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Coupling of 5-fluoro 2′-deoxyuridine to lactosaminated poly-L-lysine: an approach to a regional, non-invasive chemotherapy of liver micrometastases

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Abstract

Nucleoside analogs conjugated with galactosyl-terminating peptides selectively enter liver cells and after intracellular release from the carrier partly exit into bloodstream, resulting in higher concentrations in liver blood than in systemic circulation. The aim of the present experiments was to ascertain whether, in mice injected with non-toxic doses of a 5-fluoro 2′-deoxyuridine (FUdR) conjugate with lactosaminated poly-L-lysine (L-poly(LYS)), the drug was released by hepatic cells in high enough amounts to be pharmacologically active on neoplastic cells infiltrating the liver. We observed that L-poly(LYS)–FUdR inhibited the growth of hepatic metastases induced by intrasplenic administration of murine colon carcinoma C-26 cells. L-poly(LYS)–FUdR was not toxic for C-26 cells *in vitro*, was selectively taken up by mouse liver, and was stable in mouse blood, indicating that the effect on the metastases was due to FUdR (and/or its active metabolites) released in liver blood after the conjugate was taken up by the hepatic cells. These results suggest that L-poly(LYS)–FUdR might be useful in adjuvant chemotherapy of tumors giving liver metastases. The drug released from hepatic cells into liver blood following conjugate administration via the peripheral venous route might accomplish a locoregional, non-invasive treatment of micrometastases nourished by liver sinusoids.

Keywords: 5-Fluoro 2′-deoxyuridine; Lactosaminated poly-L-lysine; Liver targeting of drugs; Regional anticancer chemotherapy; Liver micrometastases; Antiblastic nucleoside analogs

1. Introduction

In order to reduce the side effects of antiviral NAs in treatment of chronic viral hepatitis, these drugs were coupled to galactosyl-terminating peptides that selectively enter hepatocytes through the asialoglycoprotein receptor [1–5]. The validity of this approach was supported by experiments in laboratory animals that showed a selective delivery of coupled NAs to liver cells [5] and by a clinical study in hepatitis B virus-infected patients which demonstrated an increase in the chemotherapeutic index of ara-A after coupling to L-HSA [6].

It was observed that NAs, when set free from the carrier inside hepatic cells, partly exit from these cells into bloodstream [1,7,8]. Although this release reduces the efficacy of hepatocyte targeting, it may have a useful consequence, since it can result in higher NA concentrations in hepatic blood than in the systemic circulation. This occurred in rats given an ara-A conjugate with L-poly(LYS). In these animals, blood levels of ara-A in suprahepatic veins were 2–3 times higher than those in the inferior vena cava and in aorta [9]. According to this result, coupling with a hepatotropic carrier might be a way to selectively increase the penetration of NAs not only into hepatic cells, but also into cells infiltrating the liver and nourished by hepatic sinusoids such as those of liver micrometastases [10].

The present experiments were undertaken to verify the possibility that after administration of non-toxic doses of a liver-targeted antiblastic NA, the amounts of drug released in sinusoids were high enough to be pharmacologically active on metastatic cells in liver. For this purpose, we

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Abbreviations: ara-A, adenine arabinoside; FU, 5-fluorouracil; FUdR, 5-fluoro 2′-deoxyuridine; L-HSA, lactosaminated human albumin; L-poly(LYS), lactosaminated poly-L-lysine; and NA, nucleoside analog.

