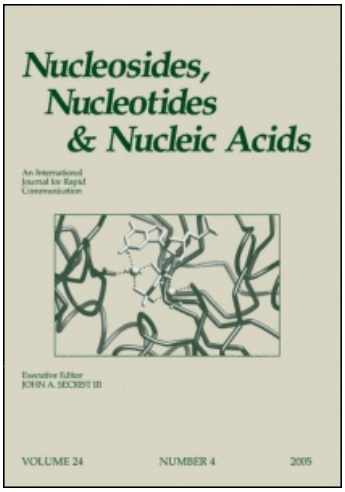

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Use of (2',5')Oligoadenylate Trimers Modified at the 2'-Terminus in Kidney Transplantation in Rabbits and Monkeys

I. A. Mikhailopulo[a]; Z. Yu. Tkachuk[b]; E. A. Baran[b]; L. V. Tkachuk[b]; A. V. Koslov[b]; I. I. Slukvin[b]; E. I. Kvasyuk[a]; T. I. Kulak[a]; G. Kh. Matsuka[b]
[a] Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, Minsk, Byelorussia [b] Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences, Kiev, Ukraine

To cite this Article Mikhailopulo, I. A. , Tkachuk, Z. Yu. , Baran, E. A. , Tkachuk, L. V. , Koslov, A. V. , Slukvin, I. I. , Kvasyuk, E. I. , Kulak, T. I. and Matsuka, G. Kh.(1995) 'Synthesis and Use of (2',5')Oligoadenylate Trimers Modified at the 2'-Terminus in Kidney Transplantation in Rabbits and Monkeys', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1105 — 1108
To link to this Article: DOI: 10.1080/15257779508012545
URL: http://dx.doi.org/10.1080/15257779508012545

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1105-1108 (1995)

SYNTHESIS AND USE OF (2',5')OLIGOADENYLATE TRIMERS MODIFIED AT THE 2'-TERMINUS IN KIDNEY TRANSPLANTATION IN RABBITS AND MONKEYS

I.A. Mikhailopulo*[1], Z.Yu. Tkachuk[2], E.A. Baran[2], L.V. Tkachuk[2], A.V. Koslov[2], I.I. Slukvin[2], E.I. Kvasyuk[1], T.I. Kulak[1], and G.Kh. Matsuka[2]

[1]Institute of Bioorganic Chemistry, Byelorussian Academy of Sciences, 220141 Minsk, Byelorussia and [2]Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences, 252143 Kiev, Ukraine

Abstract. Two core trimers, (2',5')A_2A_{RA} and (2',5')A_2A^{LA}, with 9-(2,3-anhydro-ß-D-ribofuranosyl)-adenine (A_{RA}) and its *lyxo*-isomer (A^{LA}) at the 2'(3')-terminus, respectively, were synthesized and their use as immunosuppressors in kidney transplantation in animals was investigated.

Introduction

An important role of 5'-triphosphorylated (2',5')oligoadenylates [mainly pppA2'-5'A2'-5'A, (2-5A)] in the antiviral action of interferon (IFN) is now well recognized (for a review, see [1]). It was also suggested that these oligomers and their 5'-dephosphorylated core derivatives are implicated as regulators in cell growth and/or differentiation. However, the 2-5A and its core are degraded rapidly by a (2',5')phosphodiesterase (PDase). Several studies reporting on the stability of 2-5A analogues exposed to PDase have been published. From these studies, the PDase appears to require free 2'- and/or 3'-OH group(s) at the 2'(3')-terminal residue (for a more detailed discussion, see, *e.g.*, [2]). Based on these results, we have synthesized two core trimers, (2',5')A_2A_{RA} and (2',5')A_2A^{LA}, with 9-(2,3-anhydro-ß-D-ribofuranosyl)adenine (A_{RA}) and its *lyxo*-isomer (A^{LA}) at the 2'(3')-terminus and studied their biological activities.

Results and Discussion

A phosphotriester methodology, published previously[3], was applied to synthesize the parent core trimer, (2',5')A_3, and both analogues. Determination of stability to the action of snake venom phosphodiesterase (SVPDE) gave the following $\tau 1/2$ values for the above analogues and the parent trimer, (2',5')A_3,: 16 h, 10.5 h, and 27 min. Thus, these experiments have shown that (2',5')A_3 is extremely sensitive to the action of SVPDE while the analogues synthesized are significantly more resistant.

