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Penetration of oligonucleotides into mouse organism through mucosa and skin

V.V. Vlassov, V.N. Karamyshev and L.A. Yakubov

Institute of Bioorganic Chemistry, Siberian Division of the Russian Academy of Sciences, Lavrentiev pr. 8, Novosibirsk 630090, Russian Federation

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Benzylamide 5′-^{32}P-oligonucleotide derivatives were shown to penetrate into mice organism when administered by various routes: intranasally, per os, intravaginally and per rectum. In all cases, the compounds are rapidly accumulated in blood and guts. Analysis of the radioactive material from blood and pancreas revealed intact oligonucleotides. Although concentrations of oligonucleotides in tissues differ considerably by the various methods of administration, the efficiency of delivery is sufficient to consider all the routes as being of therapeutic value. Dose effect on the efficiency of oligonucleotide penetration into mice suggests the transport to be a saturable process. Application of an oligonucleotide lotion on mice ear helices results in reproducible accumulation of radioactivity in the animal tissues. Effectiveness of oligonucleotide delivery into mouse through skin can be improved by using electrophoretic procedure.

Oligonucleotide administration; Oligonucleotide degradation; Drug delivery methods

1. INTRODUCTION

The possibility to use oligonucleotide derivatives and analogs for regulation of gene expression and suppression of viruses multiplication in tissue cultures and the data of the first animal tests [1,2] have stimulated studies on pharmacokinetics of these compounds [3,4]. We have found that oligonucleotide derivatives that are introduced into mice intravenously (i.v.) or intraperitoneally (i.p.) enter all tissues and organs of the animals except the brain; they are excreted with urine and survive in blood and organs for about 1 h [5], which seems sufficient time for them to affect the target nucleic acids. To test alternative methods of administration, we introduced oligonucleotide derivatives into mice intranasally, intravaginally, per os, per rectum, and by different methods through the undamaged skin. We have found that oligonucleotide derivatives did penetrate into the animals although with lower efficiency as compared to the i.p. route. They were found in various organs and tissues less degraded as compared to the i.p. injected compounds.

2. MATERIALS AND METHODS

The experiments were performed with the 5′-^{32}P labeled oligodeoxynucleotide pTGACCCTCTTCCCATT. The 5′-phosphate of the oligonucleotide was conjugated to benzylamine, to protect it from phosphatases [5]. Equal quantities (2 nmol, 2×10^7 cpm) of the ^{32}P-labeled benzylamide oligonucleotide derivative ($BzpE_{16}$) were introduced in Balb C mice. $BzpE_{16}$ in 200 μl of physiological salt solution was introduced intraperitoneally, intranasally (dropwise into the nose holes, total volume 5 μl), per os (dropwise into the mouth of mice, 50 μl), intravaginally (by Gilson pipette into the vagina of mice, 10 μl) and rectally (in the distal part of the rectum, 10 μl). Application onto skin was performed as follows: 10 μl of the oligonucleotide solution containing equal volumes of water, glycerol and Tween 80 was applied onto the skin of ear helices of mice. Special attention was paid to preventing the mice from licking the solution applied. Electrophoresises through skin of the animals were performed in salt solution. An aluminium cathode covered by 1.5 × 1.5 cm Whatman 3MM paper which had been soaked with 100 μl of the solution containing 0.15 M NaCl and 2 nmol (2×10^7 cpm) $BzpE_{16}$ was placed under the stomach of the immobilized mouse. An aluminium anode with the paper was put on the wet back of the mouse. Electrophoresis was run for 20 min at 2 mA. Typically the mice were decapitated 20 min after the administration of oligonucleotides. Samples of tissues were weighed and counted in a liquid scintillation counter. Blood and pancreas samples were homogenized and extracted with an equal volume of phenol. Aliquots of the aqueous phases were diluted by 0.5 vol. of glycerol and analyzed by gel electrophoresis in 20% PAAG in 7 M urea followed by autoradiography.

Correspondence address: L.A. Yakubov, Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Lavrentiev pr. 8, 630090 Novosibirsk, Russian Federation. Fax: (7) (3832) 353459.

