Mung bean sprout *(Phaseolus aureus)* nuclease and its biological and antitumor effects*

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Bovine seminal ribonuclease (BS RNase), a dimeric homolog of bovine pancreatic ribonuclease (RNase A), is known to display special biological activities namely cytotoxicity for human tumor cells. Because some plant ribonucleases have a similar mass weight and structure as the animal ribonuclease, effects of a commercial product of Mung bean *(Phaseolus aureus)* nuclease (PhA) were studied on proliferation of ML-2 human tumor cells, as well as it's aspermatogenic, embryotoxic, immunogenic, and immunosuppressive activity, and therapeutic efficiency in athymic mice bearing human melanoma tumor. Concerning the antiproliferative activity, PhA nuclease was almost non-effective *in vitro* on ML-2 cells and also immunosuppressive activity on human lymphocyte in mixed culture was very low compared to that of BS RNase. However, significant antitumor activity was detected on human melanoma tumor after intratumoral or intraperitoneal administration into the mice. Furthermore conjugate of PhA nuclease with polyethylen glycol (PEG) injected seven times at the dose of 10 μg intraperitonealy showed identical antitumor activity as that of bovine seminal ribonuclease (BS RNase) injected by the same way at ten times higher dose.

Both PhA and BS RNases exerted strong aspermatogenic effect on the width of spermatogenic layers while RNase A administration at ten times higher concentration was ineffective. PhA nuclease when compared by means of antibody cross reaction with RNase A, BS RNase and wheat leaf neutral RNase (WLN-RNase) was found to be immunologically similar to RNase A and WLN-RNase, meanwhile BS RNase showed much higher antigenicity in comparison with them.

Key words: plant ribonuclease, aspermatogenesis, embryotoxicity, antitumor effect, melanoma, mice

Many plant nucleases were studied in relation to biological processes in plants [1–6] but there has been so far no information available concerning any possible biological activity of these enzymes on mammalian cells and tissues. An important property of the nuclease I group (to which many nucleases belong) is particular specificity towards secondary structures of nucleic acids and ability to cleave different homopolymers. Despite many observations suggesting that nuclease I group is involved in many processes, which are strongly regulated during the development and influenced by many factors such as light, phytohormones, wounding, water stress and infection with some plant pathogens [2, 4], a more exact analysis of nuclease I expression was obtained as for its participation in the processes of apoptosis and plant senescence [7–11].

On the other hand, some S-like plant nucleases and ribonucleases have a similar mass weight and structure as animal ribonuclease [3, 4, 7, 12, 13]. Nucleases I consist of a single polypeptide chain cross-linked by two disulfide bonds and similar disulfide (S-S) linkage stabilizing structure is also an important feature for bovine seminal ribonuclease (BS-RNase) [13]. It might be of interest whether or not the particular structural features of nuclease I homologs could be important to cause similar biological effect in animals. For our study we have chosen the commercial product of Mung bean nuclease, isolated from bean sprouts and have decided

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to study its antitumor activity both *in vitro* and *in vivo*, spermatogenic and embryogenic toxicity, as markers of the side effects, and also immunogenic and immunosuppressive activities.

Material and methods

Ribonuclease preparation. Mung bean (*Phaseolus aureus*) nuclease (PhA) and bovine pancreatic ribonuclease (RNase A) were purchased from MP Biomedicals, LLC (USA). Bradford-Protein Assay Kit for measuring the concentration of proteins was obtained from OZ Biosciences, France. Both products, bovine seminal ribonuclease (BS-RNase) and wheat ribonuclease (WLN-RNase), were isolated and purified in our laboratory from bull seminal plasma [14] and wheat leaves [39]. Onconase (ONC) was produced in *Escherichia coli* by recombinant DNA technology and purified as described previously [15]. Activated derivative of polyethylene glycol (PEG), MW 5129 was obtained from Sherwater Polymers, Inc. (USA). Polystyren microtiter plates (Gamma a.s. České Budějovice, Czech Republic) were used for the determination of antibodies. SwAMPx (Swine anti-mice IgG with peroxidase produced by Sevapharma, Prague, Czech Republic) was used for the antibody titration and measured with Titertek Uniskan, Flow Laboratories, Irvine, UK.