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studied in mice the effect of an L-poly(LYS) conjugate of FUdR [11] on the growth of hepatic metastases of a murine colon carcinoma. FUdR is an NA active on human colorectal cancer [12], a tumor which metastasizes first to the liver. L-Poly(LYS) is a hepatotropic carrier which makes possible the preparation of conjugates which have a high drug load [13,14] and which displayed good tolerability in subchronic and chronic (26 weeks) toxicity studies in rats and monkeys.[1]

2. Materials and Methods

2.1. L-Poly(LYS)–FUdR

Coupling of galactose residues to poly-Llysine·HBr (28.5–42 kDa) (Sigma) was performed by reductive lactosamination [8]. The amount of lactose bound to poly(LYS) was determined by measuring the sugar [15] on a weighed sample of L-poly(LYS). Coupling of FUdR (Sigma) and [6-^{3}H]FUdR (Moravek) and characterization of conjugate preparations were described in [11]. To prepare the conjugate radioactive in the poly(LYS) moiety, Lpoly(LYS) was labeled with [^{3}H]formaldehyde (NEN) according to Jentoft and Dearbon [16], as reported in [13].

2.2. Animals

Female Balb/c mice 7–8 weeks old (weighing 16–19 g) and male Wistar rats (weighing 200–220 g) were used. They were obtained from Harlan Italy and were maintained in an animal facility at the Department of Experimental Pathology, Bologna, receiving humane care in accordance with the guidelines of the Italian Ministry of Health. Animals were fed a standard pellet diet *ad lib*.

2.3. Cytotoxicity of free and L-poly(LYS)-conjugated FUdR on C-26 cells cultured in vitro

The murine colon adenocarcinoma cell line C-26 is an *N*-nitroso-*N*-methylurethane-induced tumor [17]. It was obtained from Prof. P.L. Lollini, Institute of Oncology, University of Bologna. Cells were grown in Dulbecco's modified minimum essential medium, supplemented with 100 IU/mL of penicillin, 100 μg/mL of streptomycin, 2 mM glutamine, and 10% fetal bovine serum. All products were obtained from GIBCO-Life Technologies. Prior to experiments, 2×10^5 cells, suspended in 2 mL culture medium, were seeded into each well of a 6-well Nunclon multidish plate (PBI International). After 24 hr, free or L-poly(LYS)-coupled FUdR was added and cells from two wells were harvested and counted. Each dose of drug was tested in duplicate. After a further 24 hr, cells from treated cultures and from two untreated wells were separately harvested and counted, and the increase in cell number during the period in which the drugs were present was calculated. The ID_{50} (concentration producing a 50% inhibition of cell growth) was calculated from the polynomial regression of the experimental data plotted as log dose versus % inhibition. Regression was considered reliable for $r^2 > 0.9$. Incubation of the cells with drugs was not prolonged for more than 24 hr because in preliminary experiments it had been found that L-poly(LYS)–[^{3}H]FUdR, when maintained in the culture medium at 37° for 24 hr, released 3–4% of [^{3}H]FUdR. For these reasons, periods longer than 24 hr were not used, since toxicity caused by the penetration of conjugate into the cells could not be distinguished from that produced by free FUdR released into the medium.

2.4. Stability of the bond between [^{3}H]FUdR and L-poly(LYS) in blood

L-Poly(LYS)–[^{3}H]FUdR was incubated (50 μg/mL) in fresh heparinized mouse blood. At different time intervals (0, 1, 2, and 4 hrs), blood cells were removed by centrifugation. Three hundred microliters of plasma was diluted to 10 mL with NaCl 0.9% and diafiltered using a 3000-Da cut-off YM3 membrane (Amicon) which retains the conjugate. The released drug was measured by counting the radioactivity of the diafiltrate.

2.5. Distribution of the conjugate in organs of mice and in parenchymal and sinusoidal cells of rat liver

L-Poly[^{3}H](LYS)–FUdR, dissolved in NaCl 0.9%, was injected into a tail vein of mice in a volume of 200 μL/animal at the doses shown in Table 2. At different times, mice were anesthetized with ether and, after bleeding from the retro-orbital plexus, liver, spleen, heart, and a tract of intestine (8 cm long starting from pylorus) were rapidly removed and homogenized in 4 vol. (w/v) of water. Mice died while under anesthesia. For each time interval two animals were used. Because the conjugate was not precipitated by either perchloric or trichloroacetic acid, total radioactivity of plasma and homogenates was measured. The radioactive contribution given by the plasma trapped in the organs was calculated and subtracted as described in [18].

