

Experimental Study of the Antitumor Activity of Polymetalacrylates against Animal Transplantable Tumors

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ABSTRACT

The antitumor activity of the fourteen polymetalacrylates against two models of murine solid tumors (Lewis lung carcinoma and Acatol adenocarcinoma) as well as the acute toxicity of these compounds has been studied. It was shown that polyacrylates of noble metals (argent, aurum, platinum), namely argacryl (M = Ag), auracryl (M = Au) and platacryl (M = Pt) were the most effective agents among tested compounds against studied tumors. Thus, the tumor growth inhibitory effect of argacryl against Lewis lung carcinoma was equal to 90%, the life-span of treated by this compound animals has increased on 50% in comparison with control. Auracryl induced the inhibition of the Lewis lung carcinoma and Acatol adenocarcinoma development on 60 and 65%, correspondingly and the increasing of the mean life-span of animals with Lewis lung carcinoma on 20% in comparison with control. Platacryl inhibited the growth of Lewis lung carcinoma on 40% increasing the mean life-span of animals on 25% in comparison with control. In this way it was established that argacryl is the agent with the strongest antitumor activity among studied polymetalacrylates. On the basis of obtained data it seems possible to consider polymetalacrylates as a group of agents with the potential antitumor activity suitable for the further deep experimental investigation.

Keywords: Polymetalacrylates, Antitumor Activity, Transplantable Tumors of Animals

1. Introduction

The development of drugs capable of inhibiting the growth of a malignant tumor at the expense of the influence on its blood supply is one of recognized areas in the field of biomedical chemistry and experimental oncology. Modern approaches to the solution of this problem are based on the investigation of medicines causing regional embolism of vessels, feeding a tumor, or creation of drugs inhibiting neo-angiogenesis processes in tumor. Over 500 various chemical compounds possessing an anti-angiogenesis activity against some experimental models are known at the present time [1].

In this field, the metal derivatives of polyacrylic acid (polymetalacrylates) are especially promising [2-9].

A pharmacological mechanism of action of polymetalacrylates is based first of all on their unusual influence on a coagulating system of blood leading to the formation of interpolymers with blood plasma proteins [10,11].

The first representative of medicines of this type, feracryl, is allowed for a broad medical use in Russia as a blood-stopping agent [12-16]. Argacryl (an incomplete silver salt of polyacrylic acid) is later offered as an effective hemostatic with a broad spectrum of pharmacological properties [2-4]. Silver containing medicines (povargol, protargol, kollargol, argovit etc.) are successfully used in medical practice as antiseptic remedies actively suppressing a pathogenic flora and simultaneously stimulating an immune status [17].

It is well known that drugs containing complexes of metals of platinum group (cisplatin, carboplatin *et al.*) are one of the most commonly used cytostatics in the modern antitumor chemotherapy [1,18-20].

Metallic derivatives of polyacrylic acid were not previously tested as antitumor agents.

The aim of our research has been the study of the antitumor activity of polymetalacrylate derivatives containing in their structure different metals (PMA) for the

reveal of the most effective compounds interesting for a further deep experimental investigation.

2. Materials and Methods

2.1 Compounds

The fourteen metal derivatives of polyacrylic acid have been studied in the biological experiment. These incomplete metallic salts of polyacrylic acid containing metal ions (**Table 1**) correspond to the general formula $(-\text{CH}_2-\text{CH}-\text{COOH})_n(-\text{CH}_2-\text{CH}-\text{COOM})_m$, where $n = 1200-3500$; $m = 1650-6650$ and contain 4-8 mass% of a metal are colorless or colored films. They have been synthesized by the method of polymer analogous transformations of polyacrylic acid by organic and inorganic metal salts [2-9]. The molecular weight of these polymetalacrylates are in the range from 1000000 to 3000000 D. Absorption bands $1548-1540 \text{ cm}^{-1}$ ($\nu_{\text{as}} \text{ COO}^-$) и $1405-1410 \text{ cm}^{-1}$ ($\nu_{\text{s}} \text{ COO}^-$); $1694-1649 \text{ cm}^{-1}$ (COOH), a widened band in the region $3420-2554 \text{ cm}^{-1}$, shifted in a low-frequency field (associated OH).

