Sodium selenite inhibition of the reproduction of some oncogenic RNA-viruses

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Summary. In mice infected by leukemia virus Rauscher, the addition of sodium selenite to the drinking water had an inhibitory effect on spleen growth. Sodium selenite, when added to bovine leukemia virus in vitro, markedly depressed reverse transcriptase activity. The in vitro results may provide a key to explain the in vivo observations.

Selenium inhibits carcinogenesis induced by different chemical agents such as amino-azo compounds, polycyclic aromatic hydrocarbons, nitrosamines and others[1-5]. The capability of selenite to inhibit the spontaneous appearance of mammary tumors in mice has also been demonstrated[6]. The low incidence of some neoplastic diseases in the population of certain regions of North America has tentatively been ascribed to the high concentration of selenium in the environment[7]. The mechanism of the antiblastomogenic action of selenium is unknown.

In the present work we have studied: a) the influence of sodium selenite on the reproduction of the murine leukemia virus-Rauscher (MLV-R), and b) the influence of sodium selenite on the activity of RNA-dependent DNA-polymerase from bovine leukemia virus (BLV) produced by foetal lamb kidney cells (FLK) chronically infected with BLV.

Materials and methods. In the in vivo experiments we used 222 male and 71 female mice from the BALB/c strain, average weight 17-19 g. The animals were infected i.p. with 0.5 ml of 10% spleen homogenate taken from Rauscher leukemic mice. Addition of sodium selenite to the drinking water, 20 mg/l, was started 7 days before the virus inoculation or as described in table 3. On the 21st day after the virus inoculation we killed the animals and weighed their spleens, accepting spleen weight as a criterion for the progression of the disease. Furthermore, a possible virocidal effect of sodium selenite on MLV-R was estimated by prior incubation of the virus with selenite. The incubation conditions are described in table 2.

In vitro, the influence of sodium selenite on the activity of reverse transcriptase of RNA-containing, oncogenic BLV produced by chronically infected FLK cells, was investigated. As a source of the virus we used the culture medium harvested on the 3rd, 4th or 5th days after plating of the FLK cells. The cells were maintained in Eagle's-MEM with 10% foetal calf serum. Sodium selenite was added to the tissue culture medium in nontoxic doses of 0.5, 1 or 2 mg/l 24 h before the reverse transcriptase activity assay. Previously the concentration of 2 mg/l had been determined to be the highest nontoxic dose for FLK cells. The cytotoxicity was determined 24 h after the addition of the trace substance on the basis of the changes in cell morphology observed by light microscopy: roundness, granulation and detachment of single cells. We defined BLV quantitatively by the reverse transcriptase reaction according to the method of Temin and Mizutani[8] using a 100-x-virus concentrate and an incubation mixture containing all precursors of DNA synthesis, Mg^{2+}, ^{3}H-TTP and non-ionic detergents. We used exogenous synthetic template - oligo (dT):poly(rA) - Calbiochem, as described earlier[9]. We evaluated the reverse transcriptase activity by the reaction

Table 1. Sodium selenite inhibition of splenomegaly induced by murine leukemia virus Rascher

Group	Number of mice per group	Average spleen weight (g)	Inhibition of splenomegaly (%)
MLV-R (control)	5	2.85 ± 0.19[a]	39.7[b]
MLV-R + Na_2SeO_3	8	1.72 ± 0.27	p = 0.05

[a] Mean ± SE. [b] 7 identical experiments have been carried out. Each of them showed an inhibition of splenomegaly from 20% up to 39.7%.

Table 2. Absence of any effect of treating murine leukemia virus Rauscher by selenite before inoculation

Number of the experiment	Treatment	Number of animals per group	Average spleen weight (g)
1[b]	MLV-R (control)	3	1.99 ± 0.64[a]
	MLV-R + Na_2SeO_3 2 mg/l	14	2.05 ± 0.13
	MLV-R + Na_2SeO_3 5 mg/l	14	2.32 ± 0.15
	MLV-R + Na_2SeO_3 10 mg/l	10	2.09 ± 0.20
2[c]	MLV-R (control)	15	0.20 ± 0.01
	MLV-R + Na_2SeO_3 2 mg/l	20	0.27 ± 0.02
3[d]	MLV-R (control)	18	1.35 ± 0.11
	MLV-R + Na_2SeO_3 2 mg/l	18	1.23 ± 0.08
4[e]	MLV-R (control)	20	1.91 ± 0.09
	MLV-R + Na_2SeO_3 2 mg/l	18	2.01 ± 0.11

[a] Mean ± SE. [b] MLV-R was incubated 60 min at 37 °C. [c] MLV-R was incubated 24 h at 37 °C. [d] MLV-R was incubated 5 min at 45 °C. [e] MLV-R was incubated 24 h at 4 °C.

Table 3. Influence of sodium selenite on the murine leukemia virus Rauscher induced splenomegaly in mice during different stages of leukemogenesis

Number of animals per group	Duration of treatment of animals with Na_2SeO_3 (days)	Average spleen weight (g)	Inhibition of splenomegaly (%)
9	–	3.82 ± 0.33[a]	–
9	0[b]-7	3.30 ± 0.24	13.6[c]
12	7-14	3.40 ± 0.15	11.2
9	14-21	3.23 ± 0.16	15.3
12	0-21	3.20 ± 0.20	16.3
11	7-21	3.43 ± 0.17	10.2

[a] Mean ± SE. [b] The day of the inoculation of MLV-R. [c] In all cases $p > 0.05$.

