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Improvement of Fat Digestion in Rats by Dimethyl-β-cyclodextrin

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Abstract. Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB), a compound having a great water-solubility enhancing effect on lipids via inclusion complex formation was investigated as a potential bile-substituting agent *in vivo* in rats. The normal fat digestion was inhibited by ligating the bile duct. 3-H-Stearic acid or edible oil were administered orally to rats and the effect of simultaneously administered DIMEB on the lipid absorption was studied by measuring the blood radioactivity level or plasma triglyceride and free fatty acid concentrations. The lipid absorption was significantly improved by DIMEB. Accordingly, it seems to be a new fat digestion and absorption enhancing drug, i.e. a possible bile-substituting agent.

Key words: Dimethyl-β-cyclodextrin, inclusion complex, bile substituent, lipid absorption enhancement.

1. Introduction

A precondition for the digestion of fats – both by humans and animals – is the production and excretion of bile. Natural bile is a very complicated mixture, the main ingredients are the various cholic acid derivatives. The nutritional fats can be absorbed only when they are emulsified by the bile, and thereafter hydrolysed by the lipases. In some hepatic and biliary diseases either the production or the excretion of natural bile is impaired. In such cases the bile has to be substituted by drugs, which contain either cholic acid of animal origin, or other fat-emulsifying agents. According to preliminary studies, heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DIMEB) may be such a fat digestion facilitating compound.

DIMEB is a compound prepared by the selective methylation of all C(2) secondary and C(6) primary hydroxyl groups of beta-cyclodextrin. DIMEB is soluble in organic solvents and it is very soluble in cold water. 25–30% solutions of increased viscosity can be readily prepared, while a syrupy 50% solution can be prepared by prolonged stirring and shaking. DIMEB has a great solubility enhancing effect on poorly soluble compounds, e.g. lipids via inclusion complex formation [1].

Oral administration of DIMEB to male and female mice resulted in no toxic symptoms up to 3000 mg/kg. Six hours after intravenous administration only traces of DIMEB can be detected in the blood, the majority of the substance is removed from the circulation within the first two hours [1].

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2. Materials and Methods

2.1. MATERIALS

Heptakis-(2,6-di-*O*-methyl)-beta-cyclodextrin (DIMEB) (Chinoin Pharm.-Chem. Works): At least 90% of the substance corresponds to the above mentioned chemical name. The remaining 10% is a mixture of different, very closely related methylated derivatives. The heavy metal content is less than 10 ppm, the organic solvent residue is less than 100 ppm. $[\alpha]_D^{15}$: 161° (0.1% H_2O). Sodium deoxycholate (Reanal); 9,10-3-H-stearic acid (Izocommerc), specific radioactivity: >1110 GBq/mmol (labelled stearic acid was diluted with appropriate amount of non-labelled stearic acid (Reanal) before the experiments); edible oil.

2.2. METHODS

2.2.1. *Experiments with 3-H-stearic acid*

Following a 24-hour starvation, the choledocus of CFY rats weighing 160–170 g was ligated in diethyl-ether narcosis. The absorption experiments were performed 48 hours following the operation. The animals were starved for 12 hours before the treatment and during the experimental period as well. In all of the experiments, one group of rats was administered orally 3-H-stearic acid (40 to 120 mg/animal dose), the other group was given 3-H-stearic acid (40 to 120 mg/animal dose) and DIMEB (12.5 to 50 mg/animal dose) simultaneously. The materials were suspended in 1% methylcellulose solution.

Blood samples of 50 µl were taken at predetermined time intervals from the tail vein. The samples were solubilized in 0.75 ml of 1:1 Soluene-100 (Packard Instruments):isopropanol solution (v/v), and were bleached with 0.25 ml 33% hydrogen peroxide for 30 min at room temperature. Then 10 ml of a 9:1 Insta Gel (Packard):0.5 mol/l HCl (v/v) solution was added and the samples were incubated in the dark for 24 hours at room temperature. The samples were assayed for radioactivity in a Searle Nuclear Chicago Mark III 6880 apparatus. The radioactivity (dpm) measured was related to 10 ml of blood and expressed as % of total administered radioactivity.

2.2.2. *Experiments with edible oil in intact rats*

CFY rats of 205–230 g weight were administered orally 2 ml edible oil or 2 ml edible oil + 50 mg DIMEB (dissolved in 0.5 ml distilled water). The aqueous solution of DIMEB was added to edible oil at physiological temperature, and following a 5-minute vigorous shaking it was administered immediately to the animals. Blood samples were taken at 0, 1, 2.5, 4 and 6 hours after treatment by the decapitation of rats. Plasma triglyceride and free fatty acid concentrations were determined according to Van Handel [2] and Duncombe [3], respectively.

