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Gastrointestinal Absorption of [^{14}C]Poly(ethylene glycol) 4000

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ABSTRACT: Gastrointestinal absorption behavior of [^{14}C]poly(ethylene glycol) (PEG) 4000 was studied by mice. The absorption of the polymer 1 h after the oral administration was 2%-dose, though its appearance in the urine and the blood is few. On the other hand, the injection of the PEG into the duodenal ligated loop under anesthesia resulted in as high as 15%-dose absorption/h. Intraduodenal administration study revealed that the long retention of the polymer in the upper regions in the small intestines is necessary to its high absorption, and the poor absorption of the PEG seen after the oral administration seems to be brought about by its rapid transit and short retention in the upper intestines.

KEY WORDS Poly(ethylene glycol) / Intestinal Absorption / Loop / Body Distribution / Radioisotopes /

Polymeric drugs comprising a macromolecular carrier combined with drugs of small molecule have been shown to offer great advantages in the chemotherapy. A polymeric carrier prevents the glomerular filtration causing a long duration of the drugs in the blood stream. It decreases the non-specific entrance of the drugs into undesired cells by random diffusion. The administration route of the polymeric drug-carrying system is, however, strictly limited to an injection by a syringe or an instillation by drip infusion because of the poor absorption ability of the macromolecules through a gastrointestinal tract or a skin.

Poly(ethylene glycol) (PEG) is a chemically stable polymer, and has been known to be non-toxic, non-antigenic, and biologically inert. PEG is thus accepted to be a suitable drug-carrier for a certain use. This polymer, however, has also been reported to be scarcely absorbed from the intestines if its molecular weight is over a thousand.[1,2] These reports showed that the recovery of PEGs in the urine after oral administration is very few; below 2%-dose for rats,[1] and 1.3%-dose for men.[2] PEGs of molecular weight 1000—4000 are known to be rapidly excreted from the blood stream into the urine, and the gastrointestinal absorption of PEGs were evaluated to be very small by measuring their little appearance in the urine in those papers. But, it is not clarified whether PEGs absorbed from the intestines would be soon delivered into the blood stream or not, though it is premised on the evaluation.

Those experiments were, moreover, carried out by relatively high dose rate, 25 mg/rat or 5 g/man so as to facilitate the quantitative analysis of the urine by the chromatographic method.

By utilizing [^{14}C]-radiolabeled PEG, removal of the polymer from the gastrointestinal tract can be directly determined by measuring the decrease of radioactivity of the intestines, and very small amount of PEG becomes traceable.

Here we would like to show the absorption behavior of [^{14}C]PEG 4000 from the intes-

tines, containing the effect of the dosage.

EXPERIMENTAL

[^{14}C]PEG 4000 (555 MBq/g; radiochemical purity: 99.8%) was purchased from Amersham Co., Japan Ltd. Male ddY mice weighing about 30 g (6—7 weeks old) were fasted overnight prior to experiments. Water was provided *ad libitum*.

Intestinal Absorption and Body Distribution of [^{14}C]PEG 4000 after Oral Administration

[^{14}C]PEG 4000 (8 μg) in 0.4 ml of phosphate-buffered saline (PBS: pH 7.4) was administered into the stomach of the mice using a narrow metallic tube with a round-tip end. After 1 h, mice were killed and the stomach and the whole intestines were removed. The intestines were sectioned into small pieces to facilitate the radio assay.

The stomach and the intestines were burned up in the oxygen, and generated CO_2 was absorbed by monoethanolamine, which was assayed for the radioactivity with a liquid scintillation counter (Aloka LSC-3600). Absorption ratio was estimated from the difference between the administered and recovered radioactivities. For measuring the body distribution of the PEG after the oral administration, 20—30 μg of the polymer in 0.2 ml of PBS was administered. After a given time, the organs were removed and similarly radio-assayed.

Absorption of [^{14}C]PEG 4000 from the Duodenojejunal Loop

The mice were anesthetized by urethane (60 mg/mouse; i.p.), and the duodenum was exposed by abdominal incision. The 1 cm closed loop was prepared by ligation of each end. [^{14}C]PEG 4000 dissolved in 40 μl of phosphate-buffered saline was then injected into the loop with a small syringe. The loop was put back in the peritoneal cavity, and the incision was sutured. The loop was removed after a given time, and the absorption of the PEG was determined by assaying the radioactivity of the loop.