C*onjugation of PEG to PhA nuclease and BS-RNase.* As desirable point of enzyme attachment lysine and high reactive ester (N -hydroxyl-succinimidyl derivative) of PEG were used because linkage between amino groups of enzymes and PEG provides (under mild reactive condition) a stable conjugate relatively resistant to hydrolytic cleavage [16]. PEG was coupled under nitrogen atmosphere in 0.1 M sodium phosphate buffer pH 7.4 at 4 °C for 35 min. Immediately after this reaction free substances were removed by ultrafiltration through an Amicon PM-50 membrane and the conjugated product purified by size exclusion chromatography on Sephacryl S-300 in the above mentioned buffer. Eluted peaks were evaluated by UV photometry and their protein content was determined [17].

Assay of antiproliferative effect in vitro. The ML-2 cell line derived from human myeloid leukemia was used for testing anti-proliferative activity of free PhA nuclease and animal ribonucleases RNase A, BS-RNase or ONC, respectively as described previously [18]. Briefly, cells ($2x10^4$) in 0.2 ml of RPMI 1640 medium supplemented with fetal calf serum (10% v/v) were established in microtiter plates (NUNC, FB type) and cultivated in humidified atmosphere containing 5% of CO_2 for 48 h. Four hours before the termination of cultivation, samples were pulsed with 24 kBq of /6-^{3}H/-thymidine (specific activity 980 GBq/mmol, Institute for Research, Development and Application of Radioisotopes, Prague, Czech Republic). Cells were then collected with the Scatron harvester and the incorporated radioactivity was measured with the Beckman scintillation counter. The mean value of the three cultures containing a particular nuclease or ribonuclease was compared with that of untreated control cells and the percentage of inhibited proliferation calculated.

Assay of immunosuppressive activity on MLC culture. Immunosuppressive activity of PhA nuclease and other ribonucleases on human lymphocytes stimulated in mixed lymphocyte culture (MLC) was assessed as described previously [18]. Lymphocytes from two unrelated donors were mixed and cultivated in a humidified atmosphere containing CO_2 (5% v/v) at 37 °C in RPMI 1640 medium supplemented with mixed human AB serum (10% v/v). The known concentration of studied nuclease or comparative ribonuclease was added at the beginning of the experiment, and the cell mixture was incubated for 6 days. The pulsation with (6-^{3}H) thymidine and calculation of an inhibitory effect were carried out as described above.

Evaluation of therapeutic effect in nude mice. Antitumor activity of PhA nuclease, RNase A and BS-RNase, as well as PhA and BS-RNase conjugated with PEG were studied on athymic female nude mice CD-1 (Anlab-Charles River) weighing 18–20 g. Four to five mice in every experimental group were housed under aseptic condition [19]. Human melanoma was obtained from a surgical specimen cut in small pieces (3x3 mm) and transplanted to the nude mice subcutaneously on the right flank. Intratumoral (i.t.) and intraperitoneal (i.p.) treatment was initiated when the area of the transplanted tumor in mouse reached 5x5 mm. Nuclease and RNase preparations in free or PEG conjugated form were injected at the dose of 10 or 100 μg/mouse three times a week during 18 days. Tumor dimension was measured twice a week using a slide caliper (length x width x depth) [19].

Aspermatogenic activity assays. Sexually adult male ICR mice were injected a/ once in their left testis or b/ five times intraperitonealy once a week at the dose of 10 μg of PhA nuclease compared to 100 μg of RNase A or 10 μg of BS-RNase. Ten days after the intratesticular or after the last i.p. injection, the animals were sacrificed, their testicles excised and studied by histology examination. Body weight of the treated mice was determined in the course of the experiment. Degenerative effects on the testes were assessed such as decreased weight of the testes, decreased width of the spermatogenic layers and reduced seminiferous tubules diameter [20].

