and it has thus been possible to determine whether oxidation of cysteine to cystine, or reduction of cystine to cysteine, occurs in the gut lumen during absorption.

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MATERIALS AND METHODS

Absorption was measured in six normal adult volunteers and three patients with homozygous cystinuria. An excess of cystine and dibasic amino acids had been demonstrated in the urine of the cystinuric patients, and each was receiving in-patient D-penicillamine therapy, which was stopped 24 h before the study. All subjects gave their informed consent.

The subjects were intubated with a double-lumen perfusion tube incorporating a proximal occlusive balloon (Sladen & Dawson, 1970; Silk, Perrett & Clark, 1973). The tube was allowed to pass until the 30 cm perfusion segment was positioned in the upper jejunum and the final position checked radiologically. The test solutions, which were maintained at 37°C in a water bath, were infused at 20 ml min⁻¹ and contained either L-cystine (1 μmol ml⁻¹) or L-cysteine (2 μmol ml⁻¹). Both solutions were made iso-osmotic by adding sodium chloride and contained polyethylene glycol (PEG) labelled with 1 μCi of [¹⁴C]polyethylene glycol/l (New England Nuclear Corp., Boston, U.S.A.), at a concentration of 2·5 g/l. After an equilibration period of 20 min, two 5 min samples were collected. The cysteine solutions were made up immediately before the experiments. No penicillamine or penicillamine disulphides were detected in the luminal contents aspirated during the cystine and cysteine perfusion experiments.

Analytical methods

To minimize oxidation of cysteine all samples were analysed immediately on collection. Cystine was reduced to cysteine with dithiothreitol (Cleland, 1964). A portion (0·1 ml) of each sample and standard cystine solutions was mixed with 0·5 ml of freshly prepared dithiothreitol solution [10 mmol/l in buffer prepared by titrating sodium tetraborate (0·2 mol/l) with hydrochloric acid (0·1 mol/l) to pH 8·0 (measured with a Beckman Research pH Meter; Beckman Instruments Ltd, Fyffe, Scotland)], and allowed to stand for 30 min. A further 0·1 ml of sample was mixed with 0·5 ml of 0·1 mol/l hydrochloric acid to prevent oxidation of cysteine. Cystine in all solutions was estimated by the specific ninhydrin method of Gaitonde (1967) and the cystine content was calculated by difference. [¹⁴C] radioactivity was measured with a scintillation counter (Wingate, Sandberg & Phillips, 1972; Silk, Perrett, Webb & Clark, 1974).

Calculation of results

Absorption of cystine was calculated from the formula previously described (Adibi, 1971). Cysteine absorption during perfusion of seven subjects was calculated from the same formula. During cysteine perfusion of one normal subject and one patient with cystinuria, higher concentrations of cystine were detected in the intestinal aspirates than in the test solution, indicating that some intraluminal oxidation of cysteine had occurred. In these two instances, the formula was modified to take intraluminal oxidation into account, and absorption of cysteine ($A_c$) calculated as follows:

$$A_c = [A_i - (A_o + 2C_o - 2C_i)] (PEG_i/PEG_o) \times R$$

Where $A_c$ is the rate of absorption of cysteine (μmol min⁻¹ 30 cm⁻¹), $A_i$ is the concentration of cysteine (μmol ml⁻¹) in the test solution, $A_o$ is the concentration of cysteine (μmol ml⁻¹) in the intestinal aspirates, $C_o$ is the concentration of cystine (μmol ml⁻¹) in the intestinal aspirates, $C_i$ is the concentration of cystine (μmol ml⁻¹) detected in the cysteine test solution at the end of the perfusion experiments, PEG is the radioactivity (d.p.m.) of [¹⁴C]polyethylene glycol in the
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test solution, PEG, is the radioactivity (d.p.m.) of $[^{14}\text{C}]\text{PEG}$ in the intestinal aspirates and $R$ is the perfusion rate (20 ml min$^{-1}$).

