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Absorption and Excretion of Tablet Disintegrating β-Cyclodextrin Polymer in Rats

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SUMMARY

Absorption and excretion of a new tablet disintegrating agent, a cross-linked β-cyclodextrin polymer was investigated following per os *administration in the rat. The polymer, which is insoluble but swells in water, was prepared from β-cyclodextrin by reacting with [2-^{14}C]epichlorohydrin in an alkaline medium. Radioactivity of blood, urine, faeces, exhaled carbon dioxide and the gastrointestinal tract was determined by a liquid scintillation method. No radioactivity could be detected in the blood up to 24 h after the administration of the polymer. Radioactivity of urine and exhaled carbon dioxide together did not exceed 0·11% of the total administered radioactivity, 98% of which was found in the large intestine and the faeces. Therefore, it is assumed that β-cyclodextrin polymer could not be absorbed from the gastrointestinal tract.*

INTRODUCTION

Cyclodextrins (Bender & Komiyama, 1978; Saenger, 1980; Szejtli, 1982) are torus-shaped oligosaccharides, prepared by enzymatic degradation. They have the unusual property of forming inclusion complexes and so can be widely used in the drug and food industry for molecular encapsulation of many compounds. Molecular encapsula-

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tion is not the only field of possible application for cyclodextrins. They can be readily crosslinked through their hydroxyl groups producing cyclodextrin polymers of different types and properties (Zsadon & Fenyvesi, 1982). One of the β-cyclodextrin polymers, prepared by crosslinking with epichlorohydrin, can be used as a tablet-disintegrating agent due to its rapid and high swelling capacity (Szejtli *et al.*, 1981; Fenyvesi *et al.*, 1984). For such an application the crucial question is whether or not this polymer is absorbed from the gastrointestinal tract. The aim of the present work was to investigate the absorption and excretion of β-cyclodextrin polymer by rats after *per os* administration. According to previous investigations (Anderson *et al.*, 1963; Gerlóczy *et al.*, 1985), β-cyclodextrin itself was metabolized in the rat at a somewhat reduced rate compared with starch and glucose.

MATERIALS AND METHODS

Source of materials

Hyamine 10X hydroxide, Soluene-100 and Insta Gel were purchased from the Packard Instrument Co., Inc, Illinois, USA.

Preparation of β-cyclodextrin polymer

β-cyclodextrin polymer was prepared with [2-^{14}C]epichlorohydrin (Amersham International, UK) according to the method of Fenyvesi *et al.* (1983); β-cyclodextrin was dissolved in an aqueous alkaline solution, and epichlorohydrin and tetraethyleneglycol was added. The stirring of the reaction mixture was continued till gelation. The gel obtained was neutralized with hydrochloric acid solution and desalted by washing with distilled water. Specific radioactivity of the dry polymer was 11·269 kBq mg.$^{-1}$

Treatment of animals

Wistar rats (five male, six female) were administered with 401·8 mg kg^{-1} ^{14}C-labelled β-cyclodextrin polymer *per os*, using an oesophagus tube. The 400 mg kg^{-1} dose level was chosen as it was the mean dose applied

in the chronic toxicity studies of β-cyclodextrin performed on rats (Gergely *et al.*, 1982). (The doses applied were 200, 400 and 600 mg kg^{-1}, respectively.) The polymer was suspended in 10% aqueous solution of dextran in a dose volume of 2 ml. Animals were starved for 24 h before treatment and during the experimental period as well, water was provided *ad libitum*. Animals were kept in metabolic cages. Blood samples of 100 μl each were withdrawn from the tail vein of rats at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after treatment. Urine and faeces were collected for 24 h. Radioactivity of the exhaled CO_2 was determined from a single animal. Exhaled CO_2 was bubbled across three gas traps containing Hyamine 10X hydroxide:methanol solutions (1:2, 1:5, 1:10 v/v, respectively) of 50 ml each. Twenty-four hours after treatment animals were dissected and the radioactivity of the gastrointestinal tract was determined.