Absorption of [^{14}C]PEG 4000 from the Intestines after Intraduodenal Injection

The mice were anesthetized by pentobarbital sodium (2 mg/mouse; i.p.), and the duodenum was exposed by abdominal incision, to which [^{14}C]PEG 4000 in 50 μl of phosphate-buffered saline was injected by a syringe. After 2 h, the stomach and the intestines were removed, and sectioned into seven pieces; the stomach, the duodenum, the upper and lower jejuna, the upper and lower ilea, the cecum, and the colon, and each part was radio-assayed separately.

RESULTS

[^{14}C]PEG 4000 (8 μg) was orally administered to mice, and its absorption from the gastrointestines was measured.

After 1 h, 2.3 ± 0.6% of the polymer disappeared from the gastrointestines, while no radioactivity was recovered in the feces.

Body distribution of the PEG 4000 after oral administration was then studied using slightly larger amount (20—30 μg) of the polymer so as to detect the very small fraction of the polymer in the tissues. Radioactivity recovered in the blood was 0.096 ± 0.023%-dose/ml after 1 h. That appeared in the urine 1 and 18 h after the administration was 0.31 ± 0.02 and 0.73 ± 0.15%-dose, respectively. Relatively high radioactivity was recovered in the liver, the mesenteric lymph nodes, and the kidney, and the accumulation of more than 0.01%-dose was observed in no other tissues containing spleen, lung, brain, and heart (Figure 1). The radioactivity recovered in the muscle was also as low as 0.0097 ± 0.0013%-dose/g-tissue.

These results show that the absorption fraction of the poly(ethylene glycol) may not be estimated by its appearance in the urine nor in the blood stream.

In order to directly determine the absorption

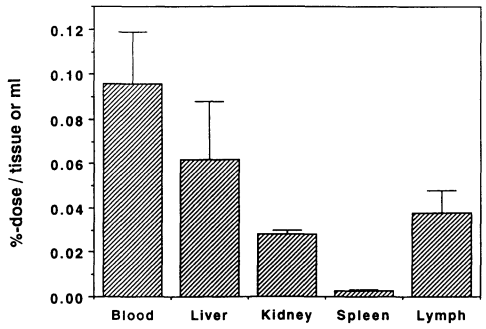

Figure 1. Body distribution of [^{14}C]PEG 4000 1 h after the oral administration (mean ± standard error).

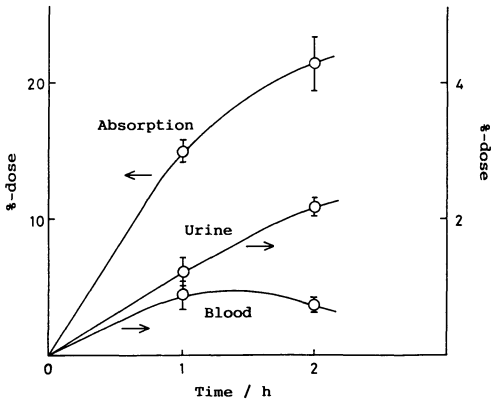

Figure 2. Absorption of [^{14}C]PEG 4000 from the duodenojejunal loop and its appearance in the blood and the urine (dose: 1.7 μg/mouse).

behavior of [^{14}C]PEG 4000 from the intestines, the *in situ* loop study was carried out. Absorption and the appearance of the polymer in the blood and the urine were illustrated in Figure 2, where shown the relatively higher absorption rate (15 and 21%-dose after 1 and 2 h, respectively) than expected by the result of the oral study. Radioactivity recovered in the urine was 1.2%-dose after 1 h, and was also higher than after the oral administration, but still much lower than the absorbed fraction.

Possibility of a leakage of the PEG solution from the loop out to the peritoneal cavity through the pinhole which may be made by the injection should be considered. The intraperitoneal administration study was then performed. The loop of the duodenum was

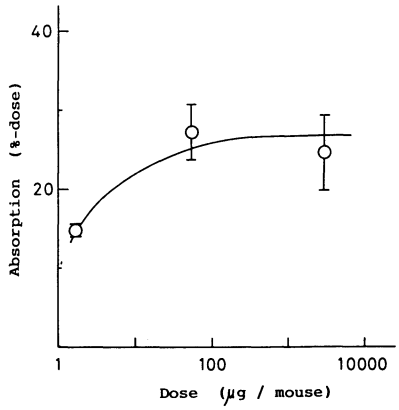

Figure 3. Correlation between absorption and the dose of [^{14}C]PEG 4000 administered into the dudenojejunal loop.