Embryotoxic effects determination. Two-cell embryos from superovulated C57/BL6 mice were flushed from oviducts approximately 36 h after mating. Embryos were cultured in CZB medium supplemented with bovine serum albumin (3 mg/ml). Cytotoxic activity of PhA nuclease, BS-RNase or ONC (10 μg/ml) and RNase A (100 μg/ml) was tested in cultured embryos kept for 72–96 h at 37.5 °C in humidified atmosphere containing CO_2 (5% v/v). Controls were prepared as above, but without addition of RNases. The development stage of the embryos was monitored with stereomicroscope [21].

Immunogenicity of tested ribonucleases. The immunogenicity (i.e. production of antibodies) of free PhA nuclease and comparative ribonucleases was determined as described

previously [21]. A non-competitive ELISA test was performed. Micro titer plate wells were coated with 25 μg of free PhA nuclease or some other ribonucleases. After washing the plates, antisera from mice treated with the above mentioned nuclease and ribonucleases and control sera from mice injected with PBS were serially diluted in wells and incubated at 37 °C for 2 h. The SwAMPX (Swine anti-mice IgG with peroxidase) conjugate diluted 1: 1000 was added and following 20 minute incubation with the substrate solution the reaction was stopped by adding 4.0 N H_2SO_4. The color reaction was measured at 450 nm on a photometer (Titertek Uniskan, Flow Laboratories, Irwine, UK). Antibody titers against PhA and other ribonucleases were detected and defined as positive when the optical density of the serum tested was found to be at least by 3 S.E.M. higher than that of controlled mice.

Histology. All mice injected intratesticularly, intratumorally and intraperitoneally were subjected to excision of testes and tumor enucleation. The small pieces of these tissues were consequently fixed in Bouin solution for histology examination. Fixed samples of tissues were embedded in paraffin blocks. Tissue slides (5 μm) were cut and stained with hematoxilin-eosin (HE).

Statistical analysis. Results are presented as a mean ± standard error mean (S.E.M.). The data were analyzed statistically using Fisher's t-test.

Results

Antiproliferative activity of PhA nuclease in vitro. The action of the plant nuclease and the comparative ribonucleases on human ML-2 cell line demonstrated in Figure 1 shows that the inhibitory effect of PhA nuclease on human tumor cell proliferation is very close to that of free RNase A and only negligible in comparison with free BS-RNase and ONC.

Immunosuppressive activity of PhA nuclease in vitro. PhA nuclease exerted only very little inhibitory activity on human lymphocyte proliferation in mixed lymphocyte culture (MLC) when compared with BS-RNase and ONC which exerted total inhibition of lymphocyte proliferation at very low concentrations (Fig. 2). Ten times higher concentration of RNase A was ineffective as reported earlier [19].

Antitumor activity of free PhA nuclease injected intratumorally (i.t.). The PhA nuclease and comparative ribonucleases (RNase A and BS RNase) were injected intratumorally into the athymic nude mice bearing human melanoma tumor and their antitumor activities were followed in the course of the three-week treatment (Fig. 3). PhA administered at a very low dose of 10 μg/mouse displayed a significant antitumor effect. Ten times higher dose of BS RNase (100 μg/mouse) was a little bit more efficient but the difference was not significant while the same dose of RNase A was nearly ineffective. PBS injected the same way as tested ribonucleases was considered as a control value (data not shown).

Antitumor activity of PEG conjugated RNases injected intraperitoneally (i.p.). In our previous experiments [19–21] we found out that RNase A after conjugation with PEG showed a significant antitumor effect in vivo after intravenous application while free RNase A was ineffective. After PEG conjugation to PhA nuclease and/or comparative

