Absorption of Oligodeoxynucleotide by Suppository from Rat Rectal Route

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Rectal absorption of 32-mer phosphorothioate deoxynucleotides (S-Oligo) in the rat was attempted with the aid of a suppository containing oleic acid and a surfactant. Although, the suppository without adjuvant did not show detectable blood levels, that containing over 10% oleic acid enabled the absorption of S-Oligo with $t_{\max}$ at 2 h postdosing.

Key words oligodeoxynucleotide; absorption; rectal route; suppository; oleic acid; rat

In recent years it has been demonstrated that oligonucleotides have a possible therapeutic effect on some diseases such as viral infections or cancers.¹⁾ In *in vivo* utilization of oligonucleotides as a therapeutic drug, biostability and delivery are important factors. Phosphorothioate oligodeoxynucleotide (S-Oligo) has been demonstrated to be relatively resistant to degradation in the body after administration.²⁻⁵⁾ Several routes such as i.v., i.p. and other systemic injection ways were employed in therapy using oligonucleotides, however, there is none that is particularly definitive for administration. Agrawal *et al.* illustrated that chemically modified S-Oligo was absorbable by an oral route and an intact form of oligomer was also observed in various tissues.⁶⁾ Generally, like other macromolecules, a larger molecular size of oligonucleotides supposedly depresses their absorption from the gastrointestinal tract. Yoshikawa *et al.* found that unsaturated fatty acid of long acyl chains enabled the gastrointestinal absorption of poorly absorbable macromolecular drugs such as interferons, especially from the colorectal routes.^{7,8)} In the gastrointestinal absorption of nucleotide oligomers, there is a possibility of first-pass effects by the liver because it has been reported that hepatic degradation of oligomers is not small.^{2,6)} The rectal route can reduce the hepatic first-pass elimination^{9,10)} and therefore is considered to be preferable for administration of nucleotide oligomer using the above mentioned absorption enhancer. We here report the possibility of S-Oligo absorption in rat by a rectal suppository which contains oleic acid as an absorption enhancer.

MATERIALS AND METHODS

Synthesis of S-Oligo The 32-mer S-Oligo (5'-CAG-TAGTCGTCATCAGTGACGACTACTGGCTA-3') was synthesized using deoxynucleoside phosphoramidites and tetraethylthiuram disulfide as a sulfurizing agent by automated DNA synthesizer. Synthesis and purification of ³⁵S-labeled S-Oligo were conducted in a similar way to non-labeled S-Oligo.

The purity of both the S-Oligos was over 92% by analysis of 20% polyacrylamide gel electrophoresis. The band products of both in the polyacrylamide gel visualized under UV shadowing were extracted in ammonium acetate and lyophilized. The specific activity of ³⁵S-labeled S-Oligo

was 0.2 μ Ci/ μ g.

Preparation of Suppositories A mixture of ³⁵S-labeled and unlabeled S-Oligo (9 mg, specific activity, 6.3 μ Ci/mg) was homogeneously incorporated at 37 °C into 0.5 ml of a melted hydrophobic suppository mass (Witepsol W35, Dynamit Nobel Chemicals, Germany) containing either: 1) no adjuvant (Suppository-1); or 2) 5% w/w of oleic acid (99% purity, NOF Co., Japan) and 1.7% w/w of HC60 (polyoxyethylated [60 mol] hydrogenated castor oil, NOF Co., Japan) (Suppository-2); 3) 10% w/w of oleic acid and 3.3% w/w of HC60, (Suppository-3); 4) 20% w/w of oleic acid and 6.7% w/w of HC60, (Suppository-4); or 5) 6.7% w/w of HC60, (Suppository-5).

Animal Experiment Male Wistar rats (body weight, 300 $\pm$ 10 g) were used for *in vivo* administration of S-Oligo. All procedures on animals were conducted in accordance with the Principles of Laboratory Animals Care of NIH. Four rats per group were anesthetized by intraperitoneal injection of pentobarbital, and thereafter the suppository of S-Oligo (dose; 30 mg/kg) was administered into the rat rectum. After insertion of the suppository, the anal canal was closed by tissue cement. Blood samples were collected periodically from the carotid artery under anesthesia and plasma was separated by centrifugation. S-Oligo levels in the plasma were determined by measuring the ³⁵S level detected by liquid scintillation spectrometry.

Gel Electrophoresis Analysis Polyacrylamide gel electrophoresis was used for the analysis of biostability of absorbed S-Oligo in the blood. Oligomers in the blood at various sampling times after administration of Suppository-4 were treated with guanidine isothiocyanate and extracted in an equal volume of phenol/chloroform (1 : 1 v/v) and 0.3 M NaCl aqueous solution. The obtained aqueous layer containing oligomers was dried and resuspended in the distilled water. This resuspended water containing oligomers was then applied on 20% polyacrylamide gel electrophoresis after which each band was excised and the radioactivity determined. By measuring the radioactivity ratio of the band of S-Oligo, the remaining percentage of intact S-Oligo in the blood was estimated.

