In a preliminary experiment in vitro, we found that selenite did not influence the oxidation of Hb produced by incubation of erythrocytes with nitrite. Therefore, the suppressive effect of selenite on methemoglobinemia may be due to increased reduction of metHb. Similar results have been obtained on the effect of selenite on methemoglobinemia produced by aniline or phenylhydrazine[4]. Nitrite is also known to cause marked hypotension by vasodilation[7]. However, we found that 3.0 mg/kg of sodium selenite, when given i.v. to rats 1 min before nitrite (20 mg/kg, i.v.), had no effect on the hypotension caused by sodium nitrite. Therefore, the protective effect of selenite against the lethal effect of nitrite seems to be due to its action on nitrite-induced methemoglobinemia.

The doses of 0.1 mg/kg and 0.5 mg/kg of selenite used in the experiments on methemoglobinemia corresponded to about 20 times and 100 times the daily requirement, respectively. The form of selenium in the diet is unknown, but it is possible that dietary selenium participates in a physiological process of reduction of metHb.

Effect of selenite on sodium nitrite poisoning in mice

$NaNO_2$ (mg/kg, i.p.)	Mortality (dead/total) Saline	Na_2SeO_3 (1.0 mg/kg)	(3.0 mg/kg, s.c.)
162	0/6		
178	7/16	1/6	0/6
195	10/15	3/6	4/16
215	5/6	4/6	3/6
236	4/5	3/6	8/16
260	6/6	5/6	5/6
LD_{50}	178.0 (159.1-199.2)	208.9 (185.5-235.3)	223.9 (183.7-272.9)
Potency ratio		1.17 (1.07-1.29*)	1.26 (1.14-1.38*)

Selenite was injected immediately after treatment with nitrite. Confidence limits ($p<0.05$) are shown in parentheses. *Significantly different from value for saline control ($p<0.05$).

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Reduction of acute toxicity of cyclophosphamide and X-rays by the new immunomodulating compound BM 12.531[1]

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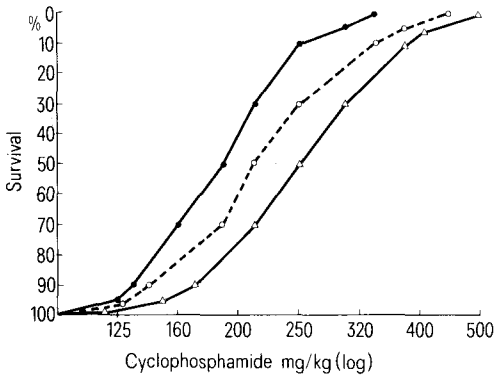
Summary. BM 12.531, the 2-[2-cyanaziridinyl-(1)]-2-[2-carbamoylaziridinyl-(1)]-propane, (prop. INN Azimexon), reduces significantly the acute toxicity of cyclophosphamide and X-rays in rats and mice, respectively. The leucopenia induced by X-rays was partially compensated by BM 12.531 in rats.

BM 12.531 (prop. INN Azimexon), 2-[2-cyanaziridinyl-(1)]-2-[2-carbamoylaziridinyl-(1)]-propane, derivative of the 2-cyanaziridine[2-6], has interesting therapeutic and immunomodulating effects[7-11]. This compound has therapeutic effects against various transplantation tumors[11] and increases resistance in bacterial, fungal and viral infections[12]. Therapeutic effects can be explained by stimulation of cellular immune functions for example T-lymphocytes[8] and macrophages[11]. The induction of leucocytosis in rats[13] by BM 12.531 and the increase of the colony-forming-units[11] in mice suggest a direct bone marrow stimulation. The aim of the present investigation was to demonstrate the influence of BM 12.531 on the acute toxicity of cyclophosphamide in rats and of X-rays in mice. Cyclophosphamide and X-rays especially damage the bone marrow and up to now no agent with a low molecular weight is known which can be used as a therapeutic drug after toxification by cyclophosphamide or X-rays.

Materials and methods. Substances. BM 12.531[5] was dissolved for administration in 0.5% methyl-cellulose or 0.9% NaCl-solution. Cyclophosphamide from the Asta Company, Chem. Werke Brackwede, Germany, was used.

For X-irradiation an X-ray apparatus set at 20 mA and 200 kV with a filter of 0.5 mm Cu was used. The dose was 70 r/min. The total dose used for rats were 175 and 350 r and for mice 650 r.

1. Influence of BM 12.531 on the leucopenia induced by X-irradiation. The experiments were carried out in mature female Sprague-Dawley rats from Wiga Company (Gassner, Sulzfeld, FRG), weighing 180-220 g. They were kept under constant temperature (23 ± 1 °C) and a constant relative humidity (55 ± 5%) over a 12-h day/night-rhythm. The animals were fed with Sniff pellet food from the



Influence of BM 12.531 on the acute toxicity of cyclophosphamide in rats (7 days after cyclophosphamide administration). ●——●, Cyclophosphamide. ○- -○, Cyclophosphamide + 3 × 10 mg/kg BM 12.531. △——△, Cyclophosphamide + 3 × 25 mg/kg BM 12.531. BM 12.531 was given 2, 24 and 48 h after cyclophosphamide administration.