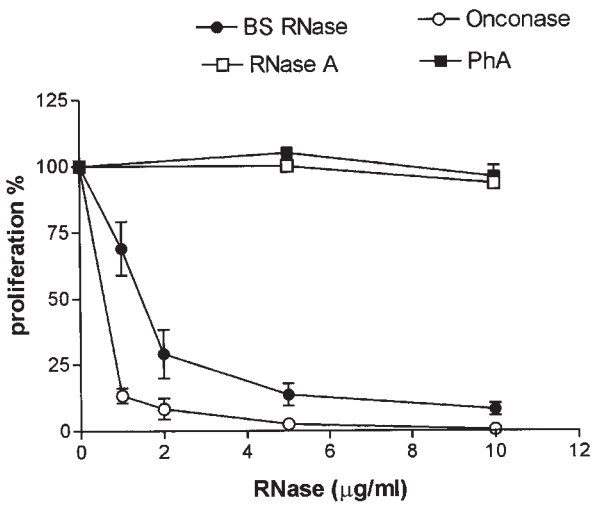

Figure 1. Effect of PhA nuclease, RNase A, BS-RNase and ONC on the proliferation of human tumor cell line ML-2 in culture. Proliferation was evaluated by the incorporation of [^{3}H]- thymidine into cellular DNA. The data represents the mean from three cultures in three experiments recorded 3 days after the addition of PhA nuclease or animal ribonucleases to the culture.

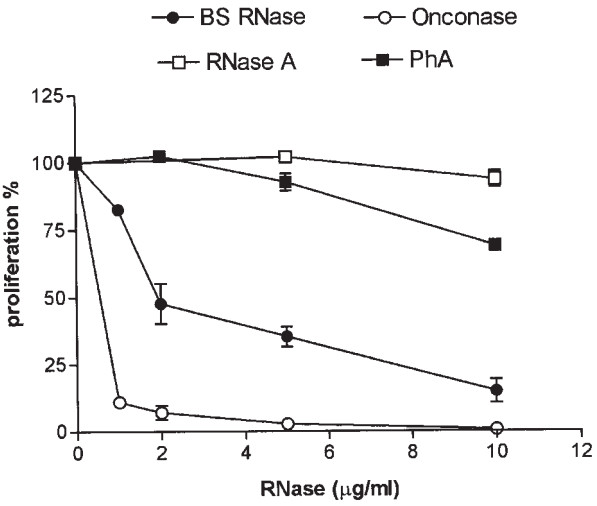

Figure 2. Effect of PhA nuclease on the proliferation of human lymphocytes in a mixed lymphocyte culture (MLC) in comparison with RNase A, BS-RNase and ONC. Cell proliferation was determined by incorporation of [^{3}H]-thymidine into cellular DNA after 6 days of incubation with the PhA or comparative ribonucleases. Each value represents the mean of the three cultures in three experiments containing an enzyme compared with that of untreated control cells.

BS-RNase, very small improvement appeared compared to the effect of free preparations demonstrated in Figure 3. As shown in Figure 4 PEG conjugates injected seven times i.p. at the dose of 10 μg of PEG-PhA or 100 μg of PEG-BS RNase into athymic nude mice proved again a significant therapeutic effect on tumor growth but no significant difference appeared to the results in Figure 3 demonstrated above. The tumor size of the mice treated with PEG conjugated PhA nuclease (10 μg) decreased from 100 to 34 percent and in the mice treated with PEG conjugated BS-RNase (100 μg) from 100 to 30 percent. The free PEG polymer injected in the same way as PEG-PhA nuclease did not differ from PBS controls (data not shown).

Aspermatogenic effect of PhA nuclease after injection into left testicles. In our previous reports BS RNase injected in the left testicles at the concentration of 100 μg/mouse exerted a very strong aspermatogenic effect [19, 21, 22]. In this experiment we used 10 times lower concentration of BS RNase comparable with the same quantity of PhA. The both PhA nuclease and BS-RNase exerted significant aspermatogenic effect on the width of the spermatogenic layers. As demonstrated in Table 1 the effect of PhA nuclease at the dose 10 μg/mouse was stronger than that of BS RNase while ten times higher concentration of RNase A was ineffective (data not shown).

