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PHENOMENON OF REFRACTIVITY IN AN ANTI-VIRAL ACTION OF SYNTHETIC POLYNUCLEOTIDES

A. S. Novokhatskii, et al

Army Medical Research Institute of Infectious Diseases
Frederick, Maryland

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PHENOMENON OF REFRACTIVITY IN AN ANTIVIRAL ACTION OF SYNTHETIC POLYNUCLEOTIDES

Article by A. S. Novokhatskiy and F. I. Yershov, Antibiotics, Vol. 18, No 8, 1973, pp 713-718

Institute of Virology imeni D. I. Ivanovskiy, USSR Academy of Medical Sciences, Moscow

Synthetic RNA including complexes of polyinosine and polycytidylic acids (polyI : C), polyguanylic and polycytidylic acids (polyG : C) and others are capable of inducing both in tissue culture and the organisms of laboratory animals two apparently interrelated phenomena [1] that is the development of the antiviral state and induction of interferon formation [2,3]. An original property of the inductors of interferon formation was discovered and studied to a great extent, to determine development both in vivo and in vitro of the refractivity to repeated stimulation of interferon development [4-7]. An investigation of the phenomenon resulted in the problem of developing optimum systems for introducing these antiviral preparations [8] as well as a search for methods of overcoming this problem [9]. However, subsequent experiments demonstrated that the antiviral effect develops also at the moment of the condition of refractivity to interferon induction and it is possible that these two phenomena have little in common [6-10]. Nevertheless, there is no information in literature dealing with studies on the refraction phase during induction of the natural antiviral state resulting from the introduction of synthetic polynucleotides. This study was designed to fill that gap to a certain degree. The results of an experimental study of the state of refractivity to repeated antiviral action of the complexes of synthetic polynucleotides -- polyI : C and polyG : C in tissue culture relative to certain RNA containing viruses are summarized below.

Material and Methods

Viruses. Sindbis virus (VS) and the Venezuelan equine encephalomyelitis (VAEV) were obtained from the Museum of Viral Strains of the Institute of Virology imeni D. I. Ivanovskiy of the USSR Academy of Medical Sciences and had undergone prior to the test 15 and 28 passages respectively on fibroblasts of chick embryos. Titration of the viruses was achieved using the agar patch method. The multiplicity of infections for all of the experiments was standard and amounted to 5-10 BOE/cell and virus multiplication occurred at 37° over a 24-hour period.

Cells. In all of the experiments we employed initially trypsin treated fibroblasts of chick embryos (FEK) which were prepared according to the standard manner.

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We employed complexes of synthetic polynucleotides -- PolyI : C of the Firm Calbiochem and Poly G: C obtained and presented as a courtesy to us by Prof. S. Ye. Bresler and his coworkers (Institute of High Molecular Compounds of the USSR Academy of Sciences, Leningrad). The cells were treated in accordance with a methodology [11] previously developed by us of a "pulse" one hour action along with polykationate DEAE-dextrane (D-d) of the Pharmacia Firm.

Statistical processing of the results was carried out by the generally recognized method of calculating the weighted mean.

Results of the Investigation

Development of refractivity depending on the dose of polynucleotide used with preliminary treatment. In the series of preliminary experiments it was established that the state of refractivity to repeated antiviral action of polynucleotides develops in the initial hours following the preliminary effect by the preparations on the cells. In connection with this, in studying features of the development of the state of refractivity under various conditions, the duration of the experiment was limited to the initial days following the preliminary effect.

The cells were treated with various doses of polyG : C from 1 to 0.001 mkg/ml. We calculated the dynamics and level of the antiviral effect over the subsequent 24 hours. Cultures processed in parallel were subjected to the action of polynucleotides a second time (concentration of polyG : C in this instance was 1 mkg/ml) and moreover this repeated treatment was carried out over 1-2 hour intervals for up to 12 hours after the initial treatment and subsequently over 4-6 hour intervals. The cultures subjected to repeated treatment from the 1st to the 12th hour were infected with VS, 24 hours after the initial treatment and the remainder after 48 hours in order to assure development of the antiviral state affected by complexes introduced at a later time. Results of the experiments presented in Figure 1, a-d show that the state of refractivity to repeated induction of the antiviral effect logically occurs during the first 8-12 hours following preliminary administration of dosages of the nucleotide complex. The maximum state of refractivity is reached at 4-6 hours, there is a gradual increase from zero hours up to this point, followed by subsequent extinction.

