EFFECTS OF CYCLOHEXIMIDE AND STREPTOVITACIN A ON PROTEIN SYNTHESIS AND GASTRIC SECRETION IN RATS

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Cycloheximide and streptovitacin A administered *in vivo* to rats display a similar dual effect on the labelling of soluble liver proteins by valine-^{14}C, and result in a similar enhancement of liver uridine kinase activity. On the other hand, in pylorus-ligated rats, both antibiotics markedly depress gastric secretion, acid output, and the level of mucoproteins and proteolytic activity in secreted juice. Streptovitacin A on a molar basis was in all cases 5~8 times more effective than cycloheximide.

The structurally related glutarimide antibiotics cycloheximide and streptovitacin A (4-hydroxycycloheximide) inhibit protein synthesis[1,2] and presumably have a common mechanism of action. However, while the inhibition of protein synthesis by cycloheximide was found to be reversible, that by streptovitacin A was completely irreversible.[8] Cycloheximide displays a polyvalent inhibitory mechanism and affects various metabolic steps in eukaryotic cells.[4~10]

The inhibition of protein synthesis *in vivo* after the administration of sublethal doses of cycloheximide is followed by a dose- and time-dependent stimulation of the incorporation of amino acids into proteins of the liver.[7,9] At 24 hours after cycloheximide treatment, an increase of uridine kinase in the liver of rats was observed.[11,12] In contrast, the same doses of cycloheximide almost completely block gastric secretion and acid output in animals with a ligated pylorus.[1] It was the aim of this study to investigate and compare the stimulatory and inhibitory actions of cycloheximide and streptovitacin A in the liver and gastrointestinal tract of rats.

Materials and Methods

Chemicals.

Cycloheximide was obtained from Calbiochem, Luzern, streptovitacin A was a gift from Dr. Z. Vaněk, Institute of Microbiology, Prague. Valine-U-^{14}C (50 μCi/μmol) and 6-azauridine-4,5-^{14}C (80 μCi/μmol) were from the Institute for Research, Production and Uses of Radioisotopes, Prague.

Labelling of liver proteins.

Groups of albino female rats kept under standard laboratory conditions were injected i.p. at 8 a.m., 60 minutes before killing. The livers were removed and the labelling of proteins in the postmitochondrial liver fraction was measured as already described.[14]

Assay of uridine kinase activity.

Enzyme was measured during a 10-minute incubation period at 37°C in cell-free liver extracts with 0.05 mM 6-azauridine-4,5-^{14}C, 66 mM Tris-HCl buffer (pH 7.4), 3 mM ATP and 1.5 mM $MgCl_2$. The separation of the reaction mixture was carried out chromatographically as described earlier.[15]

Gastric secretion and analysis of secreted juice.

Pyloric ligation was performed according to Shay *et al.*[16] The gastric juice was collected for

18 hours, filtered, its volume measured, and before analysis diluted 20 times. Hydrochloric acid was assayed by titration with 0.1 M NaOH, pepsin activity (expressed as milliequivalents of released tyrosine) was measured according to ANSON and MIRSKY[17] with 2.5% hemoglobin at pH 1.6, and total mucoproteins (expressed as mg of hexoses present in secreted juice) were assayed by the modified orcin method (WEIMER and MOSHIN[18]).

All drugs injected were dissolved in sterile saline. For dosage, see figures.

Results

In Vivo Labelling of Soluble Liver Proteins

From the study of ROTHBLUM *et al.*[7] it is known that the recovery from the inhibition of protein synthesis by sublethal doses of cycloheximide is dose- and time-dependent. At 24 hours after drug administration, the significantly higher than normal rate of amino acid incorporation into liver proteins was not due to change in either the precursor pool or the concentration of the labelled amino acid. The recent data presented by CH'IH *et al.*[19] further corroborate the original suggestion that the stimulated protein synthesis after cycloheximide administration involves gene transcription.[4,9]

The data presented in Fig. 1 indicate that cycloheximide and streptovitacin A display a similar dual effect on the labelling of soluble liver proteins. In relation to the doses of administered cycloheximide or streptovitacin A the rate of amino acid incorporation measured 2 hours after the treatment was markedly decreased. At 24 hours there was in both cases a dose-dependent increase to supranormal levels in the labelling of liver proteins.

Fig. 1. Dual effect of cycloheximide and streptovitacin A on the *in vivo* labelling of soluble proteins in rat livers by valine-^{14}C.

Drugs were administered i.p. to groups of 4~5 female rats (170~180 g); 2 or 24 hours later the animals received i.p. valine-U-^{14}C (2.5 μCi/0.025 μmol) and were killed 60 minutes thereafter.

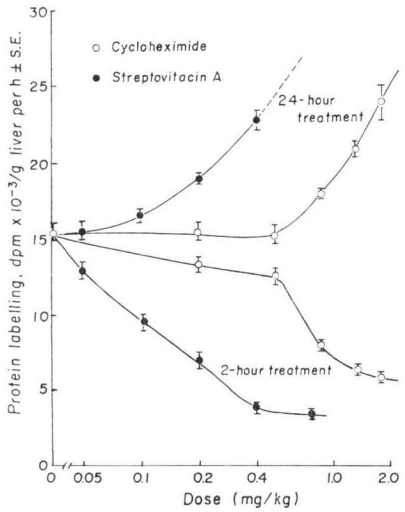
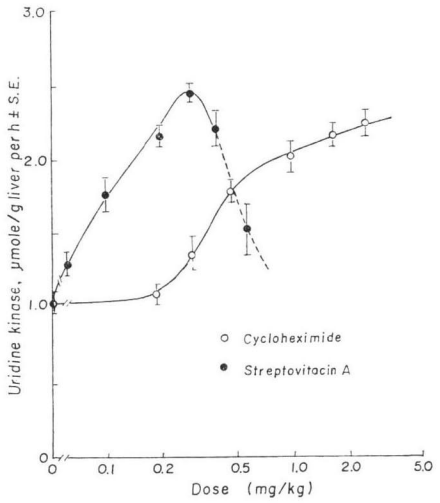


Fig. 2. Stimulation of liver uridine kinase in relation to the level of administered cycloheximide and streptovitacin A.