Compounds prepared on the ground of PMA were used as aqueous solutions injected intraperitoneally (i/p) to experimental animals (0.2-0.8 ml). Under the identification of parameters of the acute toxicity - the mean lethal dose result to the death of 50% of animals (LD_{50}) and the maximum tolerated dose not causing the death of any mice (MTD)-agents were injected one time, i/p, in the wide range of doses (from 10 to 200 mg/kg).

For the estimation of the antitumor effect, compounds were injected i/p, five times, every day starting from the next day after tumor transplantation. Daily doses for the most of agents were closed to 1/5 from MTD, but the ones for polyacrylates of noble metals (argacryl, auracryl and platacryl) were closed to 1/10 from MTD.

2.2 Laboratory Animals

Experiments have been carried out on 400 inbred mice BDF₁-first generation hybrids $f_1(\text{DBA}_2 \times \text{C}_{57}\text{Bl}/6)$ and Balb/c, males with the body weight of 18-20 g (Nursery "Stolbovaya" of the Russian Academy of Medical Sciences). The animals feeding regimen was based on the usage of the standard certificated commercial dry extruded food for rodents (PK-120-1) [21].

2.3 Experimental Models

Solid tumors, such as Lewis lung carcinoma and Acatol adenocarcinoma have been used as experimental test-systems. The transplantation of tumors has been done according to the standard procedures, subcutaneously at the right flank of mice by fragments of a tumor tissue suspended in a physiological solution of sodium chloride. The size of the inoculation material was equal to 0.3 ml [21].

2.4 The Antitumor Activity Test

Kinetics of the tumor growth in groups of treated (T) and control (C) animals as well as duration of mice life-span in both groups were studied. The coefficient of the tumor growth inhibition (TGI, %) and the increasing of the mean life-span ($\Delta\tau$, %) of treated animals in compare with control were the indicators of the antitumor activity of tested drugs. The TGI coefficient has been determined according to the correlation: $\text{TGI} = (\mathbf{P}_C - \mathbf{P}_T)/\mathbf{P}_C\%$, where \mathbf{P}_C and \mathbf{P}_T are volumes (or weights) of tumor in the control group and group of treated animals, accordingly. Two mutually perpendicular sizes of the tumor node were measured over the whole period of tumors development for the study of the kinetics of the tumor growth. The tumor volume was measured according to the formula for an ellipsoid as $\mathbf{V} = \mathbf{ab}^2/2$, where \mathbf{a} is the length, \mathbf{b} is the width and height of a tumor. The tumor weight corresponds to its volume, since the density of a tumor tissue is generally agreed to be equal to 1 g/cm^3 [21]. The increasing of the mean life-span ($\Delta\tau, \%$) of treated animals in comparison with the control was evaluated as $\Delta\tau = (\tau_C - \tau_T)/\tau_C, \%$, where τ_C and τ_T are the mean life-span of control and treated animals, accordingly. It is assumed to consider as minimal significant the value of TGI coefficient equal to 50% as well as the value of the increasing of the mean life-span ($\Delta\tau$) of treated animals in comparison with the control equal to 25% [21]. In accordance with the standard recommendations each group of treated and control animals comprised six and eight mice, correspondingly [21]. Experiments were repeated two or three times. The results of the main experiments are represented as kinetic curves of the tumor growth in groups of treated and control animals. Each dot on these curves presents the mean value of the tumor weight for 12-18 mice.

Experimental animals were observed during the whole period of the development of tumors.