3. RESULTS AND DISCUSSION

Fig. 1 shows distribution of the label among mice organs after administration of the oligonucleotide derivative by different methods. Considerable amount of the compounds entered the organism 20 min after they had been applied. Intranasal and oral routes provided 5–20% of the delivery efficiency achieved by the i.p. injection, i.e. concentration of the oligonucleotides in the blood was up to 3 μM in the former versus 15 μM in the latter case. These concentrations fit the concen-

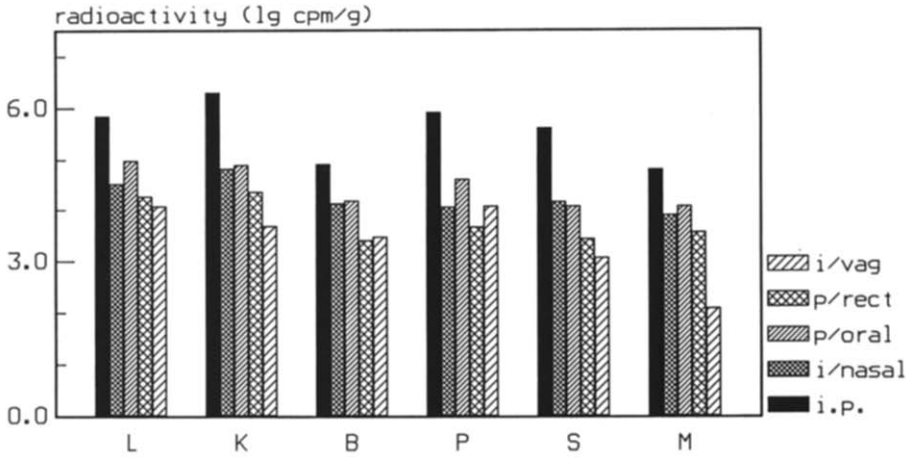

Fig. 1. Distribution of oligonucleotides in mice organism after administration by different routes. 2 nmol of the [^{32}P]-5′-benzylphosphoramide derivative of the oligonucleotide pTGACCCTCTTCCCATC ($BzpE_{16}$) (2.5 Ci/mmol) was administered. 20 min after the administration the mice were decapitated, and tissue samples were weighed and counted. L, liver sample; K, kidney sample; B, blood sample; P, pancreas sample; S, spleen sample; M, muscle sample. i.p., intraperitoneal injection; i/nasal, intranasal injection; p/oral, per os injection; i/vag, oligonucleotide was introduced into the vagina of mice; p/rect, oligonucleotide was introduced into the rectum of mice.

tration range required for the oligonucleotide derivatives to affect target viral and cellular nucleic acids [5]. By intravaginal and rectal administration of oligonucleotides, the concentration of the labeled compound in organs was 5–10 times lower than by intranasal and oral methods. Application of the aqueous oligonucleotide solution onto the skin yielded a small amount of radioactivity only in the liver and kidney. However the use of the electrophoretic procedure for oligonucleotide delivery through the skin allows to achieve considerable radioactivity in all organs (Table I). Application of a detergent lotion with oligonucleotide also gave increasing levels of the label in mouse organs.