Groups of 6~9 female rats (175~180 g) received 24 hours before killing increasing doses of both drugs. The activity of uridine kinase was measured in cell-free liver extracts.



Liver Uridine Kinase

The administration of cycloheximide to adult rats results in the enhancement of uridine kinase activity in the liver.[11] The increase of enzyme activity is not affected by adrenal secretion or uptake of

food,[12] and a similar stimulatory effect was observed after the administration of various metabolic inhibitors (5-azacytidine, gougerotin, pactamycin, adriamycin, daunorubicin and thioacetamide) affecting different steps in cellular metabolism.[20]

The stimulation of uridine kinase activity in the liver of rats after the intraperitoneal administration of increasing doses of cycloheximide and streptovitacin A is shown in Fig. 2. The increase in enzyme activity is preceded by a 6~8-hour lag-phase and reaches a maximum 24~30 hours after drug administration.

Gastric Section

Low toxicity of cycloheximide facilitated the successful clinical use of the antibiotic in patients with HODGKIN's disease.[21] However, severe nausea and vomiting as immediate side effects were observed after treatment with the drug. Recently it was found that cycloheximide administered to rats in non-toxic doses immediately after pyloric ligation results in a lower incidence of experimental gastric ulcers paralleled by a block in the secretion of gastric juice and hydrochloric acid output.[13]

The data shown in Figs. 3 and 4 indicate that a similar effect can be achieved using streptovitacin A. This antibiotic at low doses (0.5 mg per kg) depresses gastric secretion and blocks acid output (Fig. 3). Proteolytic activity of secreted juice and total hexose present (as a measure of mucoproteins) were affected by streptovitacin A as well (Fig. 4).

Fig. 3. Inhibitory effect of cycloheximide and streptovitacin A on gastric secretion and acid output in rats.

Groups of 5~8 rats received the drugs immediately after pyloric ligation 18 hours before killing.

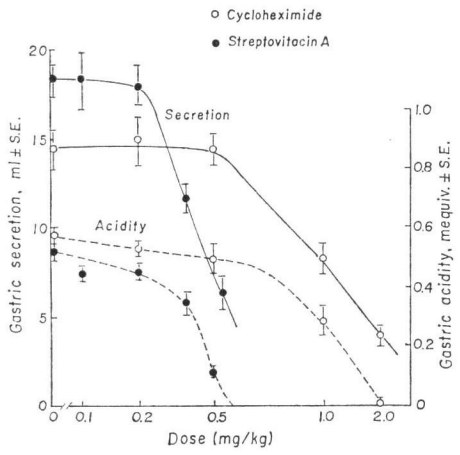
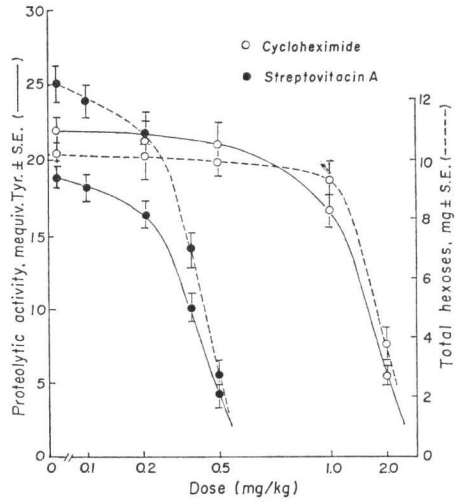


Fig. 4. Decreased activity of proteolytic enzymes and lower level of mucoproteins in gastric juice of rats following cycloheximide and streptovitacin A.

Groups of 5~8 rats received drugs as in Fig. 3.



Discussion

The adaptive mechanism of protein synthesis during and after its inhibition probably involves both the translational and transcriptional events. A mechanism by which cycloheximide activates the genome could imply the increase in template activity of chromatin mediating through an increase in the rate of synthesis of nonhistone chromosomal proteins.[4] It is likely that streptovitacin A which causes similar changes as cycloheximide in the labelling of soluble liver proteins (Fig. 1) and

a similar increase in the activity of liver uridine kinase (Fig. 2) affects the same metabolic steps in the liver. However, streptovitacin A is biologically more active[2,3] than cycloheximide, and similar changes in protein synthesis and uridine kinase activity were obtained at 1/6 to 1/10 the concentration.

The impaired gastric secretion and almost complete block in the output of hydrochloric acid observed after cycloheximide in pylorus ligated rats[13] were extended in this study to streptovitacin A. The results indicate that the biochemical parameters including acid output, proteolytic activity and the level of mucoproteins in secreted juice were almost equally decreased after cycloheximide and 4 times lower doses of streptovitacin A (Figs. 3 and 4). The poor general condition of streptovitacin A treated rats and the mortality rate suggest the preference of cycloheximide as an active antiulcer agent.

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