Two male Wistar rats fasted overnight received L-poly[^{3}H](LYS)–FUdR via the dorsal vein of the penis under ether anesthesia. One hour after conjugate administration, parenchymal and sinusoidal cells of liver were isolated by collagenase perfusion according to Seglen [19], following the procedure described in detail in [20].

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Table 1
Chemical characteristics of conjugates

Conjugate	Sugar (μg)	FUdR (μg)	% ϵ-NH_2 groups substituted by		Spec. Activity (dpm/μg)
	Conjugate (mg)	Conjugate (mg)	Sugar	FUDR	
L-poly(LYS)–FUdR	218	330	30	63	
L-poly(LYS)–[^{3}H]FUdR	197	338	26	63	5960
L-poly[^{3}H](LYS)–FUdR	213	339	30	66	2650

The percentages of substituted amino groups were calculated taking as givens that 1 mg poly(LYS) (hydrochloride form) contains 6.13 μmol lysine residues and that the M_r of lactose and FUdR were 342 and 246.2, respectively.

2.6. Induction of hepatic metastases

C-26 cells were harvested by trypsination (0.25% trypsin/0.02% Na_2 ethylenediaminotetraacetic acid) and a viable cell count was performed by trypan blue exclusion. The cell suspension was then washed in the medium and adjusted to 5×10^6 cells/mL. Mice were anesthetized by an intraperitoneal injection of ketamine/xilazine. The spleen was exteriorized by a 0.5-cm subcostal incision across the skin and abdominal muscles. One hundred microliters of tumor cell suspension was injected into the apex of the spleen using a 25G needle over a 30-sec period. After allowing 1.5 min for the tumor cells to enter the portal circulation, splenic vessels were closed with a titanium clip and the spleen removed. A second clip was applied to join the edges of abdominal muscles, and the skin was sutured with a metallic stitch.

2.7. Evaluation of metastatic growth in the liver

Conjugate administrations were performed according to the schedule reported in Table 4, beginning 24 hr after the intrasplenic inoculation of C-26 cells. Animals were killed 8 days after tumor transplant. Growth of metastases was estimated by weighing the liver and measuring the areas of metastases in histologic sections. A liver specimen from the left lateral lobe was obtained from each animal immediately after killing, fixed in 10% buffered formalin, and routinely paraffin-embedded. Four-micrometer-thick sections were then cut and stained with hematoxylin and eosin. The entity of neoplastic invasion was measured by image cytometry using a specific program (Image Pro Plus, version 3.0.2 for Windows 95, Media Cybernetics) of a computer-assisted image analysis system. The system consisted of a Panasonic PC equipped with a Sony (DXC-M3A) color camera mounted on a Zeiss light microscope. Morphometric analysis of each sample was carried out in two steps: in the first step, the area of the whole section was measured at low magnification (2×) and in the second, cancer nodules were measured at a higher magnification (10×). Measurements were performed interactively by overlying both the whole sections and the neoplastic nodule periphery with the digital cursor, and were expressed in μm^2. The ratio between the total neoplastic area per section and the total section area, expressed as a percentage (%), was then obtained for each case and was called the "metastatic index" (MI). Statistical analysis of measured data was performed using the Student's *t*-test, and statistical significance was defined at $P < 0.05$. In two experiments (nos. 1 and 5 of Table 4), histological sections were also obtained from liver right lobe. The mean MI values calculated in the two lobes were very similar, differing by less than 5%.