Computer program "Statistics 6.0" was used for the statistical analysis of experimental data. F-criterion significance test was used for the estimation of the confidence of difference between mean values of tumor weight in groups of treated and control animals. It is consider that difference between values of tumor weight in groups of treated and control animals is confidence if the estimated value "F" is more than the value of "F-criterion" known for prescribed level of significance and for the certain degrees of freedom ($f_1; f_2$) [22].

3. Results

The fourteen polimetalacrylates (PMA) including four compounds containing noble metals, such as silver, gold, platinum, palladium, have been studied in biological experiments. Acute toxicity of PMA has been defined. The antitumor activity of compounds against Lewis lung car-

cinoma and Acatol adenocarcinoma has been established.

3.1 Toxicity

All studied compounds have a good solubility in water (excluding poorly soluble palladacryl).

When studying the compounds acute toxicity it has been established that PMA LD₅₀ values vary within the limits from 15 to 200 mg/kg, while values of MTD vary in diapasons from 10 to 150 mg/kg depending from the nature of the metal (M).

From the data indicated in the **Table 1** it follows that auracryl (M = Au) and feracryl (M = Fe) have a lower order toxicity than the other studied polymetalacrylates. LD₅₀ and MTD for auracryl were equal to such values, as 150 and 100 mg/kg, the same ones for feracryl were equal to 200 and 150 mg/kg correspondingly. The same indicators for two other derivatives containing noble metals-platacryl (M = Pt) and argacryl (M = Ag) were equal to 75; 50 mg/kg and 25; 20 mg/kg respectively (**Table 1**).

The values of the LD₅₀ for the other ten compounds were varied in the range from 15 till 50 mg/kg. The values of the MTD for these compounds were varied in the range from 10 till 40 mg/kg (**Table 1**).

So as it is seen from the data represented in the **Table 1** the most toxic agents between tested compounds were

Table 1. The acute toxicity of polymetalacrylates (the single i/p administration of agents, BDF₁ mice)

##	PMA	Metal	The maximum tolerated dose (MTD) mg/kg	The mean lethal dose (LD ₅₀) mg/kg
1	Liacryl	Li	40	50
2	Rubacryl	Rb	20	30
3	Cesacryl	Cs	10	20
4	Licuacryl	Li+Cu	20	25
5	Mangacryl	Mn	30	35
6	Feracryl	Fe	150	200
7	Niacryl	Ni	10	15
8	Palladacryl	Pd	10	not defined
9	Platacryl	Pt	50	75
10	Argacryl	Ag	20	25
11	Auracryl	Au	100	150
12	Ziacryl	Zn	15	20
13	Cadacryl	Cd	30	35
14	Stanacryl	Sn	10	15

derivatives containing such metals as cesium (Cesacryl), nickel (Niacryl), stannum (Stanacryl), while the less toxic one was the ferrum derivative (Feracryl).

3.2 Antitumor Activity

The antitumor activity of PMA against Lewis lung carcinoma and Acatol adenocarcinoma was tested.

The sensitivity of Lewis lung carcinoma to PMA compounds are characterized by data represented in **Tables 2, 3** and on the **Figure 1**.

As it is seen from data below argacryl, auracryl and platacryl are the most effective among studied polymetalacrylates against this tumor model.

Thus, the tumor growth inhibitory effect of argacryl against Lewis lung carcinoma this tumor was equal to 90%. The life-span of treated by argacryl animals has increased on 50 % in comparison with control (**Tables 2, 3; Figure 1**).

In case of auracryl the coefficient of the tumor growth inhibition was equal to 60% and the increase of the mean life-span of treated animals was 20% in comparison with control (**Tables 2, 3; Figure 1**).

Platacryl inhibits the growth of Lewis lung carcinoma on 40% increasing the mean life-span of treated animals on 25% in comparison with control (**Tables 2, 3; Figure 1**).

Asaturated aqueous solution of palladacryl containing less than 2% of palladium does not inhibit the growth of the tumor studied (**Tables 2, 3**).