Table 4. Sodium selenite inhibition of the RNA-dependent DNA-polymerase of bovine leukemia virus

Concentration of Na_2SeO_3	Reverse transcriptase activity (cpm)		Inhibition (%)
	Positive control	Experiment	
0.5 mg/l	30461	14845	51
1 mg/l	12463	2439	80
2 mg/l	9046	1585	83

index representing the relation of cpm of the experiment (+template) to the control (−template) samples. At an index ⩾ 3 the reaction was considered positive.
The statistical analysis of the results was performed according to Student's t-test.
Results and discussion. The treatment of the animals with sodium selenite 7 days before the virus inoculation and during the whole experiment resulted in inhibition of splenomegaly up to 39.7% (table 1). The effect observed was independent of the sex of the animals. The treatment of non-inoculated mice with sodium selenite in drinking water did not influence the spleen weight. In some experiments any major direct virocidal action of the trace element was excluded (see table 2). The results summarized in table 3 show that sodium selenite treatment for different periods after the virus inoculation had a tendency to depress splenomegaly, though less markedly and, within the various series, non-significantly.
In order to obtain additional information on the mode of action of selenite we studied in vitro the influence of Na_2SeO_3 on the reverse transcriptase activity of BLV, another type C oncornavirus which was available. The results summarized in table 4 show a drastic depression of RNA-dependent DNA-polymerase activity. The mechanism of this effect is not clear.
Billard[10] and Oxford[11] show that selenocystine and selenocystamin inhibit the RNA-dependent RNA-polymerase of influenza A_1, A_2 and B viruses. The blocking of the enzyme is reversible. Its activity is restored by adding dithiothreitol or mercaptoethanol, which shows that the RNA-dependent RNA-polymerase is sensitive to sulfhydryl reagents. Selenium is able to displace sulphur from the sulfhydryl groups with formation of selenohydryl groups[12]. This fact may be involved in the effect observed by us.
We suppose that the sodium selenite inhibition of the splenomegaly induced in BALB/c mice with MLV-R is connected with the influence of the trace element on the reverse transcriptase activity of the virus. Possibly, the sodium selenite prevents the integration of the virus genome with the cellular DNA.
The suppressive effect of selenite on the reverse transcriptase activity may well account for its inhibition of carcinogenesis induced by different chemical agents.

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Localization of cholesterol in the Golgi apparatus of cardiac muscle cells

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Summary. Filipin (a polyene) interacts with cholesterol in membranes, producing distinctive deformations that can be detected by freeze-fracture. The distribution of filipin-induced deformations in the Golgi apparatus of cardiac myocytes suggests a role for this organelle in the transformation of cholesterol-poor membrane to cholesterol-rich membrane.

Cholesterol is an important constituent of cellular membranes, exercising a critical influence on their fluidity and permeability[2-4]. In muscle cells, cholesterol depletion of the plasma membrane leads to a significant increase in calcium and sodium influxes during depolarization[5], and the ATPase activity of sarcoplasmic reticulum (SR) is strongly inhibited by interaction of cholesterol with the calcium pump protein[6,7]. Although plasma membranes have a high cholesterol content compared with intracellular membranes[3,8-11], the mechanisms by which this difference is established and maintained are uncertain[11]. The polyene antibiotic, filipin, interacts specifically with cholesterol and related 3-β-hydroxysterols[12,13], producing distinctive deformations in membranes[14]. These deformations can be visualized by freeze-fracture electron microscopy[15,16], enabling direct localization of cholesterol in the membrane plane[17]. Using this approach in rabbit and rat cardiac muscle cells, I report here evidence for a specific distribution of cholesterol within Golgi apparatus membranes. The distal (mature) face appears cholesterol-rich, the proximal (forming) face cholesterol-poor, and the intercalary (mid-region) cisternae intermediate in cholesterol content. From these results, a Golgi apparatus function in enrichment of membrane cholesterol – as part of the process by which membranes are transformed from endoplasmic reticulum-like to plasma membrane-like – is proposed.
Materials and methods. Adult rabbits (New Zealand strain) and rats (BD IX strain) were killed by dislocation of the neck. Rabbit hearts were perfused by the Langendorff technique with Krebs-Henseleit buffer (5 min) followed by 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (5 min)[18]. Rat hearts were prepared similarly using a simple perfusion apparatus. Small (0.5 mm^3) samples of fixed left ventricle wall were immersed in the same fixative containing 100 µg filipin cm^{-3} for 3 h or 22 h in light-proof containers at 22 °C. Filipin was initially dissolved in dimethylsulphoxide (DMSO) before addition to the fixative solution; the final concentration of DMSO was 1%. Control tissue was treated in parallel with glutaraldehyde containing 1% DMSO, and with the fixative alone. Specimens were rinsed in cacodylate buffer and processed for freeze-fracture and thin-section[18] electron microscopy. For freeze-fracture, the tissue blocks were infiltrated with cacodylatebuffered 25% glycerol, frozen in melting propane,