Aspermatogenic effect of PhA nuclease after intraperitoneal injection. Intraperitoneal application of PhA nuclease and comparative ribonucleases into mice for 5 weeks displayed also aspermatogenic activity but less pronounced than that demonstrated above after intratesticular application. In any case both PhA nuclease and BS RNase exerted a significant degenerative effect on the width of the spermatogenic layers but in this case the effect of PhA did not differ from that of BS RNase (Tab. 2).

Embryotoxic effect of PhA nuclease. In our previous papers [21, 22] BS RNase and ONC at the concentration of 100 μg/ ml of cultivation medium displayed very high embryotoxicity in mice. Here, we have shown that ten times lower concentrations of both PhA nuclease and BS RNase displayed no toxicity, while the same concentration of ONC remained totally cytotoxic (Tab. 3).

Immunogenicity and immunologic relationship between PhA nuclease and comparative ribonucleases. The immunogenicity estimated as production of antibodies in the serum of mice injected with PhA nuclease and RNase A was expressed in very low titers from 0 to 320 as given in Table 4. An exception is the production of antibodies against BS-RNase in serum where the titers ranged from 1280 to 2560. All antibodies against RNases reacted with PhA nuclease in titer from10 to 40 including antibodies against BS-RNase. Antibodies produced against PhA homologous antigen reached the titer 160-320 in serum what means much less in comparison with the titer of homologous anti-BS-RNase antibodies (1280–2560). For this reason it is possible to acknowledge that PhA nuclease is significantly less immunogenic than

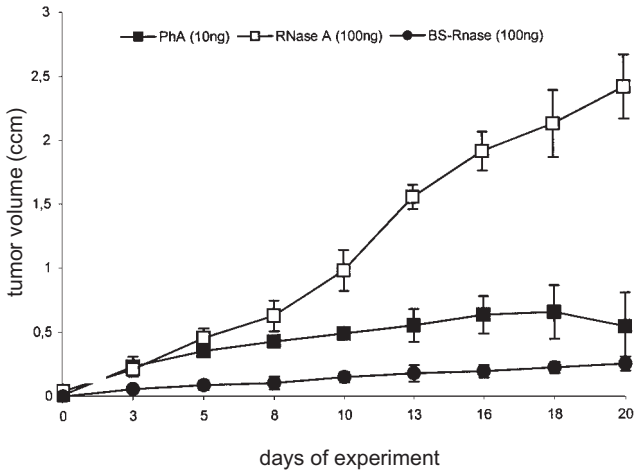

Figure 3. Human melanoma tumor growth in athymic nu/nu mice after intratumoral administration of PhA and bovine ribonucleases (RNase A and BS-RNase). Injections of PhA nuclease, RNase A and BS-RNase were given seven times at the doses of 10 μg of PhA and 100 μg of RNase A or BS RNase over a period of 20 days and tumor volume was determined twice a week.

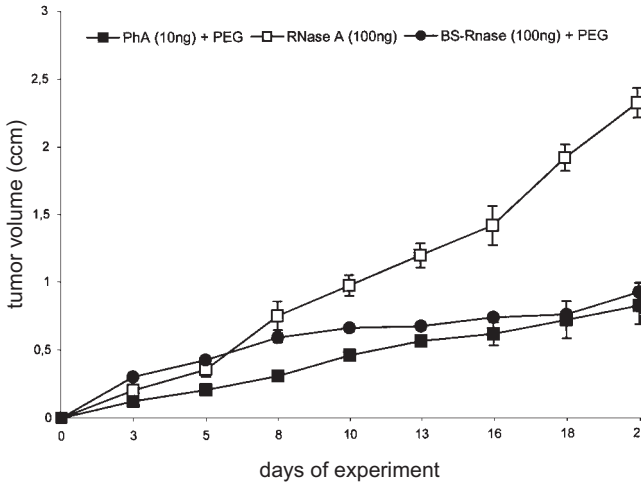

Figure 4. Human melanoma tumor growth in athymic nu/nu mice after intraperitoneal administration of PEG conjugated PhA and bovine seminal ribonuclease (BS RNase). Athymic nu/nu mice were injected 7 times over a period of 20 days at the doses of 10 μg of PEG-PhA and 100 μg of PEG-BS RNase and compared with 100 μg of free RNase A.