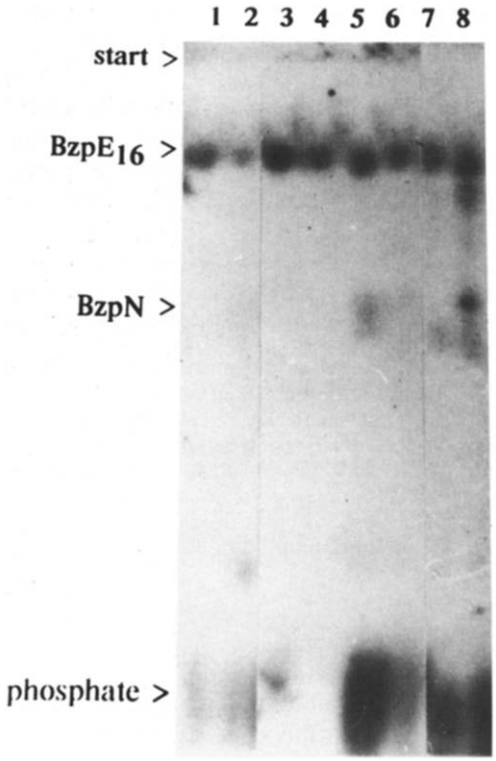

Fig. 2. Stability of oligonucleotide derivatives introduced in mice by different routes. The experimental conditions were as described in the legend to Fig. 1 and section 2. The radioactivity of the blood samples (odd-numbered lanes on the gel) and pancreas samples (even-numbered lanes) was analyzed by electrophoresis under denaturing conditions (20% PAAG, 7 M urea). (1,2) intranasal administration (3,4) intravaginal administration; (5,6) administration per os; (7,8) i.p. injection. $BzpE_{16}$, position of the initial oligonucleotide derivative; BzpN, position of the mononucleotide derivative.

Whatever method of administration was used, the oligonucleotide distribution between organs was more-or-less the same. It differed considerably from the distribution of inorganic phosphate when most radioactivity remained in the bloodstream even after a few hours post injection (data not shown). It means that there is one mechanism of transport of the oligonucleotides into organs: transportation by the bloodstream followed by uptake by the cells, which probably includes specific oligonucleotide binding to cellular receptors [6]. Recently we have shown the existence of such cellular receptors in some organs of mice and have found that the

Table I

Penetration of ^{32}P-oligonucleotides through skin (cpm/mg)

Tissues*	L	K	B	P	S	I	M
Water solution	0.12	0.54	–	–	–	–	–
Detergent lotion	0.5	0.5	0.6	0.6	0.25	0.25	0.35
Electrophoretic procedure	2.4	3.5	0.1	2.7	0.2	0.1	2.2

*The oligonucleotides were administered as described in section 2. L, liver; K, kidney; B, blood; P, pancreas; S, spleen; I, intestines; M, muscle.

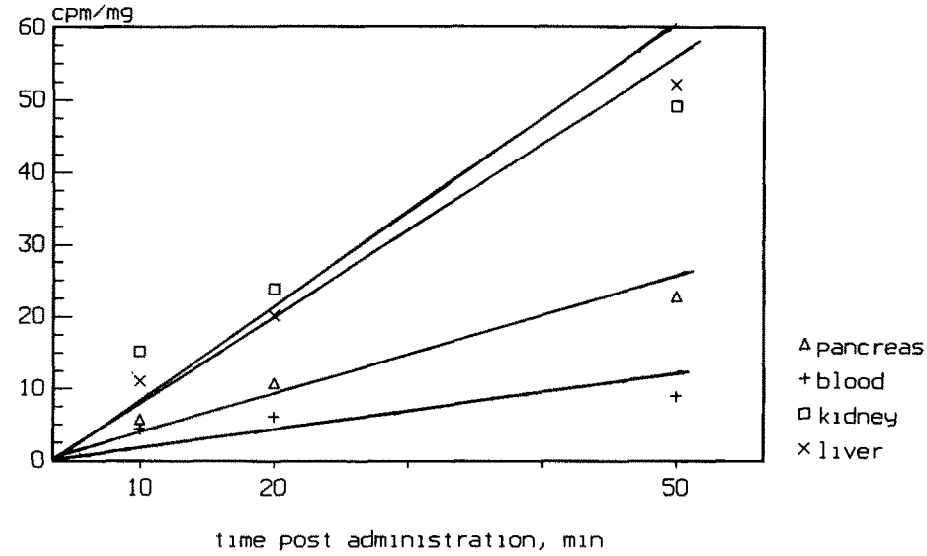

Fig. 3. Time course of oligonucleotide accumulation in mice organs after the nasal administration. Experimental conditions were as described in the legend to Fig. 1 except for the times.

number of receptors correlated with the quantity of the labeled oligonucleotide internalized by the organs [5].

