INFLUENCE OF CYCLODEXTRINS ON NITROSATION OF DRUGS

K.-H. Frömming, V. Wedelich, W. Mehnert
Pharmaceutical Institute of the Free University of Berlin
Königin-Luise-Str. 2+4, 1000 Berlin 33, FRG

ABSTRACT. The World Health Organization issued a nitrosation procedure (NAP Test) which allows to carry out nitrosation under standard conditions. It has proved that the in vitro reaction rates of the fast nitrosatable drugs piperazine, cimetidine and ethambutol are not influenced by α-, ß- and γ-cyclodextrin. On the contrary, ß-, γ-cyclodextrin and heptakis-2,6-di-O-methyl-ß-cyclodextrin enhance the nitrosation of the slower nitrosatable l-ephedrine and fencamfamine significantly. This possible reaction must be considered if nitrosatable drugs are formulated with cyclodextrins to be administered to human beings.

Key words: Nitrosation reactions: α-, ß-, γ-cyclodextrin, dimethyl-ß-cyclodextrin; piperazine; ethambutol; cimetidine; l-ephedrine; fencamfamine.

1. Introduction

As solid inclusion compounds, unstable drug molecules can be stabilized. Dissolved cyclodextrins (CDs) influence the chemical stability of drugs in a different way. Hydrolytic or oxidative reactions can be accelerated or inhibited.

No experiments are known as to how far nitrosation of amine drugs can be influenced by CDs. Nitrosamines as reaction products can have carcinogenic effects. The in vivo formation of nitrosamines is theoretically possible with all compounds containing nitrosatable amino groups if conditions are favourable for such reactions. Nitrate is a normal constituent of human saliva; its concentration depends largely on the nitrate intake in food.

In 1978 the WHO issued an in vitro Nitrosation Assay Procedure (NAP Test) which allows to carry out nitrosation under standard conditions (1). This NAP Test should be used preferentially as a reference test to discriminate between compounds which represent a potential risk and the majority of drugs with low reactivity.

It was our purpose to examine whether the in vitro nitrosation

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reaction rate of some drugs can be influenced by α-, ß- and γ-CDs. In some cases a simple methyl derivative, heptakis-(2,6-di-O-methyl)-ß-CD (2,6-DIMEB) has been used, too.

2. Materials and Methods

α-cyclodextrin: Aldrich Europe, Beerse; ß-cyclodextrin: Chinoin Co., Budapest; γ-cyclodextrin: Nihon Shokuhin Kako Co., Tokyo. Heptakis-(2,6-di-O-methyl)-ß-cyclodextrin (2,6-DIMEB): According to Szejtli et al. (2). Fp 311°. The NMR spectrum corresponds to the specifications given by Casu et al. (3). Piperazine: Ferak Co., Berlin; Ethambutol dihydrochloride: Cyanamid GmbH, Wolfratshausen; Cimetidine: Smith Kline & French Labor. Ltd., Welwyn Garden City; l-ephedrine sulfate: Serva Co., Heidelberg; Fencamfamine hydrochloride: E. Merck Co., Darmstadt. ^{1}H-NMR spectra were taken on a 250 MHz Bruker spectrometer WM 250 (Bruker Co., Karlsruhe).

Nitrosation procedures

The nitrosation conditions are derived from NAP Test (tab. I).

Table I. Nitrosation Conditions

	Drug (mmol/l)	Molar Ratio Drug:CD	Nitrite (mmol/l)	pH
NAP Test	10	-	40	3-4
Piperazine	10	1:2	40	3,0
Ethambutol	10	1:2	40	3,1
Cimetidine	5	1:1	20	1,1
l-Ephedrine	20	1:1	40	3,2
Fencamfamine	20	1:1	40	3,6

In the case of ephedrine and fencamfamine the drug:nitrite molar ratios were enhanced to increase the nitrosation rate.