Assay of samples for radioactivity

Blood samples: Samples of 100 μl were solubilized in 1·5 ml of Soluene-100:isopropanol = 1:1 (v/v) solution and were bleached with 0·3 ml 30% hydrogen peroxide for 30 min at room temperature. Then 10 ml of Insta Gel:0·5 M HCl = 1:1 (v/v) solution was added to the samples.

Urine samples: Samples of 20–40 μl urine were pipetted into 10 ml Insta Gel.

Faeces samples: Faeces were homogenized in 50 ml of 30% isopropanol, then a 0·5 ml aliquot was pipetted into 6 ml Insta Gel.

Exhaled carbon dioxide: Samples of 0·5, 1 and 2 ml Hyamine 10X hydroxide:methanol solutions were pipetted into 10 ml Insta Gel.

Gastrointestinal content: The stomach, the small intestine and the large intestine were rinsed three times with physiological saline. The total volume was 40, 40 and 50 ml, respectively. In order to gain a 30% isopropanol solution, the appropriate amount of isopropanol was added. Rinsing fluids were homogenized and a 0·5 ml aliquot of them was combined with 10 ml Insta Gel.

Stomach and intestine walls: Wet stomach, small intestine and large intestine were weighed and 40 to 50 mg samples were taken. Soluene-100 was added to each tissue sample at 0·01 ml mg^{-1} ratio. After 24 h incubation at room temperature, the completely lysed samples were combined with the corresponding amount of isopropanol, then samples

were bleached with 0·1 ml 30% hydrogen peroxide for 30 min. Nine millilitres of Insta Gel:0·5 M HCl (9:1) mixture was added to the samples.

Samples were incubated in the dark for 24 h, at room temperature, to eliminate the effect of light. After the incubation, the samples were assayed for radioactivity in a Nuclear Chicago Mark III 6880 liquid scintillation counter.

Sensitivity of blood radioactivity measurements

Double the level of the average blood background radioactivity (107 dpm), namely 214 dpm, can be detected with confidence in 100 μl of blood withdrawn from the tail vein of rats. If the detected radioactivity is below 214 dpm it is not certain if it originated from the substance absorbed or if it is due to the background radioactivity. Thus, if 100 μl of blood contains 214 dpm, 1 ml of blood contains 2140 dpm radioactivity, and 2140 dpm is 0·053% of the total administered radioactivity of 40 751 499 dpm. When 60·27 mg of polymer (with a radioactivity content of 40 751 499 dpm) is administered to rats, then 0·053% is equivalent to a weight of 3·19 μg. That is to say levels of less than 3·19 μg polymer in 1 ml of blood (3 μg polymer in 1 g of blood, i.e. 3 ppm) cannot be detected with confidence. Consequently, the sensitivity of our method is 3 ppm.

RESULTS AND DISCUSSION

In the first experiment Wistar rats (two male and three female, serial No. of animals: 1, 2, 3, 4 and 11) with an average weight of 200 g were administered with 401·8 mg kg^{-1} (80·36 mg per animal, 54 335 333 dpm per animal = 902·8 kBq per animal) ^{14}C-labelled β-cyclodextrin polymer *per os*. In the second experiment, three male and three female rats (serial No. of rats: 5, 6, 7, 8, 9, 10) with an average weight of 150 g were administered with 401·8 mg kg^{-1} (60·27 mg per animal, 40 751 499 dpm per animal = 679·2 kBq per animal) ^{14}C-labelled polymer *per os*. Animals Nos 1 and 2, Nos 3 and 4, Nos 5, 6 and 7 and Nos 8, 9 and 10 were placed in the same cage, respectively, while animal No. 11 was kept alone in a metabolic cage equipped with three gas traps for the collection of exhaled carbon dioxide. Radioactivity level in the blood of animals Nos 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 was measured at 1, 2, 3, 4,

5, 6, 8, 10, 12 and 24 h after administration. The individual radioactivity values relating to 10 ml blood were expressed as % of the total administered radioactivity. No radioactivity could be detected in blood during the 24-h observation period. As the sensitivity of our method was 3 ppm, it can be concluded that after *per os* administration of 401·8 mg kg^{-1} β-cyclodextrin polymer, less than 3 ppm polymer may be absorbed if it is absorbed at all, from the gastrointestinal tract.