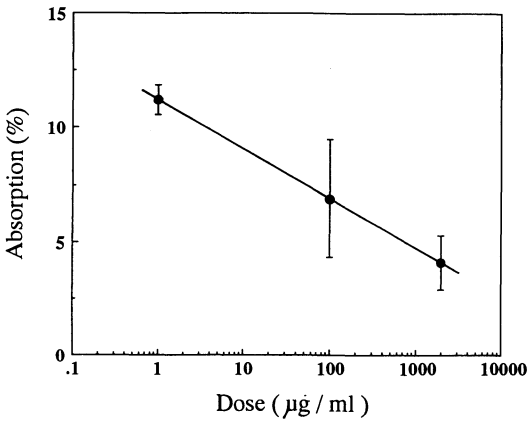

Figure 4. Absorption of [^{14}C]PEG 4000 2 h after the intraduodenal administration.

prepared similarly to the loop study, and the PEG solution was poured into the peritoneal cavity around the loop (not into the loop).

Radioactivity recovered in the urine after 1 h was 22 ± 5%-dose, showing efficient delivery and excretion of the PEG from the peritoneal cavity to the urine. This shows an impossibility of the attribution of the high absorption in the loop study only to the leakage of the PEG solution out of the loop judging from the low radioactivity in the urine after the injection of PEG into the loop.

The effect of the dose on the absorbance was tested by the similar loop method. As shown

in Figure 3, the absorption slightly increased with the increasing dose, and no active transporting manner appeared.

The absorption behavior of the PEG from the intestines was then investigated by the intraduodenal injection study; the polymer was directly injected to the duodenum of mice anesthetized by pentobarbital, and after 2 h,