The use, during preliminary processing, of a high dose of polyG : C (1 mkg/ml) leading to a suppression of the propagation of the model virus up to the background hinders the clarification of the state of refractivity even if in the given case it does appear, inasmuch as by the 24th hour i. e. up to the time of culture infection, their antiviral resistance in any event reaches the maximum potential level (see Figure 1, a).

The gradual reduction of the polynucleotide dose employed in the first administration promotes a more definitive calculation of the state of refractivity and the dynamics and level of its development. The fact that this phenomenon is also clearly expressed when used in the first administration

of very small doses of polynucleotides which in fact do not result in a noticeable suppression of VS reproduction is characteristic (see Figure 1, d). It is particularly important to note two conditions: 1) the state of refractivity by duration did not exceed 10-12 hours for any of the experiments i. e. the period in which the development and growth take place of the antiviral resistance induced with the initial introduction of the complex of polynucleotides (data on dynamics of the development of the antiviral action of synthetic polynucleotides is presently being readied for publication); 2) repeated introduction of the preparation during the period of the maximum developed state of refractivity was almost completely ineffective (see Figure 1, b, c), but never led to a drop in the antiviral action of the initially introduced dose of the preparation.

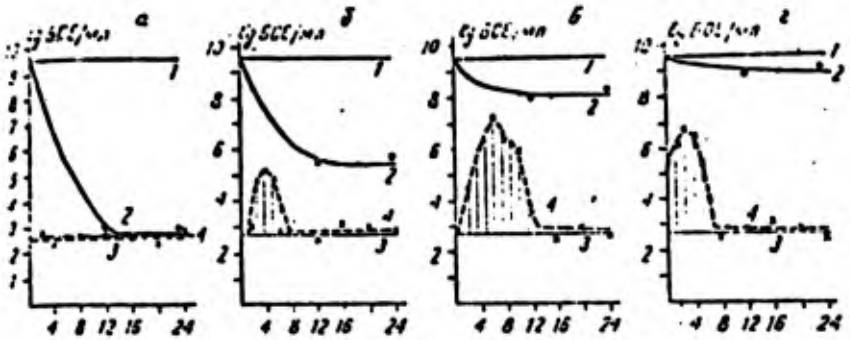

Figure 1. Phenomenon of refractivity to antiviral action of synthetic polynucleotides when using various doses of polyG : C for the initial treatment of FEK cells. Doses of polyG : C during initial treatment 1 (a), 0.1 (b), 0.01 (c), 0.001 mkg/ml (d), with repeated introduction of 1 mkg/ml.

Along the axis of the abscissa -- time (in hours) from the point of the initial treatment of cells with polyG : C; along the ordinate axis -- VS activity (in lg BOE/ml). 1 -- Virus control; 2 -- dynamics of the development of resistivity to viral infection induced as a result of the initial treatment of cells with polynucleotides; 3 -- control level of the suppression of VS multiplication induced as a result of the effect of a dose of polyG : C employed for repeated introduction; 4 -- real suppression of VS multiplication as a result of the introduction, over various periods of time, of a second dose of polyG : C against the background of the development of the antiviral effect on the first processing of cells with the preparation.

Effect on the State of Refractivity of a Dose of Polynucleotides Used during Repeated Introduction. In setting up this series of experiments we used the complex polyI : C and model virus VEL. Initial processing of cells was carried out with a dose of polyI : C equal to 0.01 mkg/ml, assuring the opportunity for a sufficiently precise exposure of the phenomenon of refractivity. With repeated introduction we used doses of polyI : C 0.1 and 0.3 mkg/ml. The methodology for preparing the experiments is identical to that described above.