Table 1. Influence of BM 12.531 on the leucocyte number of rats pretreated with 175 R (n = 10)

Day	Number of leucocytes ($\times 10^3/\mu l$)						
	0	4	6	8	11	13	15
Control	11.6±0.8	2.2±0.2	3.9±0.4	2.9±0.2	4.6±0.3	5.2±0.5	6.9±0.3
BM 12.531 50 mg/kg i.p. 3 times a week	11.8±0.6	2.3±0.2	5.9±0.4	5.5±0.4	5.1±0.3	5.6±0.4	6.9±0.3
Significance compared to control (t-test)	n.s.	n.s.	−3.77/17 $p<0.001$	−8.44/17 $p<0.001$	−1.17/18	n.s.	n.s.

n.s., not significant.

Table 2. Influence of BM 12.531 on the leucocyte number of rats pretreated with 350 R (n = 10)

Day	Number of leucocytes ($\times 10^3/\mu l$)									
	0	2	4	7	9	11	14	16	18	23
Control	10.5±0.8	1.7±0.2	1.4±0.1	2.0±0.1	2.9±0.3	2.8±0.4	4.0±0.3	6.0±0.6	6.5±0.8	7.9±0.6
BM 12.531 50 mg/kg i.p. 3 times a week	10.3±0.4	1.9±0.2	1.7±0.1	3.2±0.3	3.8±0.2	3.2±0.4	5.9±0.5	7.5±0.5	10.7±1.3	7.8±0.6
Significance compared to control (t-test)	n.s.	n.s.	−1.6/17 $0.25<p<0.1$ n.s.	−3.81/18 $p<0.002$	−2.31/18 $p<0.05$	−0.7/18 n.s.	−3.08/17 $p<0.01$	−2.2/18 $p<0.05$	−2.79/18 $p<0.02$	n.s.

n.s., not significant.

Table 3. Lethality of x-irradiated mice (650 R) after treatment with BM 12.531

	Dead 20th day	30th day	Significance compared to control (30th day)
Control	16/20	19/20	-
5 mg/kg twice a week	13/20	13/20	n.s.
50 mg/kg twice a week	8/20	11/20	$\alpha = 0.1504$

n.s., not significant.

Intermast Company, Soest, FRG, and water ad libitum. Groups of 10 rats were treated on the 1st day with a dose of 175 r and 350 r, respectively. On day 4 and at intervals of 2 or 3 days after irradiation blood was collected with a heparinized lance capillary from the retroorbital venous plexus and the leucocytes were counted by means of a Coulter Counter®. BM 12.531 was given in a dose of 50 mg/kg i.p., dissolved in 0.9% NaCl. First application was 3 h after irradiation. This was repeated 3 times a week. The control group was treated with an equal volume of 0.9% NaCl-solution. Statistical analysis was carried out using the Student's t-test.

2. Influence of BM 12.531 on mortality of mice treated with X-rays. Female NMRI-mice, 22–27 g (Wiga, Gassner, Sulzfeld, FRG) were used. They were kept under the same conditions as described above. The animals were irradiated with 650 rad. BM 12.531 was given in doses of 50 and 5 mg/kg i.p., dissolved in 0.9% NaCl-solution. The 1st treatment was made 3 h after irradiation and was repeated twice a week. The control group was treated with an equal volume of 0.9% NaCl-solution. A statistical analysis was made by means of the tied linear rank test[14].

3. Reduction of acute toxicity of cyclophosphamide by BM 12.531 in rats. The LD_{50} of cyclophosphamide was calculated by the method of Litchfield and Wilcoxon[15]. 10 mature Sprague-Dawley rats (5 female and 5 male, Ivanovas GmbH & Co., Kisslegg, FRG), weighing 150–220 g, were used for each dose group. Doses of cyclophosphamide were 320, 200, 160 or 125 mg/kg. Cyclophosphamide was given i.v., dissolved in saline solution. BM 12.531, dissolved in 0.5% tylose was administered orally in doses of 3×10 mg/kg or 3×25 mg/kg, 2, 24 and 48 h after cyclophosphamide injection. The LD_{50} was calculated 7 days after cyclophosphamide administrations by means of probit analysis. The dose-response relationship from mortality was plotted graphically.

Results and discussion. X-irradiation (175 r) reduced the number of leucocytes in rats (table 1). If BM 12.531 was given 3 times a week at a dose of 50 mg/kg a clear effect on the number of leucocytes could be demonstrated: on the 6th and 8th days after irradiation a significant difference in the number of leucocytes compared with the untreated control could be demonstrated. When the dose of radiation was increased to 350 r (table 2) a significant compensation of the leucopenia induced by the X-irradiation could be demonstrated on the 7th, 9th, 14th, 16th and 18th days after administration. Similiar effects were demonstrated after administration of cyclophosphamide[13].