**RESULTS**

No significant absorption of cystine was detected during perfusion of the cystinuric patients with L-cystine (Fig. 1a). During perfusion of the normal subjects mean cystine absorption was $6.7 \pm 1.3$ SEM $\mu$mol min$^{-1}$ $30\text{ cm}^{-1}$. No cysteine was detected in the luminal contents aspirated during perfusion of the normal subjects or the cystinuric patients.

Cysteine was absorbed at the same rate ($30.6, 22.8, 31.1\ \mu$mol min$^{-1}\ 30\text{ cm}^{-1}$) during perfusion of the three cystinuric patients with L-cysteine as during perfusion of normal subjects (mean $24.9 \pm 2.9$ SEM $\mu$mol min$^{-1}$ $30\text{ cm}^{-1}$; Fig. 1b). At the end of the experiments some cystine was detected in the cysteine test solutions, which were incubated at 37°C during the perfusion procedure; $0.16 \pm 0.03\ \mu$mol of cystine ml$^{-1}$ (mean $\pm$ SEM) was detected at the end of the perfusions in the normal subjects and $0.17, 0.04$ and $0.18\ \mu$mol ml$^{-1}$ at the end of the perfusions in the cystinuric patients, indicating that $15.1 \pm 2.7\%$ SEM and $17.4\%, 3.5\%$ and $18.0\%$ of the cysteine content of the test solutions had been oxidized *in vitro* during the course of the perfusions in the normal subjects and three cystinuric patients respectively. There was no significant difference in the concentrations of cystine detected in the luminal contents aspirated during cysteine perfusion of the normal subjects ($0.10 \pm 0.04$ SEM $\mu$mol ml$^{-1}$) and the three
cystinuric patients (0.17, 0.07 and 0.11 μmol ml⁻¹) compared with the concentrations detected in the test solutions which had been incubated at 37°C in vitro during the time of the perfusion experiments. There was thus no evidence that more oxidation of cysteine occurred in the gut lumen during perfusion of either the normal subjects or the patients with cystinuria than when the amino acid solution was incubated in vitro.

DISCUSSION

The present findings confirm that there is an intestinal transport defect for cystine in homozygous cystinuria whereas cysteine absorption is normal, which must imply that under the described experimental conditions L-cystine and L-cysteine are absorbed by separate transport processes. This is the first time that mucosal uptake of these amino acids in vivo has been directly quantified in man, and the conclusions are similar to those reached when oral load tests (London & Foley, 1965; Foley & London, 1965) and studies in vitro on jejunal biopsy material (McCarthy et al., 1964; Thier et al., 1965; Rosenberg et al., 1967) were carried out.

It has been shown during the active transport of cystine into rat jejunal everted sacs and segments in vitro that some reduction of cystine to cysteine in the mucosal medium occurs at low concentrations (Crawhall & Segal, 1967; Crawhall & Davis, 1969). This was not apparent during the present perfusion experiments in vivo, but it should be remembered that different experimental techniques have been used in the studies. Also the reduction might have been obscured by the oxidation of cysteine to cystine, which was shown to occur in the control solutions, and which occurred to about the same extent in vivo.

It seemed unlikely from the results of the cysteine perfusions in the normal subjects that significant oxidation of cysteine to cystine occurred, by virtue of the presence of the amino acid in the gut lumen before absorption, and because there was no difference in the concentration of cystine detected in the cysteine aspirates compared with the concentrations of cystine detected in the cysteine test solutions. This conclusion is supported by the results of the perfusions in the cystinuric patients because, despite the transport defect for cystine, there was still no increase in the concentrations of cystine in the cysteine aspirates compared with the concentrations detected in the cysteine test solutions.

Thus the results of the perfusion studies in the cystinuric patients clearly demonstrate that cystine and cysteine are absorbed by separate transport processes, and that no significant interconversion occurs in the luminal phase of absorption.

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REFERENCES

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