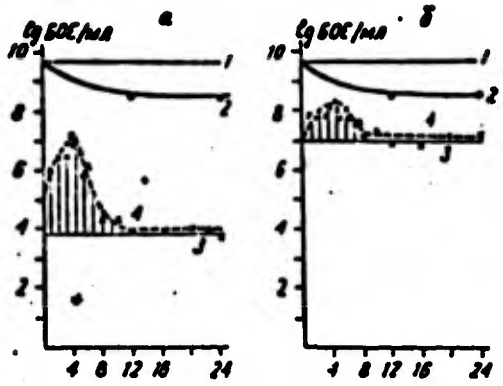

Figure 2. Phenomenon of refractivity to antiviral action of synthetic polynucleotides in the case of employing various doses of polyI : C for secondary introduction of FEK cultures. The dose of polyI : C during the first treatment of cells was 0.01 mkg/ml and during the second introduction (a) 0.1 and (b) 0.03 mkg/ml. Cultures were infected with VAEL.

Keys are the same as for Figure 1.

The results (Figure 2, a, b) showed a direct relationship in the level of development of the state of refractivity with the dose of polynucleotides during repeated introduction. The duration, general dynamics of development and extinction of the state of refractivity are maintained without any significant changes when using various doses of the preparation.

Antiviral State of Cultures with Repeated Introduction of Polynucleotides much Later Following Initial Treatment. We studied the level of antiviral resistance of cellular cultures following repeated introduction of various doses of polynucleotides after 24 and 48 hours following initial processing of the cells with various doses of the complexes. We used complexes such as polyI : C as well as polyG : C and the model viruses VS and VAEV. Results of the 5 series of experiments, in each of which the experimental point was obtained as a result of the combination of at least 3 parallel tests, were statistically processed and generalized in the table. Following the conclusion of the period of refractivity and at much later stages of the development of the antiviral effect, each subsequent introduction of the polynucleotides acts independently of the preliminary treatment of the cells with the preparation. The effect of repeated doses of polynucleotides develops from the level of antiviral resistance which the cellular cultures achieved under the effects of the initial treatment.

In comparing results of the various series it became apparent that the noted regularities do not depend on the nature of polynucleotides (polyI : C crossed with polyG : C in their variants) and the type of the model virus (VS in comparison with VAEV).

Discussion of Results

Results obtained by us show that in studying refractivity to antiviral

action of synthetic polynucleotides during their repeated introduction into the tissue culture we come up against phenomena differing from the phenomenon of refractivity to repeated induction of interferon.

Suppression of VS Multiplication with Repeated Introduction of the Polynucleotide PolyG : C Complex into FEK Cultures

Доза полиГ:Ц при первом введении	Доза полиГ:Ц при втором введении	3 Активность ВС в культуральной жидкости через 24 часа после заражения			
		4 заражение через			
		48 часов после первого введения полиГ:Ц (5)		72 часа после первого введения полиГ:Ц (8)	
MKG/ML		6 без повторного введения полиГ:Ц	полиГ:Ц введен повторно за 24 часа до заражения (7)	6 без повторного введения полиГ:Ц	полиГ:Ц введен повторно за 24 часа до заражения (7)
1		4,30±0,92		5,96±1,42	
	1,0		3,84±0,69		4,30±0,57
	0,1		4,88±0,29		5,56±1,12
	0,01		5,06±0,68		6,20±1,00
0,1		5,8±2,02		6,44±2,16	
	1,0		3,70±0,29		3,62±0,24
	0,1		5,36±1,00		4,86±0,50
	0,01		6,52±1,42		6,14±1,00
0,01		8,08±1,02		8,10±1,02	
	1,0		4,86±1,16		3,94±0,60
	0,1		6,92±0,40		5,72±1,20
	0,01		7,62±1,13		7,70±0,90

Key: 1. Dose of polyG : C with first introduction;
2. Dose of polyG : C with second introduction;
3. VS activity in a liquid culture medium 24 hours after infection;
4. Infected after;
5. 48 hours following initial introduction of polyG : C;
6. Without repeated introduction of polyG :C;
7. PolyG : C introduced repeatedly after 24 hours prior to infection;
8. 72 hours following initial introduction of polyG : C;

Note: Viral activity is given in 1 g BOE/ml. Viral control is equal to 9.7 1 g BOE/ml.

Refractivity to repeated interferon reaction both in tissue culture [6] and with animal models [8, 12] develops after a specific period of time following the conclusion of the processing of maximum quantities of interferon. We noted a significant number of general features during the development of the phenomenon of refractivity to the induction of additional amounts of interferon with the use of viral and synthetic inductors of interferon formation [4-7]. At the same time we demonstrated experimentally the presence of essential differences in the mechanisms of tolerance induced by preparations polyI : C and the Newcastle Virus [9].