Piperazine:

Solution I: piperazine 0,9475 g, HCl (37%), 3,0 ml. water to 1000 ml; solution II: sodium nitrite 3,036 g to 100 ml water. In all experiments distilled water is used. 0,200 ml solution II (37°) are transferred to 2,0 ml solution I (37°) in a small screwed 4,5-ml plastic tube and mixed vigorously. At 0,5, 1, 2, 4, 6, 8, 10 and 20 min 0,200 ml-samples are withdrawn and transferred to a 25-ml volumetric flask which contains 0,200 ml potassium hydroxide solution (2%) in 10 ml water. After dilution to volume with water the samples are analyzed spectrophotometrically at the absorption maximum of nitrosopiperazine at 240 nm.

In the experiments with CDs 2,0 ml solution I contained 42,8 mg α-CD, 50,0 mg ß-CD, and 57,1 mg γ-CD, respectively.

Ethambutol:

As described for piperazine. Solution I contains 3,0492 g ethambutol dihydrochloride in 1000 ml water, acidified with 1 ml HCl (37%). 10 samples are withdrawn between 0,5 and 60 min. The content of dinitroso-ethambutol is assayed spectrophotometrically at 240 nm.

Cimetidine:

200 µl of a solution of 1,656 g sodium nitrite in 100 ml water (37°) are added to 2 ml of a solution of 1,5144 g cimetidine in 1000 ml water (37°) which is in a cuvette. By adding of 0,200 ml 1 N HCl the reaction is started. The absorption of nitrosocimetidine is determined at 305 nm between 1.5 and 140 min (19 samples). For the preparation of CD containing reaction mixtures the following amounts are weighed into the empty cuvette: 11,7 mg α-CD, 13,6 mg ß-CD, 15,6 mg γ-CD, 16,0 mg 2,6-DIMEB, respectively.

l-Ephedrine:

As described for piperazine. Solution I contains 4,7135 g l-ephedrine sulfate in 1000 ml water, acidified with 1,0 ml HCl (37%). 9 samples are withdrawn between 0.5 and 240 min and determined spectrophotometrically at 240 nm.

Fencamfamine:

As described for piperazine. Solution I contains 2,7697 g fencamfamine hydrochloride in 500 ml water, acidified with 0,5 ml HCl (37%). Solution II contains 0,200 ml 3,036 per cent aqueous sodium nitrite solution. The volumetric flask contains 0,200 ml potassium hydroxide solution (0,3%). Because of the formation of a badly soluble precipitate in presence of γ-CD the corresponding sample for the assay of the nitroso-compound has the following composition: methanol 10,0 ml, potassium hydroxide solution (0,3%) 0,200 ml, reaction solution 0,200 ml, water to 25,0 ml. 9 samples are withdrawn between 0,5 and 240 min and assayed spectrophotometrically at 240 nm.

Nitroso-fencamfamine-γ-cyclodextrin:

As described for fencamfamine. A sample with 57,1 mg γ-CD is nitrosated for 180 min. The plastic tube is centrifuged for 3 min and the liquid phase withdrawn. The precipitate is dried at 40° on filter paper. Yield 11,2 mg. In a solution of deuterated DMSO the substance could be identified by NMR as a N-nitrosofencamfamine-γ-CD inclusion compound.