It should be mentioned that some tested compounds such as cadacryl, rubacryl, feracryl induced the increasing of the mean life-span of treated mice with Lewis lung carcinoma on 53, 39 and 31% in compare to control correspondingly, but didn't influence on the rate of tumors growth (**Tables 2, 3**).

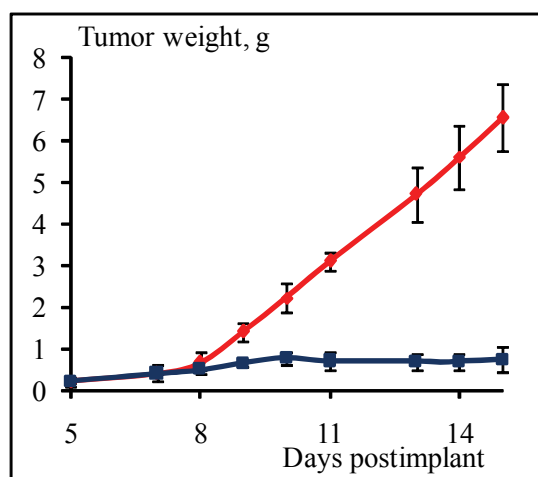
The obtained results prove that derivatives of noble metals (M = Ag, Au, Pt), especially argacryl, are the most effective among studied polymetalacrylates against Lewis lung carcinoma.

The development of the other studied tumor - Acatol adenocarcinoma was the most effectively inhibited by auracryl – the value of the TGI coefficient for this agent was equal to 65% (**Table 4, Figure 2**).

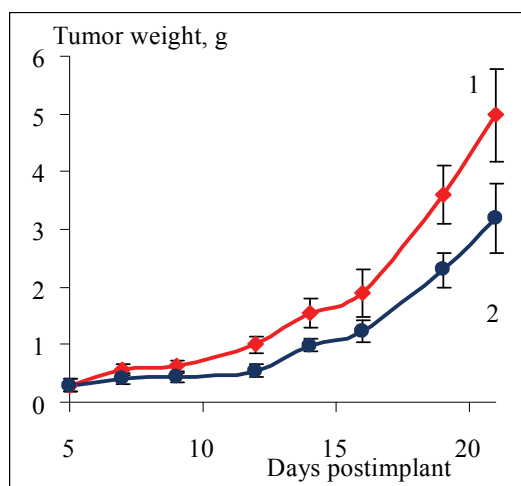
Platacryl and argacryl inhibited the growth of Acatol adenocarcinoma on 55% and 50% in comparison with control, accordingly (**Table 4, Figure 2**).

Therefore, the results of these experiments prove that compounds argacryl, auracryl and platacryl pronounced the significant antitumor effect against some murine solid tumors such as Lewis lung carcinoma and Acatol adenocarcinoma.

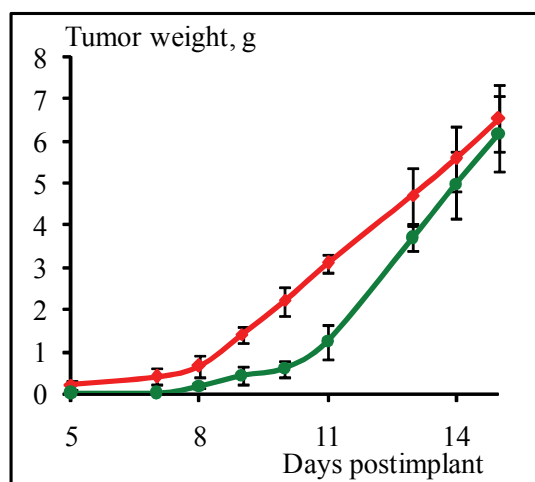
The received data allows to consider polymetalacrylates as a new group of compounds with a potential antitumor activity. Antitumor effect of polymetalacrylates containing noble metals (Ag, Au, Pt) has been first estab-