Fig. 2 shows results of electrophoretic analysis of the radioactive material from blood and pancreas of mice 20 min after the administration of the labeled oligonucleotide derivatives. In all samples undegraded oligonucleotides are present in reasonable amounts: 10–70% of total radioactivity. Accumulation of the ^{32}P inorganic phosphate in the blood is observed when the oligonucleotides are injected i.p. and per os. It can be explained by the increased activity of macrophages and nucleases in the peritoneal cavity and intestines and by the fact that the oligonucleotides reach the blood already partially degraded. It should be noted that formation of large amounts of inorganic phosphate without considerable degradation of the remaining oligonucleotide points to high phosphoramidase activity, which detaches the benzylamide residue from the oligonucleotide and unmasks the 5′-^{32}P phosphate to phosphatase attack. In the pancreas, the degradation of the oligonucleotides seems somewhat slower than in the blood. This obviously means that the pancreatic cells select preferentially undegraded oligonucleotides from the blood. Here we suspect functioning of the oligonucleotide binding receptors on pancreatic cells, which can select undegraded oligonucleotides. It was found that the CD4 receptor binds long oligonucleotides more tightly than the short ones, and that the oligonucleotides shorter than 5-mer do not bind to the receptor at all [7]. A less pronounced degradation of the oligonucleoti-

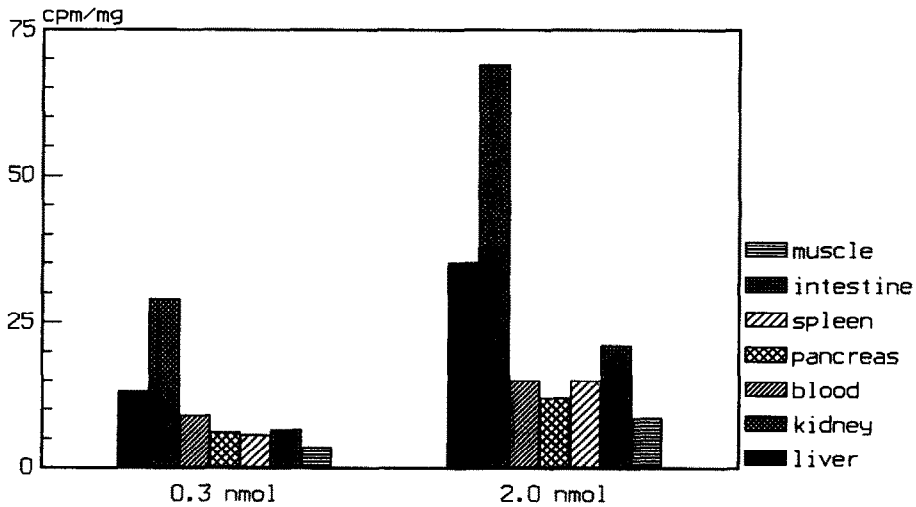

Fig. 4. Dose dependence of efficiency of oligonucleotides accumulation in mouse organs after nasal administration. Experimental conditions were as described in the legend to Fig. 1 with the exception of the doses. The same volume (5 μl) of the compound was introduced each time.

des was observed in the case of the intravaginal application.

The intranasal absorption of oligonucleotides is rather slow at high oligonucleotide doses (5 nmol per animal). Increase in the absorbed radioactivity has been observed for up to 2 h after the application (Fig. 4). The less efficient uptake at high doses and non-linear dependence of the absorbed material on the doses applied (Fig. 5) may suggest functioning of a saturable uptake mechanism.

The described experiments evidence that the oligonucleotides can penetrate into mice through the nasal, rectal and vaginal mucosa and even through the skin. Taking into account the existence of specific nucleic acid binding cellular receptors [6,8] we suggest that the mechanism of oligonucleotide penetration be transcytosis through the mucosa cells. This makes one optimistic about the prospects of the in vivo use of natural oligonucleotides. Apparently, topical application of oligonucleotides to treatment of local lesions caused by viruses like the papilloma virus can be envisioned.

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