3. Results

3.1. Chemical and biological properties of L-poly(LYS)–FUdR

The chemical characteristics of L-poly(LYS)–FUdR preparations used in the present experiments are reported in Table 1. In the experiments *in vitro*, free FUdR produced a 50% inhibition of the growth of colon 26 tumor cells at a concentration of 1.0 ng/mL. The conjugate did not display any effect on these cells even at a concentration of 30.3 ng/mL (corresponding to 10 ng of coupled FUdR), which was the highest tested. When L-poly(LYS)–[^{3}H]FUdR was incubated at 37° in mouse blood, only 1.8, 2.7, and 4.8% of the coupled drug was released from the carrier after 1, 2, and 4 hr, respectively. This is in agreement with previous findings on the stability of the bond between NAs and L-poly(LYS) in blood from different mammals [7,8,21,22]. Table 2 shows that L-poly[^{3}H](LYS)–FUdR, intravenously administered to mice, was selectively taken up by the liver. This result fits with data in mice and rats i.v. injected with an L-poly(LYS) conjugate of ara-A [9,13]. The contribution by parenchymal and sinusoidal cells to liver conjugate uptake was studied in rats. One hour after i.v. administration of L-poly[^{3}H](LYS)–FUdR (14.7 μg/g, corresponding to 5 μg FUdR and 39,000 dpm/g), radioactivity values per mg cell proteins measured in parenchymal and sinusoidal cells isolated by collagenase perfusion (see Materials and methods) were 2052 ± 167 and 1641 ± 116, respectively. Penetration of a galactosyl-terminating conjugate into liver sinusoidal cells was previously observed in rats injected with L-HSA–ara-A [20,23]. The mechanism of entry of L-poly(LYS)–FUdR and L-HSA–ara-A into sinusoidal cells is not known; it might be a consequence of a non-specific adsorption endocytosis. Toxicity of conjugate and of free FUdR was

Table 2
Plasma and organ radioactivity in mice after administration of L-poly[^{3}H](LYS)–FUdR

Coupled FUdR (μg/g)	Time (hr)	Radioactivity (dpm/g/sp. act.)				
		Plasma[a]	Liver	Spleen	Intestine	Heart
2.5[b]	1	1.2 ± 0.1	67.4 ± 2.0	3.0 ± 0.2	1.9 ± 0.3	1.7 ± 0.1
2.5	2	1.4 ± 0.1	61.8 ± 2.9	3.4 ± 0.1	2.3 ± 0.0	2.2 ± 0.0
5	0.25	38.2 ± 11.4	63.0 ± 5.2	7.0 ± 0.7	3.3 ± 0.2	6.5 ± 2.5
5	0.5	15.1 ± 1.5	85.0 ± 24.4	6.5 ± 1.8	6.3 ± 0.2	6.3 ± 0.2
5	1	2.4 ± 0.9	95.4 ± 1.9	7.4 ± 0.8	4.1 ± 0.8	4.3 ± 0.3
5	2	3.3 ± 0.1	107.0 ± 5.2	5.7 ± 0.4	4.9 ± 0.3	4.4 ± 0.4
5	4	4.0 ± 0.3	110.0 ± 3.6	6.5 ± 0.7	6.4 ± 0.2	6.2 ± 0.2
7	1	2.9 ± 0.1	135.6 ± 1.4	7.8 ± 0.0	4.9 ± 1.1	4.0 ± 0.1
7	2	3.3 ± 0.2	130.6 ± 4.9	6.5 ± 0.3	4.1 ± 0.2	4.1 ± 0.2

Experiments were performed as described in Materials and Methods. Data are mean values ± SE.
[a] dpm/mL/sp. act.
[b] 2.5 μg/g of coupled FUdR corresponds to 7.6 μg/g of conjugate.

evaluated in healthy mice treated according to the schedule reported in Table 3. Toxicity was assessed by determining the changes in body weight and in peripheral leukocyte number. L-Poly(LYS)-coupled FUdR, up to a dose of 7 μg/g (corresponding to 21.2 μg/g of conjugate) per single administration, did not produce statistically significant changes in these parameters. A significant decrease in body weight was observed in animals treated with a dose of 10 μg/g. Free FUdR produced a significant decrease in body weight at a dose of 25 μg/g.