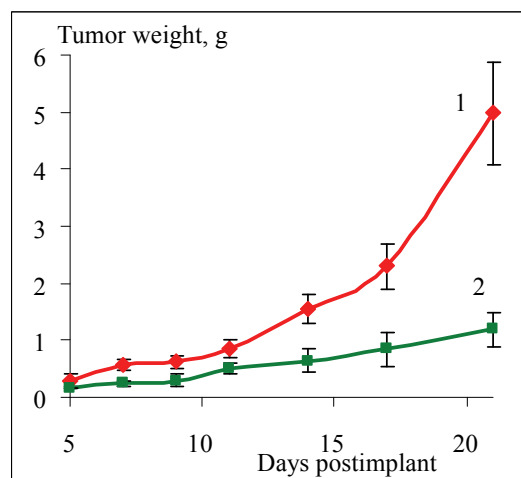
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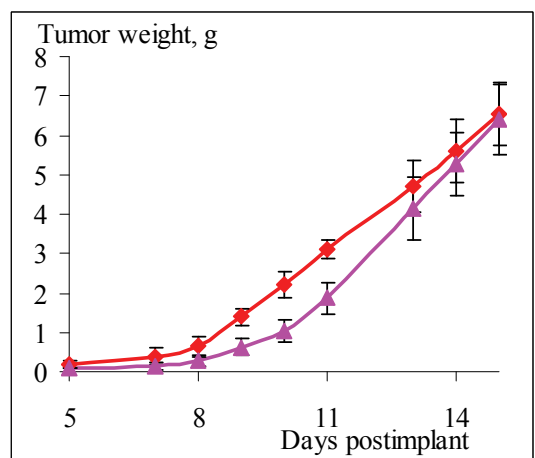
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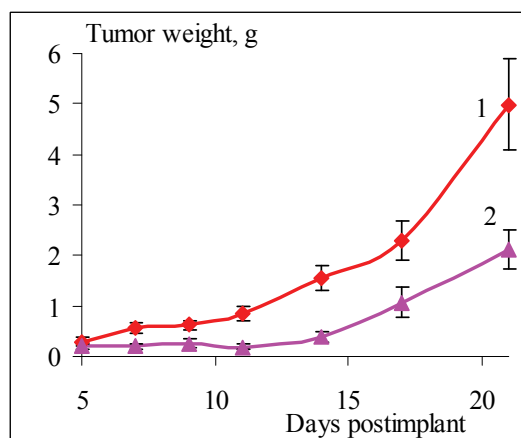
(b)



(b)



(c)



(c)

Figure 1. Antitumor activity of Argacryl (a) Auracryl, (b) Platacryl, (c) against Lewis lung carcinoma (intraperitoneal administration of compounds, 5 times, days 1-5); 1—control, 2—corresponding compound; Argacryl—2 mg/kg/day, Auracryl—10 mg/kg/day, Platacryl—4 mg/kg/day

Figure 2. Antitumor activity of Argacryl (a) Auracryl, (b) Platacryl, (c) against Acatol adenocarcinoma (intraperitoneal administration of compounds, 5 times, days 1-5); 1—control, 2—corresponding compound; Argacryl—1 mg/kg/day, Auracryl—10 mg/kg/day, Platacryl—4 mg/kg/day

Table 2. Antitumor activity of polymetalacrylates (PMA) against Lewis lung carcinoma (BDF₁ mice)