Further, in order to account for the total administered radioactivity, urine and faeces were collected in the metabolic cages for 24 h, and in the case of one rat (serial No. 11) the radioactivity of the exhaled carbon dioxide was measured. At the end of the experiment, rats were dissected and the radioactivity of the gastrointestinal tract was determined. The radioactivity values of urine, faeces, exhaled carbon dioxide, stomach and small and large intestine are shown in Table 1. Radioactivity values are expressed as a percentage of the total administered radioactivity. Practically no radioactivity could be detected in the stomach at 24 h after administration of the polymer. A very small amount of radioactivity (0·17% of the total administered radioactivity) was detected in the small intestine. Almost the whole of the total administered radioactivity was found in the large intestine and the faeces (58·29% and 39·97%, respectively). Urine contained 0·04% of the administered radioactivity, while 0·07% was found in the exhaled carbon dioxide. In total, 98·5% of the administered radioactivity was accounted for.

These results show that β-cyclodextrin polymer was not absorbed from the stomach and the small intestine. It is very probable that there is no absorption from the large intestine (as a result of the digestion of the polymer by bacteria) as well, since a considerable amount of the radioactive material is in the large intestine at 10–12 h following *per os* administration to starved rats. Gerlóczy *et al.* (1984) found that approximately 50% of radioactive β-cyclodextrin was in the large intestine 10 h after *per os* administration of a 300 mg kg^{-1} dose. However, the possibility that a very limited amount of the degradation products of the polymer is absorbed between the 24th and 48th hours following the *per os* treatment cannot be eliminated.

The small amount of radioactivity in the urine and the exhaled carbon dioxide (0·11% together) can be explained by the fact that it is practically impossible to prepare a radioactive material with 100% purity.

TABLE 1

Distribution of Radioactivity in Rats 24 h after *per os* Administration of 401·8 mg kg^{-1} ^{14}C-Labelled β-Cyclodextrin Polymer

	Radioactivity (%)[a]											
	Serial No. of rats											
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*[b]	$\bar{X} \pm SD$
Stomach (wall and content together)	0·00	0·00	0·05	0·02	0·00	0·01	0·00	0·06	0·00	0·02	0·03	0·02 ± 0·05
Small intestine (wall and content together)	0·67	0·09	0·07	0·00	0·10	0·12	0·08	0·08	0·45	0·06	0·11	0·17 ± 0·13
Large intestine (wall and content together)	37·71	65·54	59·93	63·49	62·83	58·15	59·62	64·56	51·22	60·35	55·80	58·29 ± 8·31
Urine	0·03	0·03	0·04	0·04	0·04	0·04	0·04	0·04	0·04	0·04	0·10	0·04 ± 0·005
Faeces[c]	42·82	42·82	37·41	37·41	40·12	40·12	40·12	39·61	39·61	39·61	–	39·97 ± 1·82
Exhaled CO_2	–	–	–	–	–	–	–	–	–	–	0·07	–
Total	81·23	108·48	97·50	100·96	103·09	98·44	99·86	104·35	91·32	100·08	98·5	98·53 ± 7·57

[a] Radioactivity is expressed as % of total administered radioactivity.

[b] Faeces of animal No. 11 was lost so this animal is not included in $\bar{X}$

[c] Faeces of animals Nos 1 and 2, 3 and 4, 5, 6 and 7, 8, 9 and 10 were collected and analysed together.

It is probable that β-cyclodextrin polymer has no toxic effects. Long term toxicity studies of β-cyclodextrin (Gergely *et al.*, 1982) proved that β-cyclodextrin was not toxic orally. Consequently, the polymer would be toxic only if absorbed in its intact form; and the radioactivity measurements suggest that this is not the case.

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