In vitro investigation of the lymphocyte blast-transformation reaction (BTR) under the influence of mitogens makes it possible to use this model system to select (2',5')A_3 analogues which suppress division of lymphocytes. Using Concanavalin A (Con A; a mitogen for T-lymphocytes) or lipopolysaccharide (LPS; a mitogen for B-lymphocytes) as mitogens, the effects of the above analogues on the division of T- and B-lymphocytes of mouse splenocytes have been investigated. Differences in the actions of the parent (2',5')A_3 and the analogues were observed. The former suppresses the division of B-lymphocytes more effectively, while the (2',5')A_2A_{RA} suppresses the T-lymphocytes division to a greater extent, but shows a weaker effect on the division of B-lymphocytes (data not shown). The reaction shows a concentration dependence (Table 1). The ability of low level concentrations of the analogues to inhibit BTR under the influence of Con A and LPS, and to suppress the division of T-helper and T-killer cells in the organism for an extended time, indicates their utility in the treatment of a variety of diseases. This activity relates particularly to the treatment of diseases related to disturbance of T-cellular immunity: the autoimmune disorders, viral diseases, lymphocytic tumors, prophylaxis of transplant rejection after bone marrow transplantation, and treatment of graft *vs.* host diseases.

Table 1. *In vitro* Blast-transformation of murine lymphocytes treated with (2',5')A_2A_{RA} and mitogens.

Control	DNA synthesis at [^{3}H]-thymidine (counts/min)				
	(2',5')A_2A_{RA} concentration (M)*				
	$5x10^{-6}$	$5x10^{-7}$	$5x10^{-8}$	$5x10^{-9}$	$5x10^{-10}$
Con A (5 μg/mL)					
5,428	1,733	3,865	5,383	1,599	777
[5,108]	*[4,732]*	*[2,237]*	*[1,355]*	*[6,915]*	*[1,425]*
LPS (0.1 μg/mL)					
10,766	7,665	6,018	4,767	7,348	672
[5,513]	*[131]*	*[292]*	*[655]*	*[3,342]*	*[1,213]*

*) The data for (2',5')A_3 are given in brackets.

Table 2. Effect of single intravenous administration of (2',5')A_2A_{RA} on the immune system of *Macaque Rh.* monkeys at a dose of 25 μg/kg (data for *lyxo*-isomer are given in brackets).

Type of assay	Days following the administration of the trimer						
	0	1	2	4	8	12	21
IgG (g/L)	7.5	7.4	5.2	-	14.5	-	14.5
IgA (g/L)	2.8	2.0	1.1	-	0.53	-	0.38
IgM (g/L)	0.5	0.6	1.0	-	1.4	-	1.2
T-helpers (%)	16	20	10	4	5.3	7	23
	[26.2]		*[27]*	*[25.3]*			
T-suppressors (%)	47	40	21	11	11	14	46
	[53.1]		*[44]*	*[43.4]*			
T-killers (%)	9.9	12	8	4	4	4	6.4

It was further shown in experiments with mice that both analogues exhibited no toxicological effects at doses up to 1,000 μg/kg of the body weight. The above data prompted us to use (2',5')A_2A_{RA} and (2',5')A_2A^{LA} as immunosuppressors in kidney transplantation in animals.

It has been shown that intravenous (*iv*) injection of (2',5')A_2A_{RA} to rabbits at a dose of 5 μg/kg of the body weight daily assured normal functioning of the transplanted kidney for a period of 3 months (four animals of ten). The rabbits were alive even on the transplanted kidney alone after subsequent removal of their healthy kidney which initially functioned along with the transplanted organ. The lymphocyte BTR in post-transplanted rabbits (group with positive results) stimulated with Con A was suppressed almost 10-fold within 2 weeks of the post-operational period.

Immunosuppressive activity of (2',5')A_2A_{RA} compared to its *lyxo*-isomer, (2',5')A_2A^{LA}, and the parent trimer, (2',5')A_3, was investigated in 4 year-old monkeys. The levels of the principal subpopulations of T-lymphocytes, IgA, IgG, IgM, and the amount of α- and γ-IFN and interleukin-2 (IL-2) after administration of trimers at the dosage of 25 and 50 μg/kg of the body weight was investigated. As indicated in Tables 2 and 3, a single *iv* injection of (2',5')A_2A_{RA} results in a reduction of the subpopulation of T-lymphocytes; the effect of (2',5')A_2A^{LA} with respect to the T-helpers and T-suppressors was considerable lower.

Table 3. Effect of single intravenous administration of (2',5')A_2A_{RA} on the immune system of *Macaque Rh.* monkeys at a dose of 50 μg/kg (data for *lyxo*-isomer are given in brackets).

Type of assay	Days following the administration of the trimer 0	1	2	4	8	12	21
IgG (g/L)	9.6	7.8	15.0	-	14.8	-	13.2
IgA (g/L)	2.8	2.7	0.8	-	0.9	-	0.4
IgM (g/L)	0.6	0.5	1.1	-	0.9	-	0.8
T-helpers (%)	30	29	15	1.2	5.0	7.0	30.6
	[22]		*[20.5]*	*[18.4]*			
T-suppressors (%)	43	31	19	12	7.4	13	44
	[42]		*[44]*	*[43.4]*			
T-killers (%)	12	9	7	1.5	4.4	9	19.3

Table 4. Effect of single intravenous administration of (2',5')A_3 on the immune system of *Macaque Rh* monkeys at a dose of 500 μg/kg.