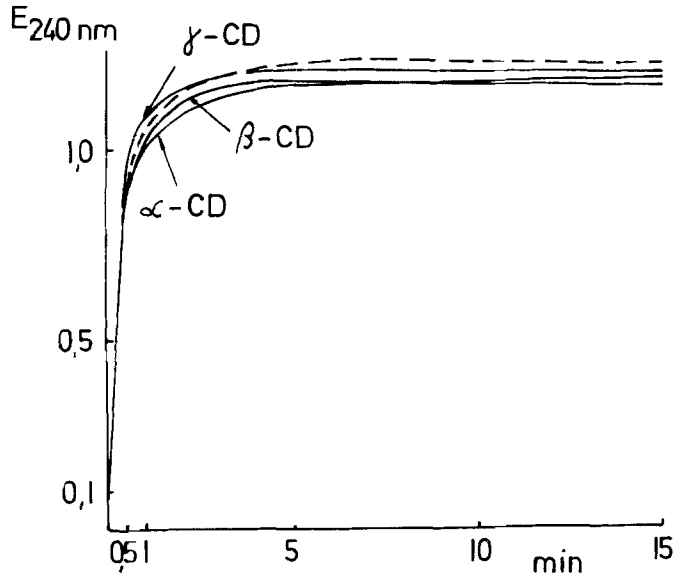

fig. 1: In vitro nitrosation of piperazine in presence of cyclodextrins

3. Results

The nitrosation behaviours of piperazine, ethambutol, cimetidine, l-ephedrine and fencamfamine without CDs and in presence of CDs were determined.

3.1. Piperazine, ethambutol, cimetidine

Piperazine (4), ethambutol (4) and cimetidine (5) can form nitroso-compounds in a fast reaction. α-, ß- and γ-CD and 2,6-DIMEB (for cimetidine) did not influence these reactions. This is shown in fig. 1 for piperazine. The pH values of these reactions were between 3 and 4. Only the NAP Test with cimetidine was carried out at pH 1,1 because the reaction rate decreases very much with increasing pH values (5).

Piperazine can form a soluble ß-CD inclusion compound which could be proved by NMR spectroscopy. The protons H-3 and H-5 which are located in the interior of the cavity show a high-field shift whereas the protons at the outside are not affected. Only weak interactions can be seen with α-CD, no interactions are observed with γ-CD. A determination of inclusion formation between ethambutol and CDs was not possible because of overlapping of proton signals. The strong hydrophilic protonated ring of cimetidine does not form an inclusion compound with the three CDs.

Different behaviours could be observed with l-ephedrine and fencamfamine.