The study of the interferon formation resulting from numerous injections of polyI : C into mice suggested that resistance to viral infection does not absolutely parallel interferon titres with subsequent introduction of the preparation [8] although it was shown somewhat earlier that with a one time introduction of polyI : C intranasaly into mice the degree of their protection against viral infection was found to be in a direct mathematical relationship to the discovered interferon titres [1].

Subsequently, tests with the initial cells of rabbit kidneys established that polyI : C results in resistance to the virus of vesicular stomatitis without inducing measurable amounts of interferon in a dose which does not lead to development of refractivity but conversely to stimulation of the production of interferon during repeated introduction of polynucleotides [6]. The authors have concluded that antiviral activity and the ability to bring on refractivity for secondary production of interferon are not linked.

In studying the relationship between synthesis of interferon serum and the appearance of antiviral resistance during multiple injection of mice with bifilar RNA obtained from Penicillium culture containing large quantities of virus-like particles we found that following the third injection of the preparation interferon induction was practically nonexistent while antiviral resistance was maintained throughout the entire course of the injections at a higher level than the antiviral action occurring following the one time injection which stimulated formation of high titres of the interferon serum. Comparable results were obtained when using the Sendai Virus as an inductor of interferon formation [10].

However, as our studies have shown refractivity is found while studying the antiviral action as well of the repeatedly injected bifiliar synthetic RNA. This phenomenon, however, is observed only in the early hours following the initial action of the polynucleotides and is rapid, it does not have any inhibiting effects on development of the antiviral effect with repeated introduction of the preparations and ends approximately at the same time when the antiviral effects of the first injection of the polynucleotide complexes attain their maximum.

The refractivity mechanism to the repeated antiviral action of synthetic polynucleotides is unclear. Apparently, it is logical to fully separate it from refractivity to the induction of interferon formation. It should be noted that in our experiments development of refractivity against antiviral action occurred in a time span corresponding to interferon formation in the cells and its movement into the trophic medium. The dynamics of the development and conclusion of refractivity against antiviral action of the interferon coincided very closely with the graph of the derived intensity of interferon synthesis in FEK cultures.

Data obtained recently on the nature of the interaction between the antiviral action of synthetic polynucleotides and interferon products and the contradictory role in this process of the appearance of refraction to repeated interferon induction as well as the results of our studies may point out the necessity of clarifying recommendations for a regime of the optimum conduct of these antiviral preparations.

It may be that a more productive method will be the course of investigating the phenomenon of refractivity to the repeated induction of the antiviral state. Results of the study of this phenomenon and of the factors affecting it will undoubtedly facilitate a clarification of the rational application of bifilar RNA of various types as antiviral preparations.

The results presented above of the experimental study show that (under the conditions employed by us) a possible and most effective repeated injection of polynucleotides occurred when it was carried out immediately following the conclusion of the growth of the antiviral effect from the previous effect on the cells with complexes of synthetic polynucleotides.

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REFRACTERY PHENOMENON IN ANTIVIRAL EFFECT OF SYNTHETIC POLYNUCLEOTIDES

A. S. Novokhatsky, F. I. Ershov

D. I. Ivanovsky Institute of Virology of the USSR Academy of Medical Sciences, Moscow

New data on the development of resistance to the antiviral effect of synthetic polynucleotide complexes on thier repeated use were obtained in tissue culture on a model of RNA-containing viruses. Unlike the refractery phenomenon to repeated induction of interferon the refractery phenomenon to the antiviral effect of polynucleotides developted during the first hours of the cell treatment with the drugs. The duration of the refractery phenomenon with respect to the antiviral effect did not exceed that of the increasement of the antiviral effect induced by the first administration of the synthetic polynucleotides. At later periods the antiviral effect on repeated administration of the polynucleotides developed independently of the previous drug administration. The development of the refractery phenomenon with respect to the antiviral effect of the polynucleotides did not probably depend on the type of the synthetic inductor, since it was not affected by a cross use of such complexes as polyI : C and polyG : C.