3.2. Effect of L-poly(LYS)–FUdR on liver metastases

The histological features of C-26 hepatic metastases were studied 3, 6, and 8 days after intrasplenic injection of tumor cells in Balb/c mice. Three days after cell inoculation, nests of very few cancer cells were observed inside the branches of portal veins within the smallest portal tracts. Neoplastic cells were sometimes visible within the sinusoids immediately adjacent to the portal tracts. Cell nests of 4 to 5 cancer cells were also observed at a distance from the portal tracts, always inside the sinusoids. Only rarely were a greater number of neoplastic cells observed within portal vein branches. Six days after cancer cell injection, a large part of hepatic tissue appeared to have been replaced by large islands and solid cords of polygonal or spindle-shaped cancer cells. Biliary ducts and small arterial vessels were frequently present within these neoplastic structures. Portal veins were no longer detectable, while intralobular veins

Table 3
Effect of free and L-poly(LYS)-coupled FUdR on body weight and leukocyte number

Compound	Daily dose of FUdR (μg/g)	Relative body weight (%)[a]	Number of leukocytes ($\times 10^6$/mL)[b]
Saline		101.1 ± 0.8	10.22 ± 0.67
Free FUdR	4	98.5 ± 1.2 NS	11.40 ± 1.06 NS[c]
	7	99.5 ± 1.6 NS	13.21 ± 2.49 NS
	10	99.2 ± 0.6 NS	13.10 ± 1.89 NS
	17.5	98.4 ± 0.9 NS ($P = 0.055$)	10.84 ± 1.11 NS
	25	97.6 ± 0.8 $P = 0.015$	12.53 ± 2.13 NS
	50	94.5 ± 1.0 $P = 0.000$	9.33 ± 0.97 NS
Coupled FudR	2	99.5 ± 0.8 NS	ND
	3	99.3 ± 2.0 NS	ND
	4	99.1 ± 1.2 NS	12.18 ± 1.95 NS
	5	98.2 ± 1.1 NS	10.66 ± 0.74 NS
	7	103.3 ± 0.6 NS	9.08 ± 0.48 NS
	10	98.0 ± 0.2 $P = 0.006$	10.26 ± 1.12 NS
	15	93.3 ± 2.8 $P = 0.028$	7.28 ± 0.65 $P = 0.014$

Compounds were i.v. injected every other day in a volume of 200 μL. Four administrations were performed. Animals were weighed and leukocytes counted one day after the last administration. NS, not significant; ND, not determined.
[a] Animal weight is expressed as percentage of the initial value. Values are means ± SE of five mice.
[b] In untreated female Balb/c mice, the number of leukocytes $\times 10^6$/mL of blood was 10.60 ± 0.61.
[c] Results (in drug-treated versus saline-injected animals) were evaluated by means of Student's *t*-test and statistical significance was defined as $P < 0.05$.

Table 4
Effect of free and L-poly(LYS)-coupled FUdR on liver metastases

Experiment	Compound	Daily dose of FUdR (μg/g)	Increase of liver weight (g)[a]	Metastatic index (%)[b]
1	Saline		1.66 ± 0.20	47.68 ± 9.77
	Coupled FUdR	5	0.90 ± 0.06 $P = 0.003$	19.86 ± 4.39 $P = 0.023$
2	Saline		0.99 ± 0.16	52.90 ± 7.08
	Coupled FUdR	2.5	0.62 ± 0.14 NS	17.19 ± 4.33 $P = 0.001$
		7	0.31 ± 0.08 $P = 0.002$	14.27 ± 1.62 $P = 0.000$
3	Saline		0.88 ± 0.26	55.24 ± 10.71
	Coupled FUdR	1.25	0.39 ± 0.12 NS	15.32 ± 7.62 $P = 0.010$
		0.62	0.39 ± 0.13 NS	23.38 ± 4.60 $P = 0.015$
4	Saline		1.69 ± 0.24	52.67 ± 9.39
	Coupled FUdR	0.3	1.38 ± 0.23 NS	49.79 ± 8.51 NS
		0.15	1.28 ± 0.17 NS	45.06 ± 9.05 NS
5	Saline		1.52 ± 0.14	46.76 ± 8.74
	L-HSA[c]		1.24 ± 0.26 NS	45.51 ± 6.26 NS
6	Saline		0.65 ± 0.20	25.13 ± 7.66
	Free FUdR	2.5	0.85 ± 0.23 NS	37.33 ± 8.35 NS
		5	0.85 ± 0.20 NS	17.39 ± 3.06 NS
7	Saline		2.19 ± 0.30	44.06 ± 8.08
	Free FUdR	5	1.68 ± 0.29 NS	46.35 ± 7.97 NS
		10	1.55 ± 0.11 NS	41.79 ± 4.14 NS