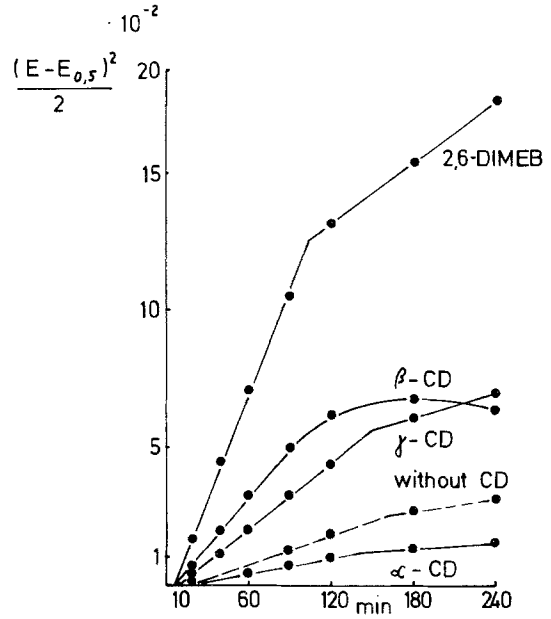

fig. 2: In vitro nitrosation of ephedrine in presence of cyclodextrins

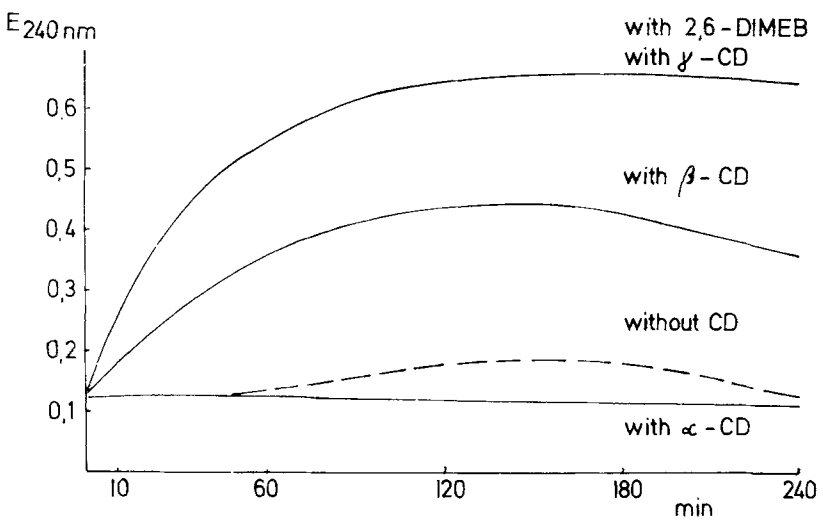

fig. 3: In vitro nitrosation of fencamfamine in presence of cyclodextrins

3.2. l-Ephedrine

Contrary to the results with the three other drugs, the nitrosation of ephedrine to nitrosoephedrine takes place slowly. After 24 hours the reaction was not yet finished. Addition of CDs resulted in remarkable changes of reaction rates. α-CD decelerated the nitrosation rate whereas ß-, γ-CD and 2,6-DIMEB increased the reactions remarkably. 2,6-DIMEB has the greatest effect (fig. 2).

The increase of the initial reaction rates was in the following relative order:

$$k_{\alpha} : k_{without} < k_{\gamma} < k_{\beta} < k_{DIMEB} = 0{,}6 : 1 : 2{,}3 : 3{,}4 : 7{,}4$$

The maximum formed nitrosamine concentrations are different, too.

ß-CD did not only increase the nitrosation of ephedrine, but also favoured the decomposition of the reaction product. During the first 90 minutes all reactions follow a first order process. After this time the reaction in presence of ß-CD gets nonlinear. The formation of a small amount of a precipitate could be observed.

All reaction curves were highly significantly different at all sampling times: $p < 0{,}001$, except 10 min (without CD/α-CD), 180 and 240 min (ß-/γ-CD), 6 determinations.

l-ephedrine forms a soluble ß-CD inclusion compound (6). By NMR spectroscopy we could prove the formation of soluble inclusion compounds with ß-, γ-CD and with 2,6-DIMEB, but not with α-CD.

3.3. Fencamfamine

Nitrosation of fencamfamine without CDs starts slowly and proceeds only with a small reaction rate. Maximum absorption values are reached after about 150 minutes. From this time a faster decomposition reaction superimposes the nitrosation (fig.3). Presence of α-CD inhibits the nitrosation completely. The other CDs increased the nitrosation considerably; ß-CD has the smallest, γ- and 2,6-DIMEB have the greatest effects. In presence of γ-CD the formation of a precipitate was observed. This could be identified as inclusion compound of nitrosofencamfamine.

The hydrophobic fencamfamine forms soluble inclusion compounds with ß-, γ-CD and 2,6-DIMEB, not with the α-isomer. According to the NMR spectrum the fencamfamine complexes with ß-CD and 2,6-DIMEB are especially stable.

4. Conclusion:

Addition of CDs cannot generally avoid or inhibit the in vitro nitrosation of the five drugs. The reaction rates of the fast nitrosatable drugs piperazine, ethambutol and cimetidine are not influenced by the three CDs. But, ß-, γ-CD and 2,6-DIMEB catalyze significantly the nitrosation of the slower nitrosatable ephedrine and fencamfamine. This must be taken into consideration if nitrosatable drugs should be administered with CDs. Some inhibitory effect could be seen in these

two cases with α-CD, but there is no relation with inclusion formation of drug molecules.

We have to assume that the nitrosatable amino groups of the five drugs are not located in the cavity of the CD ring. Because of the location outside of the cavity the nitrosation process can proceed in presence of CDs.

Beyond it, the nitrosation rate can be enhanced if an inclusion between CD and the nitroso-product is possible.

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