3. Results

3.1. THE EFFECT OF DIMEB ON THE ABSORPTION OF ORALLY ADMINISTERED 3-H-STEARIC ACID IN BILE DEFICIENT RATS

The free fatty acid absorption enhancing effect of DIMEB was investigated in rats suf-

fering from an experimental bile deficiency. 3-H-Stearic acid or 3-H-stearic acid and DIMEB were administered orally to the animals and the blood radioactivity level was determined. The blood radioactivity values versus time are demonstrated in Figure 1.

Blood radioactivity level was considerably higher (2 to 15 fold) in DIMEB-treated groups than in those administered 3-H-stearic acid alone. The absorption enhancing effect of DIMEB was most apparent in the first two hours. On the contrary, a slow increase of blood radioactivity was observed in the case of animals treated with 3-H-stearic acid and sodium deoxycholate. In this case, a more apparent increase was observed only after the 6th hour.

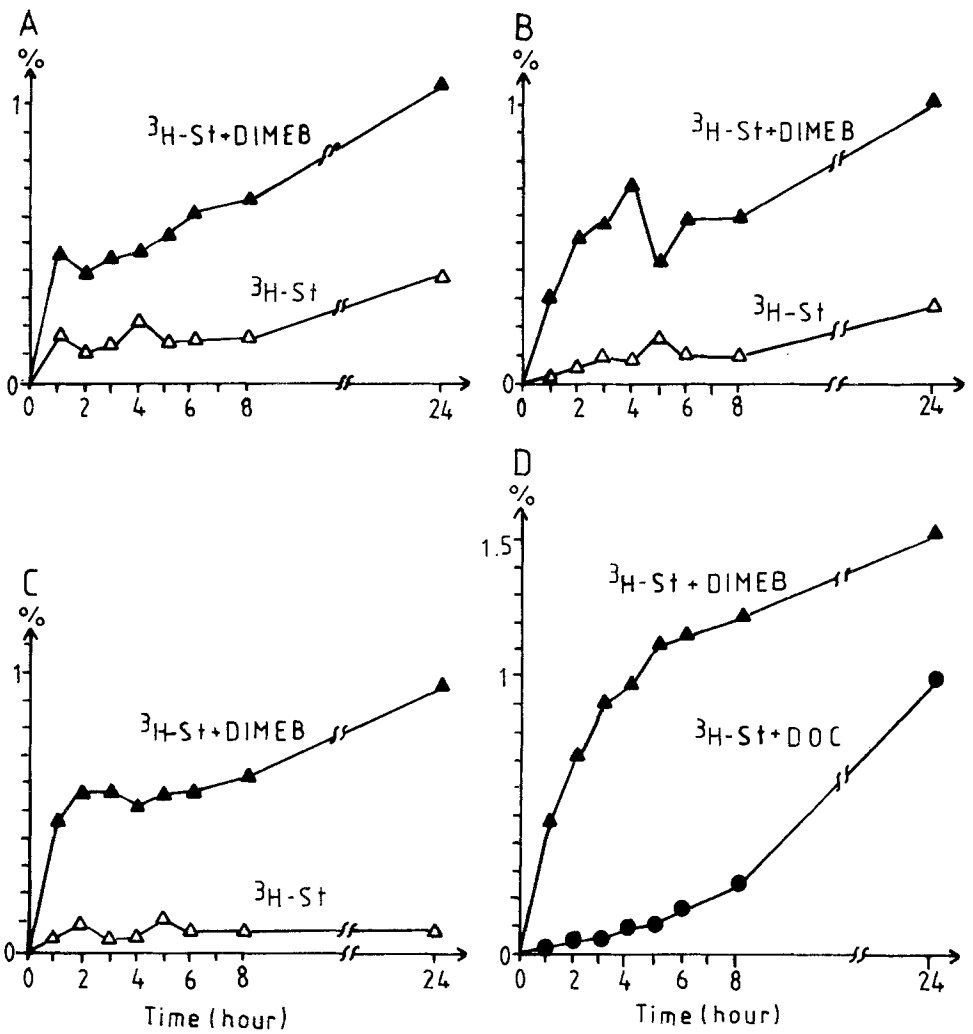

Fig. 1. Absorption of orally administered 3-H-stearic acid in the presence and absence of DIMEB in bile deficient rats. Blood radioactivity values are expressed in % of total administered radioactivity and related to 10 ml of blood. Key: (△——△)3-H-St: 3-H-stearic acid; (▲——▲) 3-H-St + DIMEB: 3-H-stearic acid + DIMEB; (●——●) 3-H-St + DOC: 3-H-stearic acid + sodium deoxycholate. Doses: *A*: 3-H-St: 40 mg/animal, 3-H-St + DIMEB: 40 mg + 12,5 mg/animal, resp. *B*: 3-H-St: 40 mg/animal, 3-H-St + DIMEB:: 40 mg + 25 mg/animal, resp. *C*: 3-H-St: 120 mg/animal, 3-H-St + DIMEB: 120 mg + 50 mg/animal, resp. *D*: 3-H-St + DIMEB: 40 mg + 25 mg/animal, resp., 3-H-St + DOC: 40 mg + 25 mg/animal, resp.