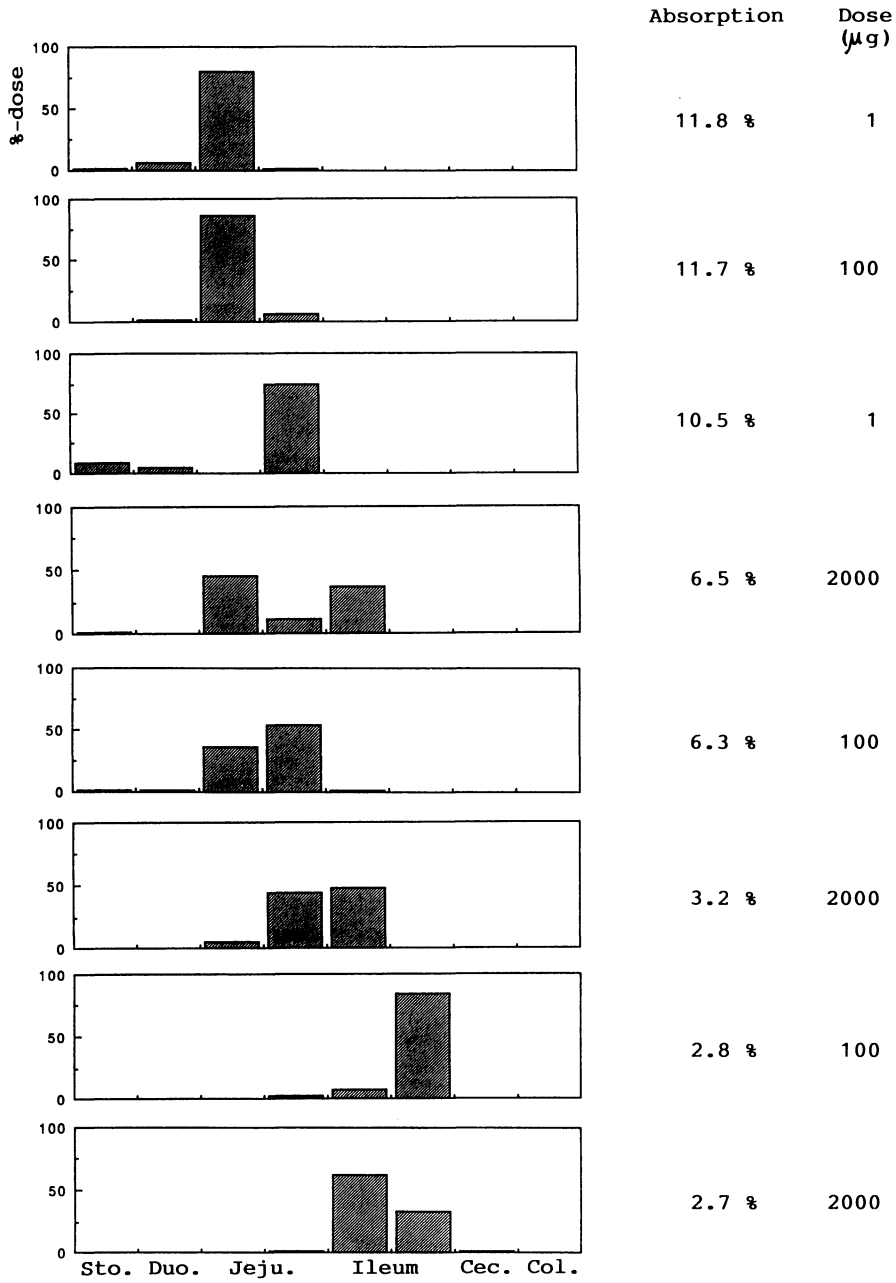

Figure 5. Distribution of [^{14}C]PEG 4000 in the gastrointestines and its absorption 2 h after the intraduodenal administration.

each section of the gastrointestines was radio-assayed. The absorption fraction was determined from the difference between the dose and the recovery in the whole gastrointestines. The correlation between the dose and the absorption was shown in Figure 4. The absorption fraction after the intraduodenal injection decreased with the dose contrary to the data obtained by the loop study (Figure 3).

The distribution of the radioactivity along the gastrointestines was then illustrated for individual mouse, and ranged according to the absorption fraction (Figure 5). Good correlation between the absorption and the distribution of the polymer in the intestines can be seen in Figure 5. The absorption of PEG seems to much more depend on its transit in the intestines than the dosed amount, and retention in the upper rigions of the small intestines appears to bring about the high absorption of the polymer.

DISCUSSION

Oral Administration Study

Gastrointestinal absorption rate of macromolecules such as PEG and dextran has often been estimated by assaying the appearance of the polymer in the urine, because of its convenience. In this study, we employed [^{14}C]labeled PEG 4000 which allows the direct and quantitative analysis even for the very small amount ($<0.005\,\mu$g) of the polymer. First we examined the absorption of the [^{14}C]PEG 4000 after oral administration at a dose of $8\,\mu$g/mouse. As high as 2.3% of the dosed radioactivity disappeared in 1 h, which is already slightly larger than the fractions reported to be recovered in the urine 6 h after the oral administration to rats.[1]

Body distribution of the PEG after the oral administration (dosed at 20—30 μg/mouse) was then examined. The radioactivity recovered in the blood was very low (0.1%-dose), and the appearance of the polymer in the urine was also as few as 0.3 and 0.7%-dose after 1 and 18 h, respectively. The rapid and efficient clearance of PEGs from the blood stream into the urine has been reported.[1,3] [^{14}C]PEG 4000 was also very quickly removed from the blood. The polymer remained in the blood only 2%-dose/ml 15 min after the intravenous injection. This fast blood clearance may also be attributed to the low concentration of the PEG in the blood after oral administration. The radioactivity recovered in the urine after the oral administration was indeed much higher than that in the blood, but was still smaller than the disappearing fraction as mentioned above.

Macromolecules are known to be often delivered into the lymph instead of the blood after the intestinal absorption.[4] The mesenteric lymph nodes were then collected, and were radioassayed. Radioactivity of the lymph nodes was 0.038 ± 0.010%-dose, being as high as that in the same weight of the blood. These results also indicate that the PEG absorbed from the intestines would not be soon all delivered into the blood stream, but partly into the lymph and may be dispersed in the whole body, which would cause the low concentration of the polymer in the urine.