Type of assay	Days following the administration of the trimer 0	2	4
T-helpers (%)	22.1	32.3	37.2
T-suppressors (%)	33.2	36.0	44.2
T-killers (%)	8	12	12

Table 5. Effect of single intravenous administration of (2',5')A_2A_{RA} on the immune system of *Macaque Rh.* monkeys at doses of 25 and 50 [given in brackets] μg/kg.

Type of assay*	Days following the administration of the trimer 0	1	2	12	21
α-IFN	8	8	4	4	4
(plasma)	*[16]*	*[32]*	*[4]*	*[4]*	*[4]*
γ-IFN	4	16	16	16	8
(lymphs)	*[4]*	*[8]*	*[32]*	*[32]*	*[8]*
α-IFN	16	32	32	32	16
(lymphs)	*[32]*	*[32]*	*[64]*	*[64]*	*[32]*
IL-2	4	2	2	2	4
	[8]	*[4]*	*[4]*	*[4]*	*[4]*

*) Units per 10,000 lymphocytes.

Table 6. Effect of intravenous administrations of (2',5')A_2A_{RA} (50 μg/kg) on the immune system of *Macaque Rh.* monkeys after kidney transplantation.

Type of assay	2 days pre-oper.	Days post-operation 1	5	8	13	18
IgG (g/L)	13	-	12.2	12.8	-	-
IgA (g/L)	0.4	-	2.1	2.5	-	-
IgM (g/L)	0.8	-	0.8	0.9	-	-
T-helpers (%)	30	8	39.9	32	34.7	14.5
T-suppressors (%)	44	40.6	44	47	47.7	57.5
T-killers (%)	19	5	19	22	22.6	16

The results of experiments with (2',5')A_3 are shown in Table 4. It was observed that this trimer exhibited no immunosuppressive activity at a dose of 500 μg/kg. Moreover, single *iv* injection of (2',5')A_3 increased in about 50% the quantity of the subpopulation of T-helpers and T-killers, which contribute to the rejection of the transplant organs.

An investigation of the action of (2',5')A_2A_{RA} on the immune system of monkeys showed that this analogue suppressed 2-fold the production of IL-2 and stimulates the level of α-IFN (2-fold) and γ-IFN (8-fold) in blood lymphocytes for a period of two weeks (Table 5). Suppressing production of IL-2 with T-helper lymphocytes, (2',5')A_2A_{RA} suppressed proliferation of T-killer cells.

The experiments with kidney cross-allotransplantation were performed on 4 year-old *Macaque Rh.* monkeys. Each group consisted of 3 animals and they were observed for a period of 3 consecutive months. For the first group, (2',5')A_2A_{RA} was administrated *iv* at a dose of 50 μg/kg two days before the operation and at the second, sixth, and twelfth days after the operation and then every sixth day. The data show that the trimer selectively inhibits the subpopulation of T-lymphocytes, which have been documented to be critical in the rejection of transplants. After five days the quantity of T-killers and T-helpers returned back to normal and at 18 days their numbers had been reduced (Table 6). After transplantation kidney functions were restored within 10 h.

The administration schedule of (2',5')A_2A_{RA} was modified slightly for the second group of monkeys. The preparation has been injected two days before the operation and every other day at a concentration of 50 mg/kg during the entire post-transplantation period. It was found that the normal function of the kidney was reinstituted in the transplanted monkeys within 10 h. Immunological analyses showed that 7 days after surgery, the quantity of T-suppressors increased from 44% (pre surgical level) to 49%. The number of T-helpers dropped correspondingly from 37% to 7%. An analogous suppression effect has been observed for the T-killers. During the entire post-operative period, this activity tendency continued.

In conclusion, the results seem to indicate that (2',5')A_2A_{RA} at a dose of 50 μg/kg effectively prevents the rejection of the transplanting organ, insures the normal functioning of transplanted kidney and slows down the growth of the T-helper and T-killer cells in experimental animals during the post-operative period.

Acknowledgment. I.A.M. is deeply thankful to the Alexander von Humboldt-Stiftung (Bonn-Bad Godesberg, Germany) for partial financial support of this work.

REFERENCES

1. Torrence, P.F. Biological response modifiers. New approaches to disease intervention. (Torrence, P.F., ed.) **1985**, pp. 77-105, Academic Press, Orlando.
2. Van Den Boogaart, J.E.; Kalinichenko, E.N.; Podkopaeva, T.L.; Mikhailopulo, I.A.; Altona, C. *Eur. J. Biochem.* **1994**, *221*, 759-768.
3. Kvasyuk, E.I.; Kulak, T.I.; Khripach, N.B.; Mikhailopulo, I.A.; Uhlmann, E.; Charubala, R.; Pfleiderer, W. *Synthesis* **1987**, No. 6, 535-541.