Compounds were i.v. injected every other day in a volume of 200 μL starting the day after inoculation of tumor cells. Four administrations were performed. Seven animals were used for each group (saline- or coupled FUdR-treated mice). Animals were killed one day after the last injection. NS, not significant.

[a] The weight of liver was measured and that of non-tumor-bearing Balb/c mice (0.90 ± 0.02 g) was subtracted.

[b] Metastatic index is the ratio between the total neoplastic area per section and the total section area, expressed as a percentage.

[c] Human albumin with 25 lactose residues. L-HSA was injected at the daily dose of 7.2 μg/g, corresponding to 0.8 μg/g of lactose. The same amount of sugar was administered by injecting L-poly(LYS)–FUdR at the daily dose of 3.8 μg/g (corresponding to 1.25 μg/g of FUdR) (exp. no. 3).

were still visible. Sometimes cancer cells appeared to branch out from the periphery of the neoplastic islands and cords, invading the sinusoids of the surrounding hepatic tissues. Neoplastic infiltration of intralobular veins rarely occurred. Eight days after cancer cell injection, the time at which the effect of L-poly(LYS)–FUdR was measured, the morphological pattern of metastatic invasion was superimposable to that at six days. Free and coupled FUdR were administered according to the schedule used for the toxicity experiments (Table 3), starting the day after inoculation of tumor cells. At this stage, neoplastic cells do not form a tumor tissue and some are located within sinusoids, reproducing the situation of human hepatic micrometastases, which are fed by sinusoidal blood and are the target of the possible chemotherapeutic activity of L-poly(LYS)–FUdR (see Discussion). In mice treated with the conjugate, metastatic invasion was greatly reduced (Table 4) (Figs. 1 and 2). The morphological aspects of neoplastic invasion did not differ from those observed in saline-injected mice. Antitumor activity of conjugate was observed at the dose of 1.9 μg/g, corresponding to 0.62 μg/g of coupled FUdR. The absence of a dose–response effect with higher doses of conjugate (see Table 4) cannot at present be explained. In tumor-bearing mice, at all doses used, the conjugate did not cause changes in body weight with a statistically significant difference from those of the animals injected with saline. The role of lactose in inhibiting growth of metastases was studied by treating tumor-bearing mice with L-HSA (exp. no. 5). L-Poly(LYS) was not used in this experiment since this carrier displays high toxicity when not conjugated with a phosphorylated NA [21]. The finding that L-HSA was ineffective indicated that the inhibition caused by L-poly(LYS)–FUdR was not due to the sugar moiety. Free FUdR was administered at the daily doses of 2.5, 5, and 10 μg/g. Even at the highest dose, the drug did not reduce the tumor growth.

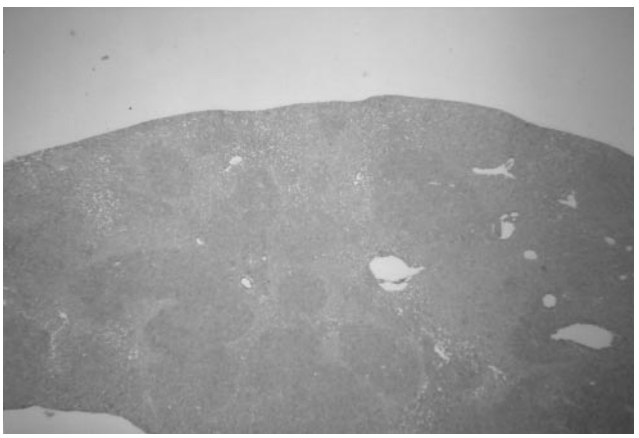

Fig. 1. Histological liver section from a tumor-bearing mouse injected with saline (Table 4, exp. no. 1). Section was stained with hematoxylin and eosin. Metastatic areas are easily detectable, being more intensively stained than normal liver parenchyma.