Loop Study

Duodenal ligated loop was prepared, and the PEG 4000 ($1.7\,\mu$g) was injected into the loop. The absorption was determined by measuring the radioactivity of the closed loop remained after a given time. The absorption rate of PEG from the duodenal loop was much higher than expected by the oral administration study, and 15 and 20% of the dosed radioactivity disappeared from the loop after 1 and 2 h, respectively. Recovery of the PEG in the tissue or the urine was also much lower than the absorbed amount.

An apprehension of leakage of the PEG solution out of the loop through the pinhole which might be made by the injection syringe should be concerned. The ligated loop was then prepared similarly to the loop study and put

back in the peritoneal cavity, and the PEG solution was not injected into the loop but poured around the loop. Relatively high fraction (22%) was recovered in the urine after 1 h. If the disappearance of PEG from the loop was caused only by the leakage through the pinhole, the 15% absorption at 1 h post injection should have resulted in over 3% recovery in the urine. The experimental data in the loop study ($1.2 \pm 0.4\%$ in the urine) would seem to deny the above possibility and the disappearance of PEG is thought to be due to the absorption of the polymer through the intestinal epithelia.

Dose dependence on the absorption of the polymer from the loop was investigated. Absorption increased from 15 to 25%-dose/h as the dose increased from 2 μg to >50 μg. This relatively higher absorption at high doses seems to be due to the histopathological change in the epithelium by the concentrated solution of PEG as was reported for the poly(acrylic acid) gel,[6] which made a higher absorption of phenol red from the polymer-treated rectal mucosa in rats.

Intraduodenal Injection Study

The absorption study by the ligated loops is known to sometimes result in the enhanced absorption ability than in physiological conditions.[5] [^{14}C]PEG 4000 was then injected into the intact duodenum of the mice anesthetized by pentobarbital.

Figure 4 shows the relationship between the dose and the absorption fraction of the PEG 2 h after the injection. The relatively high absorption from the intestines of the PEG was seen with a small dose, and negative correlation was observed between the absorption and the dose in contrast to the loop study. Radioactivity in each part of the intestines was illustrated for individual mouse, and ranged by the absorption fraction in Figure 5. In those graphs, good correlation can be seen between the absorption and the intestinal transit of the polymer.

For the mice in which the transit of the PEG through the intestines was slow and the polymer remains in the upper regions of the small intestines, relatively high absorption was observed almost regardless of the dose. And higher dose tends to transit rapidly in the gut, giving rise to the small absorption fraction, and also the negative correlation in Figure 4.

These experiments showed that PEG 4000 would potentially have an absorbability from the small intestines itself, but the absorption rate is not so high that the fairly long retention of the polymer in the upper regions of the small intestines, where facile absorption would be expected, is necessary for its high absorption.

Relatively high absorption seen in the loop study may be attributed to the long time-contact of the polymer with the duodenal surfaces in addition to the not physiological conditions of the ligated loops.

In summary, when [^{14}C]PEG 4000 was orally administered, 2.3% of the polymer was absorbed from the gastrointestines after 1 h, though its appearance in the blood and the urine is very few. The polymer was, however, absorbed in much higher ratio from the ligated duodenal loops. Intraduodenal injection study under anesthesia revealed that gastrointestinal transit of the PEG much affected its absorption, and very low dose (1 μg/mouse) into the duodenum resulted in the high retention of the polymar in the upper regions of the small intestines and also in high absorption ($>10\%$/2h).

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REFERENCES

1. M. D. Donovan, G. L. Flynn, and G. L. Amidon, *Pharm. Res.*, **7**, 863 (1990).

2. P. G. Jackson, M. H. Lessof, R. W. R. Baker, J. Ferrett, and D. M. MacDonald, *Lancet*, 1285 (1981).
3. D. J. Larwood and F. C. Szoka, *J. Labelled Compounds and Radiopharm.*, **21**, 603 (1984).
4. H. Yoshikawa, K. Takada, and S. Muranishi, *J. Pharmaco-Dyn.*, **7**, 1 (1984).
5. T. Yamamoto, *Arch. Histol. Jpn.*, **45**, 1 (1982).
6. K. Morimoto, T. Iwamoto, and K. Morisaka, *J. Pharmaco-Dyn.*, **10**, 85 (1987).