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RESULTS AND DISCUSSION

S-Oligo levels in the blood after suppository administration were illustrated in Fig. 1. The suppository containing neither oleic acid nor HC60 (Suppository-1) did not yield detectable S-Oligo levels in the blood. However, S-Oligo incorporated in a suppository with oleic acid and HC60 yielded detectable S-Oligo concentrations according to the dose dependence of adjuvant. Thus, Suppository-2 containing a lower dose of adjuvant (oleic acid; 5% w/w, HC60; 1.7% w/w) slightly enhanced the rectal absorption; however, the middle dose (10% w/w oleic acid and 3.3% w/w HC60) in Suppository-3 and the high dose (20% w/w of oleic acid and 6.7% w/w of HC60) in Suppository-4 enabled the absorption of S-Oligo from rat rectal route. Two hours after administration of Suppository-3 the peak blood level of S-Oligo was detected and thereafter it decreased relatively rapidly. Suppository-4 also showed the peak concentration of S-Oligo in the blood at 2 h after administration, and this peak plasma level was approximately 1.6 times higher than that by Suppository-3. After the peak of S-Oligo level by Suppository-4, oligomer concentration decreased gradually. Suppository-5 containing surfactant alone at a dose the same as that in Suppository-4 was administered, but there was no S-Oligo detected (not shown). This finding suggests that HC60 itself has less absorption enhancing ability.

In our preliminary data on i.v. bolus injection to rats,¹¹⁾ the blood radioactivity of S-Oligo decreased in a two exponential manner with the half-lives of 0.46 and 6.32 h. With *AUC* of this intravenous injection as 100%, the calculated bioavailability of S-Oligo from the data in Fig. 1 was raised by increase in the dose of adjuvant, and Suppositories-3 and -4 indicated bioavailability of 3.1 and 6.9%, respectively.

Figure 2 shows the biostability of absorbed S-Oligo in the blood for up to 8 h after dosing of Suppository-4 as determined by polyacrylamide gel electrophoresis. At 1 h

after administration of the suppository, approximately 70% of the S-Oligo remained intact, and at 8 h about 60% of absorbed S-Oligo was detected as an intact form.

Regarding the stability of oligonucleotides in the body, phosphorothioate deoxynucleotides are reportedly far more stable in the blood after administration by various injection routes than phosphodiester deoxynucleotides.²⁻⁵⁾ However, precise manner of their degradation has not been elucidated, and various factors are believed to affect their metabolism, such as base length, structure, dose, route of administration and species.⁶⁾ In rat after i.v. injection of an antisense S-Oligo, Zhang *et al.* indicated the presence of intact oligomer and its degradative products in the blood by gel electrophoresis, but no quantitative inspection was made.¹²⁾ In our study here, about 40% S-Oligo was degraded in the plasma at 8 h after administration of Suppository-4 (Fig. 2). We do not have data on degradation of S-Oligo using the i.v. route and thus are not able to compare the biostability of S-Oligo in plasma between the two pathways. In a quantitative analysis of biostability, Agrawal *et al.* indicated that about 85% of intravenously administered S-Oligo remained intact in the plasma of mice at 24 h postdosing.²⁾ To argue the biostability of S-Oligo by suppository administration in detail, degradation data in suppository, rectal lumen and intestinal tissue are also necessary.

Unsaturated fatty acids with a carbon chain length from 12 to 20 and their monoglycerides solubilized by less harmful surfactant were demonstrated to act on the gastrointestinal barrier as enhancers; this was found by Muranishi and co-workers and reviewed by him.^{13,14)} In these unsaturated lipid, oleic acid or linoleic acid possess an especially strong promotional effect on the intestinal absorption of poorly absorbable chemicals including macromolecules. Muranishi mentioned the following factors in reference to the mechanism of enhanced colorectal absorption by these solubilized unsaturated fatty acids¹⁴⁾: 1) turbulence of lipid bilayer and increase in its fluidity of mucous membrane and 2) interaction of enhancer with SH proteins at apical membrane and tight junction of epithelial cells which changes their permeation. Molecular weight of the S-Oligo used here was approximately 10 kDa. Regarding the pathways of en-

Fig. 1. S-Oligo Concentration in Rat Plasma after Administration of Suppository by Rectal Route

○, Suppository-1 (no adjuvant); ●, Suppository-2 (5% w/w oleic acid and 1.7% w/w HC60); ■, Suppository-3 (10% w/w oleic acid and 3.3% HC60); ▲, Suppository-4 (20% w/w oleic acid and 6.7% w/w HC60). Results are expressed as the mean $\pm$ S.D. of four experiments. S.D. is indicated unless smaller than the point as plotted.

Fig. 2. Remaining Percentage of Intact S-Oligo in Rat Plasma after Administration of Suppository-4 by Rectal Route

Each value represents the mean $\pm$ S.E. of four experiments.

hanced permeated macromolecules by such lipid in the rat colon, Masuda *et al.* suggested the contribution of both transcellular and paracellular routes at a proportion deeply dependent on the molecular weight of the macromolecules.¹⁵⁾ In addition to these mechanisms, it is clear that the enhancing ability of oleic acid is reversible and its effect is immediately eliminated after its removal from the intestinal lumen; this indicates that the acid is a harmless absorption promoter for intestinal mucosa.¹⁴⁾

These reports used the administration of solution, and therefore the mechanism of enhanced absorption and transport route of S-Oligo by suppository as reported here is unknown. In suppository administration, the process of the dissolution step of drug and adjuvant have to be considered. With reference to the absorption promotion by the rectal suppository, Murakami *et al.* reported the possibility that a release pattern of a drug and adjuvants, which is dependent on the melting point of the suppository and viscosity of the melted base, affects the mode of absorption of the drug.¹⁶⁾ Subsequent investigations will be needed to determine: (1) the mechanism of absorption enhancement including dissolution behavior of S-Oligo and adjuvants from the suppository and (2) stability of S-Oligo in the suppository and each stage of its transport from the rectal lumen to circulation.

Consequently, the results here demonstrate the feasibility of practical rectal absorption of S-Oligo in the rat with the aid of a suppository containing absorption enhancer. We will also attempt further promotion of oligonucleotide absorption by evaluating various components of the enhancer adjuvant as well as the suppository

base.

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