BS-RNase. The mutual immuno-reactivity of PhA nuclease with WLN RNase and also with animal RNase A and BS-RNase constitutes supposition about the existence of some common antigens between these phytogenetically different species.

Histology of tumor cells growing in athymic nu/nu mice in-

Table 1. Aspermatogenic activity after injection of PhA nuclease, RNase A and BS RNase into the left testes of mice

Substances injected dose in μg/mouse	No. of mice	Index weight of testes ± S.E.M.		Width of spermatog. Layers ± S.E.M.		Diameter of seminiferous tubuli ± S.E.M.	
		Injected testes	Non-inj. testes	Injected testes	Non-inj. testes	Injected testes	Non-inj. testes
PBS	5	42 ± 8	40 ± 6	60 ± 7	62 ± 9	151 ± 7	152 ± 12
RNase A 100	5	41 ± 5	43 ± 3	64 ± 4	62 ± 7	151 ± 6	154 ± 5
BS Rnase 10	4	39 ± 4	41 ± 3	24 ± 6^{**a}	61 ± 7	137 ± 9*	153 ± 5
PhA 10	4	22 ± 4**	44 ± 3	5 ± 9^{**a}	49 ± 7	98 ± 22**	144 ± 5

[a] – differences between width of spermatogenic layers after injections of BS RNase (24±6) and PhA (5±9) are significant ($p<0.01$). *significant at $p<0.05$, **significant at $p<0.01$

Table 2. Aspermatogenic activity of PhA nuclease, RNase A and BS RNase after their repeated intraperitoneal application into the mice

Substances injected dose in μg/mouse	No. of mice	Index weight of testes ± S.E.M.	Width of spermatogenic layers in μm ± S.E.M.	Diameter of seminiferous tubules of testes in μm ± S.E.M.	Body weight in g	
					Before	After experiment
PBS	5	93 ± 6	65 ± 8	163 ± 6	23 ± 1	25 ± 1
RNase A 100	5	90 ± 6	66 ± 11	164 ± 9	22 ± 1	25 ± 1
BS Rnase 10	5	101 ± 6	32 ± 5**	141 ± 8**	25 ± 1	24 ± 1
PhA 10	5	90 ± 7	43 ± 2**	159 ± 4	24 ± 1	24 ± 1

Mung bean nuclease (PhA), bovine pancreatic ribonuclease (RNase A) and bovine seminal ribonuclease (BS RNase) were injected once a week in the course of following five weeks and their aspermatogenic effects in the both testes of a mice were determined. **significant at $p<0.01$

Table 3. Embryotoxic activity of PhA nuclease, RNase A, BS RNase and Onconase in mouse embryos

Ribonuclease (μg/ml)	No. of mice embryos	Number of embryos in cell stages			
		Blastocysts	Expanded blastocysts	Total blastocysts	Blastocysts %
PBS	12	7	1	8	66
RNase A 100	10	2	4	6	60
BS RNase 10	11	3	3	6	55
Onconase 10	14	0	0	0	0**
PhA 10	13	7	0	7	54

Development of mice embryos after 72 hours incubation with mung bean nuclease (PhA), bovine pancreatic ribonuclease (RNase A), bovine seminal ribonuclease (BS RNase) and Onconase. Indexes 10 and 100 mean μg/ml of an RNase. **significant at $p<0.01$

jected by PEG conjugated PhA nuclease. Histology of melanoma tumors growing in athymic nu/nu mice confirmed the degenerative events in tumor after seven i.p. injections of 10 μg of PEG-PhA. The same procedure with 100 μg of free RNase A did not show any degenerative effects (Fig. 5).