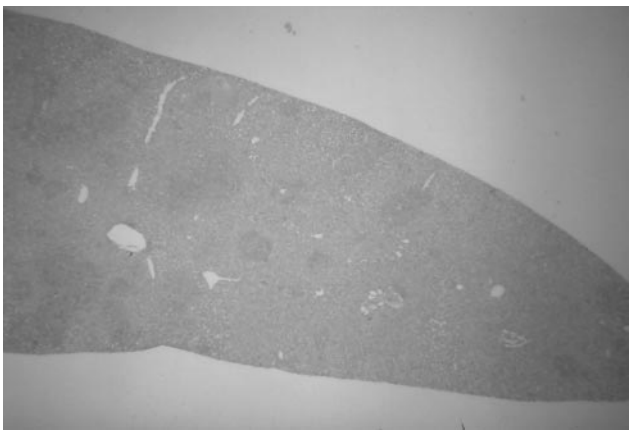

Fig. 2. Histological liver section from a tumor-bearing mouse injected with L-poly(LYS)–FUdR (Table 4, exp. no. 1). Section was stained with hematoxylin and eosin. Note the smaller metastatic areas compared to those in liver of saline-injected mouse.

4. Discussion

Coupling to L-poly(LYS) substantially enhanced the efficacy of FUdR against the hepatic metastases of C-26 cancer cells. Conjugated FUdR at the daily dose of 0.62 μg/g, which was >11 times smaller than the highest (7 μg/g) not producing toxic effects in healthy mice, inhibited the growth of the metastases. Free FUdR, even at a daily dose of 10 μg/g, which was 1–2 times lower than the non-toxic dose (17.5 μg/g), had no effect on metastases. The findings that L-poly(LYS)–FUdR was not toxic for C-26 cells *in vitro*, was selectively taken up by mouse liver and was stable in mouse blood indicated that the inhibitory activity on the growth of hepatic metastases was the consequence of FUdR (and/or its active metabolite FU [12]) release into bloodstream after the conjugate had been taken up by the hepatic cells.

The release of NAs from liver cells after administration of their hepatotropic conjugates was previously described [1,7–9]. The present results demonstrate for the first time that NAs can exit from hepatic cells in quantities sufficient to be pharmacologically active on cells infiltrating the liver. Although our experimental model may not reproduce the growth pattern of human hepatic metastases, the present findings encourage further experiments aimed at verifying whether L-poly(LYS)–FUdR might be a candidate for the treatment of small avascular hepatic metastases nourished by sinusoids (micrometastases) [10]. FUdR and FU are the most widely used chemotherapeutic agents for therapy against human colon carcinoma cells [12]. Their release into liver blood following conjugate administration could reproduce the locoregional treatment performed by the intraportal infusion of drugs [24–26], a procedure which was specifically developed for the therapy of micrometastases [24]. Administration of the conjugate by the peripheral venous route would have the advantage of avoiding risks of catheter complications and permitting repeated cycles of treatment starting prior to surgery. Established hepatic metastases larger than 200 μm are vascularized and cannot be a target for L-poly(LYS)–FUdR, since their central areas are mainly supplied by the hepatic artery [10] and they thus do not receive sinusoidal blood containing the drug released by hepatocytes. However, since the peripheral cells of these metastases are partly supplied by the adjacent liver sinusoids [10], L-poly(LYS)–FUdR might also improve the outcome of treatments directed at interrupting their arterial flow or at delivering tumoricidal agents via the hepatic artery.

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