Therefore, the absorption of stearic acid was considerably enhanced due to the presence of DIMEB in bile duct-ligated rats. In all probability, the absorption enhancing mechanism of DIMEB differs from that of sodium deoxycholate.

3.2. THE EFFECT OF DIMEB ON THE ABSORPTION OF EDIBLE OIL IN INTACT RATS

Two ml edible oil was administered to intact rats orally in the presence or absence of DIMEB. The absorption process was followed by measuring the plasma triglyceride and free fatty acid concentration, as demonstrated in Figure 2.

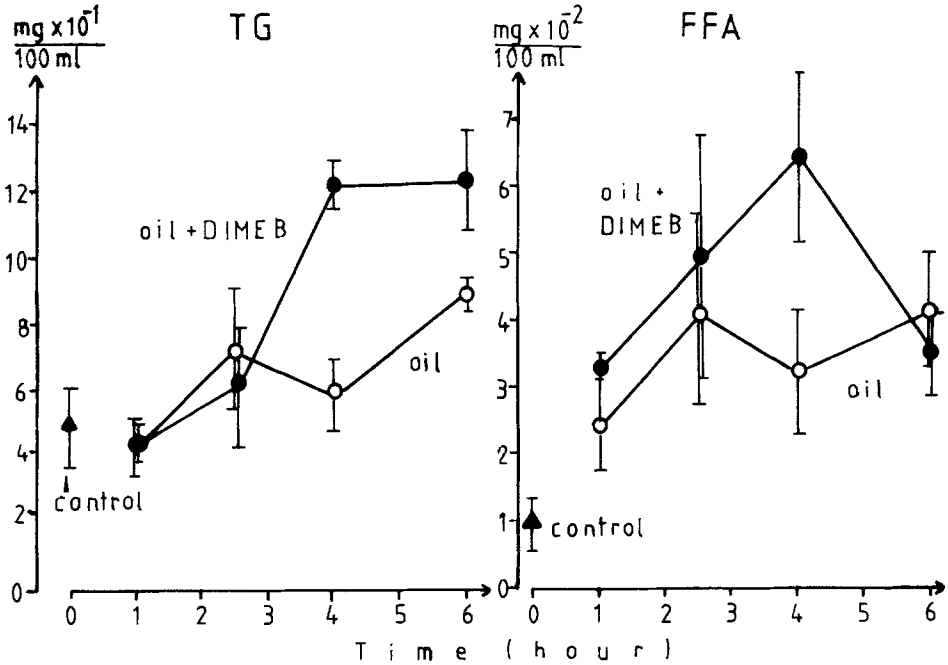

Fig. 2. Plasma triglyceride (TG) and free fatty acid (FFA) concentration of intact rats following oral administration of edible oil in the presence or absence of DIMEB. Key: (▲) control, animals were bled at the beginning of the treatment; (○——○) 2 ml edible oil/animal; (●——●) 2 ml edible oil + 50 mg DIMEB (dissolved in 0.5 ml water)/animal.

Four hours following the treatment there is no significant difference in the plasma triglyceride concentration of the control and the oil-treated group. At the same time, a significant increase can be observed in the DIMEB treated group. There is a significant difference between the oil-treated and the oil + DIMEB treated group even at the 6th hour. A similar tendency was observed by measuring the free fatty acid concentration. Therefore, DIMEB promoted the lipid absorption in healthy, intact rats.

4. Discussion

It is well known that the presence of bile is of capital importance in lipid absorption. In some hepatic and biliary diseases either the production or the excretion of bile is impaired. However, in some cases it is unadvisable to administer bile acids to the

patients. Bile acids should be substituted by a compound being no bile-like regarding the chemical structure, but being capable of solubilizing lipids to a similar extent to bile.

DIMEB seems to be a suitable material for this purpose. It is poorly absorbed orally and is probably non-toxic [1]. The solubility of lipids is highly enhanced by DIMEB via inclusion complex formation [1]. According to rat experiments, DIMEB promoted the absorption of edible oil even in intact rats having a normal bile supply (Fig. 2). Plasma triglyceride and free fatty acid concentrations were significantly increased in the presence of DIMEB 4 hours following the treatment. In the case of bile-deficient, choledocus ligated rats, the absorption of a free fatty acid, stearic acid, was considerably increased (2 to 15 fold) by DIMEB mainly in the first two hours (Fig. 1). Comparing the effect of DIMEB and sodium deoxycholate in a preliminary experiment, it was found that the degree of absorption enhancement effect was similar but the mechanism of action is probably quite different (Fig. 1).

According to these results, DIMEB seems to be a possible bile-substituting agent.

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