Discussion

Plant nucleases I, major plant sugar nonspecific endonucleases form rather heterogenous group of catalytically related enzymes [2, 6, 23]. Their activity has been reported in a number of plant species like pollen extracellular nuclease from evolutionary old *Pinus nigra* [24] through various duo-cot species like tobacco [25–28], mung bean [29], sugar cane [30], tomato [31], zinnia [32], hop [24, 33], alfalfa [34] to various mono-cot plants like maize [24], wheat [35] and barley [10, 11]. This selected list of species demonstrates clearly that sugar non-specific nuclease I is an universal enzyme widely spread in higher plants.

An important property that suggests intimate heterogeneity within the nuclease I group is the specificity towards secondary structures of nucleic acids and the ability to cleave different homopolymers. On one hand, there are enzymes single strand specific only (like mung bean nuclease), and on the other hand enzymes having ability to cleave different secondary structures including double-stranded DNA or supercoiled plasmid DNA [6] as well as dsRNA with limited activity [25, 33].

Many plant nucleases and ribonucleases have been studied in plants in relation to gametophytic self-incompatibility function, senescence, phosphate starvation, plant diseases, seed development or germination and root, flower or fruit development [2, 4, 23]. It is therefore such a situation that there is lack of research concerning possible biological activity of RNA and DNA-degrading enzymes from higher plants on mammalian cells and tissues. Despite of very limited literary data available so far, antiproliferative activity from roots of sanchi ginseng (*Panax notoginseng*) [36], ribosome inactivating protein from mature seeds of pumpkin (*Cucurbita maschata*) [37] and/or ribotoxin with ribonuclease activity from mature seeds of oriental arbovital (*Biota orientalis*) [38] respectively, were isolated. All of these substances were studied under *in vitro* conditions. Since some S-like plant RNases and nucleases have similar molecular mass as animal ribonucleases [4, 7, 12] and nuclease I consists of a single polypeptid chain cross linked by two disulfide bonds as bovine seminal BS-RNase [13], we decided to compare mung bean nuclease with bovine RNase A and BS-RNase and also with wheat neutral RNase (WLN-RNase) [39].

We supposed that Mung bean nuclease as a representative of nucleases strongly single strand specific, might be suitable for utilization in some biologic and antitumor studies in animals. However the first experiments *in vitro* on human tumor cell lines did not show any remarkable antiproliferative effect. In comparison with bovine BS-RNase the antiproliferative activity of this nuclease was negligible similarly to

that we had found also in studies with WLN-RNase [39]. Also the immunosuppressive activity of PhA nuclease tested *in vitro* on human mixed lymphocyte cultures was found to be negligible compared to that of ONC and BS-RNase which caused actually total inhibition. It was of interest that the antitumor action of PhA nuclease tested *in vivo* on human melanoma tumor was successful. However, similarly to the results with wheat ribonuclease (WLN-RNase) [39] the improved antitumor efficiency of PhA nuclease conjugated to PEG was also observed. It could be suggested that this situation simulated that of PEG-conjugated RNase A, which was also *in vitro* totally ineffective and acquired antitumor activity after conjugation with PEG [22], PHPMA (polyhydroxypropyl metacrylamid) [40] and/or hyaluronidase [21]. The activated ability of these polymers for enhancing the antitumor effect of RNase A and probably also for PhA, might be attributed to the existence of three probably positive mechanisms of actions. First, it can protect the PhA nuclease against the action of cytoplasmic nuclease inhibitor and increase its cytotoxic activity. Second, the half life of the plant enzyme in blood circulation is prolonged, and thus the possibility to reach the site of action has been improved, and third, the modification of nuclease enzyme by a polymer increases its resistance to proteolytic attack and prevents binding of antibodies directed against PhA [19, 22]. These three actions are, of course, in some respect rather hypothetical since we know only very little about nuclease inhibitors from literature and from our contemporary experiments. Besides the positive effect of PEG polymer on PhA nuclease antitumor activity we have to bear in mind that this enzyme has been also active in its free form injected intratumorally. According to the problem concerning the preparation of a more concentrated product, we decided to test the antitumor activity of PhA at ten times lower concentration than we had used with BS-RNase. The experiments *in vivo* confirmed that 10 μg of PhA nuclease exerted the same antitumor activity as 100 μg of BS-RNase.