##	PMA	Daily dose (mg/kg)	The mean tumor weight (g) P ± SD	Day after tumor transplantation	The coefficient of the tumor growth inhibition TGI, %	F _{0.001} -criterion significance test (f ₁ =15; f ₂ =11)
1	Liacyrl	10	6.5 ± 0.4	15	1	-
2	Rubacryl	2	4.3 ± 1.1	15	35	-
3	Cesacryl	2	6.6 ± 1.1	15	0	-
4	Licuacryl	4	5.0 ± 1.1	15	24	-
5	Mangacryl	5	6.6 ± 1.3	15	0	-
6	Feracryl	20	5.4 ± 0.8	15	18	-
7	Niacryl	2	6.3 ± 1.9	15	5	-
8	Palladacryl	2	6.3 ± 1.4	15	5	-
9	Platacryl*)	4	1.9 ± 0.3	11	40*)	5.44>4.25
10	Argacryl	2	0.7 ± 0.3	15	90	7.10>4.25
11	Auracryl*)	10	1.2 ± 0.3	11	60*)	5.44>4.25
12	Ziacryl	1	5.4 ± 1.2	15	18	-
13	Cadacryl	5	4.6 ± 1.2	15	31	-
	Control*)	-	3.1 ± 0.7	11	-	-
	Control	-	6.6 ± 0.8	15	-	-

Compounds were injected i/p, five times, every day starting from the next day after tumor transplantation.

*) The coefficient of the tumor growth inhibition (TGI, %) for platacryl and auracryl was evaluated on the 11th day after tumor transplantation, for all other agents - on the 15th day after tumor transplantation.

Table 3. The influence of polymetalacrylates (PMA) upon the mean life-span (Δτ,%) of animals with Lewis lung carcinoma (BDF₁mice)

##	PMA	Daily dose (mg/kg)	The mean life-span of mice (days) τ ± SD	The change of the mean life-span of treated mice in compare to control (Δτ,%)
1	Liacyrl	10	30.8 ± 5.9	23
2	Rubacryl	2	34.8 ± 2.1	39
3	Cesacryl	2	32.3 ± 1.5	29
4	Licuacryl	4	25.8 ± 2.6	3
5	Mangacryl	5	30.2 ± 2.5	20
6	Feracryl	20	32.8 ± 4.5	31
7	Niacryl	2	25.0 ± 5.8	0
8	Palladacryl	5	24.7 ± 2.5	0
9	Platacryl	4	31.3 ± 4.2	25
10	Argacryl	2	36.5 ± 2.6	46
11	Auracryl	10	30.0 ± 4.3	20
12	Ziacryl	1	24.8 ± 2.6	0
13	Cadacryl	5	38.3 ± 1.2	53
	Control	-	25.0 ± 2.8	-

Table 4. Antitumor activity of polymetalacrylates against Acatol adenocarcinoma (Balb/c mice)

##	PMA	Daily dose (mg/kg)	The mean tumor weight (g) P±SD	The coefficient of the tumor growth inhibition TGI, %	F _{0.001} -criterion significance test (f ₁ = 15; f ₂ = 11)
1	Liacyrl	10	3.6 ± 1.7	28	-
2	Rubacryl	2	3.2 ± 1.2	36	-
3	Cesacryl	2	4.4 ± 1.8	12	-
4	Licuacryl	4	4.1 ± 2.6	18	-
5	Mangacryl	5	4.7 ± 1.5	7	-
6	Feracryl	20	4.9 ± 1.5	1	-
7	Niacryl	2	5.1 ± 1.2	0	-
8	Palladacryl	2	4.8 ± 0.6	0	-
9	Platacryl	4	2.1 ± 0.4	58	5.06 > 4.25
10	Argacryl	2	3.2 ± 0.4	36	5.06 > 4.25
11	Auracryl	10	1.2 ± 0.3	76	9.00 > 4.25
12	Ziacryl	1	3.5 ± 1.5	30	-
13	Cadacryl	5	5.2 ± 1.3	0	-
	Control	-	5.0 ± 0.9	-	-

Compounds were injected i/p, five times, every day starting from the next day after tumor transplantation.

The coefficient of the tumor growth inhibition TGI, % was evaluated on the 21st day after tumor transplantation.

lished. Thus, these compounds may be recommended for the further advanced experimental studies as potential antitumor agents.

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