Table 4. Production of antibodies in mice injected with various plant and animal RNases and their reciprocal immunological cross-reactions with injected enzymes

Enzyme	PhA nuclease	PhA nuclease	PhA nucleas	PhA nucleas
Antibody type	anti – PhA	anti – WLN	anti–RNase A	anti–BS RNase
Titer	160 – 320	20 – 40	20 – 40	10 – 20
Enzyme	WLN–RNase	WLN–RNase	WLN–RNase	WLN–RNase
Antibody type	anti – PhA	anti – WLN	anti–RNase A	anti–BS RNase
Titer	40 – 80	160 – 320	20 – 40	80 – 160
Enzyme	RNase A	RNase A	RNase A	RNase A
Antibody type	anti – PhA	anti – WLN	anti–RNase A	anti–BS RNase
Titer	0 – 10	40 – 80	160 – 320	10 – 20
Enzyme	BS RNase	BS RNase	BS RNase	BS RNase
Antibody type	anti – PhA	anti – WLN	anti– RNase A	anti–BS RNase
Titer	160 – 320	80 – 160	80 – 160	1280 – 2560

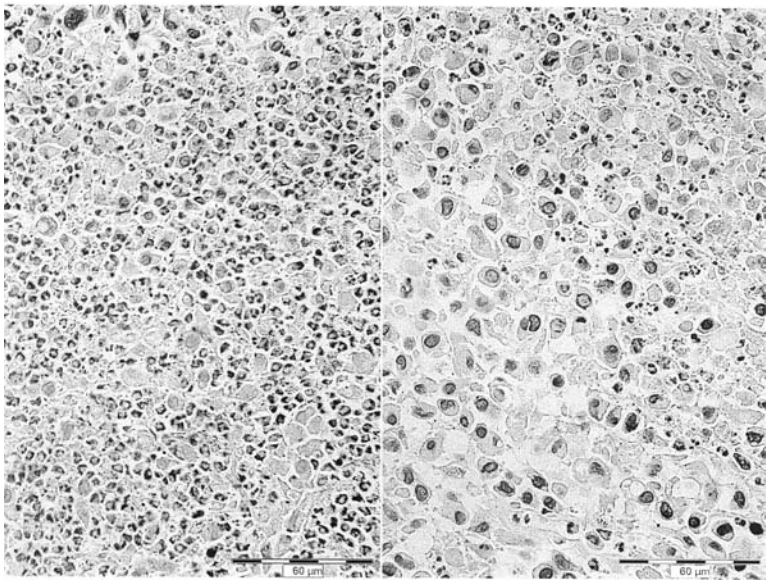

Figure 5. Effect of PEG-PhA nuclease and free RNase A on human melanoma tumor. Athymic nu/nu mice bearing human melanoma tumors were injected intraperitoneally (i.p.) seven times at the dose of 10 μg of PEG-PhA (left part) or 100 μg of RNase A (right part of the picture). On the left side the tumor cells are degenerated and the tumor size is much smaller in comparison with tumors from mice injected by RNase A. On the contrary the tumor cells on the right side are without substantial degeneration and tumor size is significantly greater in comparison with that growing in mice injected with PhA nuclease.

What concerns the immunogenicity and antigenic relationship between PhA nuclease and RNase A and BS-RNase, it may be suggested that certain antigenic similarity exists between PhA and animal ribonucleases, mainly with BS-RNase. The similar results were obtained also in a study on wheat leaf neutral ribunuclease and some animal ribonucleases [39]. In both cases bovine seminal enzyme was significantly more immunogenic than plant nucleases. It was proved by antibody production and differences in antigenic reactivity of homologous antibodies with PhA nuclease and BS-RNase.

If these statements resulting from our findings could be confirmed in further experiments it would be possible that some other nucleolytic enzymes would be developed which could be more suitable for antitumor studies than animal and plant ribonucleases.

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