The irradiation of mice with a dose of 650 r kills a high percentage of the treated mice till the 20th day (16/20). After treatment with 50 mg/kg BM 12.531, twice per week, i.p., the lethality was reduced (8/20). A slight effect could be demonstrated after administration of the lower dose of 5 mg/kg (twice/week, i.p.) (table 3).

Results presented in the figure show clearly that BM 12.531 is able to reduce also the acute toxicity of cyclophosphamide. The LD_{50} of cyclophosphamide is increased from 190.3 mg/kg (166.6–212.4, cyclophosphamide alone, curve 1) to 219.5 mg/kg (190.9–250.9, cyclophosphamide $+3\times 10$ mg/kg BM 12.531, curve 2) and to 249.3 mg/kg (218.8–293.9, cyclophosphamide $+3\times 25$ mg/kg BM 12.531, curve 3). Curve 3 is significantly different from curve 1 (p 0.05 level probit analysis). Curve 2 does not differ significantly from curve 1.

These investigations clearly demonstrate that BM 12.531 can restore the immune system after it has been damaged with cyclophosphamide or X-irradiation. Because of the high bone marrow toxicity of cyclophosphamide and X-rays we suggest that the good therapeutic effects of BM 12.531 are caused by direct stimulation of the bone marrow. Furthermore it could be demonstrated that the number of CFU_s[11] is increased in mice after treatment with BM 12.531. Unpublished data of Braun et al.[16] have shown

that also the number of CFU_c in mice is increased after administration of BM 12.531. Because of the bone marrow toxicity of many drugs used in cancer chemotherapy and of therapeutic radiation, BM 12.531 might be an efficient adjuvant in cancer chemotherapy. Moreover BM 12.531 might be a valuable drug after radiation accidents.

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High K^+ content explains the abolition of the action potential in amphibian sciatic nerve in vitro by *Lathyrus sativus* seed extract

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Summary. The action potential in amphibian sciatic nerve in vitro has been reported to be abolished by the topical application of *Lathyrus sativus* seed extract. We have confirmed this effect, but find that it is probably caused by the high K^+ content of such seed extracts and that organic neurotoxins are not implicated.

The consumption of *Lathyrus sativus* seed by man causes neurolathyrism[2,3]. A number of toxic substances occur in these seeds[4-6] and one of them, β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP), is believed to be the main cause of neurolathyrism, but the evidence is inconclusive[7]. A report that the action potential in toad sciatic nerve in vitro was abolished by the topical application of *L. sativus* seed extract[8] suggested a comparatively simple experimental system for evaluating the relative importance of the numerous neurotoxins in *L. sativus* seed.

Materials and methods. Frog sciatic nerves *(Rana pipiens)* were dissected and desheathed. Action potentials were recorded by placing the nerve on silver wire electrodes, and stimulating supramaximally. Nerves were bathed in Ringer solution containing NaCl (115 mM), KCl (2 mM), $CaCl_2$ (2 mM), glucose (11 mM) and Tris (10 mM) at 20 °C.

Ground, decorticized, *L. sativus* seed (200 g) was extracted in 1.2 l 30% v/v aqueous ethanol and heated for 2 h at 85 °C under reflux[8]. The mixture was cooled and centrifuged at 10,000 × g for 15 min at 20 °C; the supernatant fluid was concentrated to approximately 60 ml and dialyzed against 3 changes of 350 ml water. The dialysate was freeze-dried and dissolved in water to give a final volume of 16 ml.

Results. When frog sciatic nerves were bathed in a 5% v/v dilution of this extract with Ringer solution (equivalent to 0.625 g seed/ml), the action potential was abolished within 30 min, while control nerves, bathed in Ringer solution alone, were unaffected. Affected nerves recovered after bathing in Ringer solution for 20 min. The concentrations of some ions in the seed extract were determined by flame photometry; the concentrations of β-ODAP and the corresponding α-isomer derived from it[9] were determined by quantitative paper electrophoresis at pH 1.9[9,10]. The results are shown in table 1.

The $[K^+]$ of the diluted seed extract was sufficiently high to suggest that it might abolish the nerve impulse[11] and this was confirmed by bathing nerves in Ringer solution containing a $[K^+]$ concentration which simulated that in the diluted seed extract.

To investigate further whether K^+ or organic neurotoxins were responsible for the activity of the seed extract, a number of solutions was prepared and tested on the nerve preparation. K^+ was removed from a sample of seed extract by precipitation with sodium cobaltinitrite at 0 °C. A

Table 1. Content of some substances in decorticized *L. sativus* seed

Substance	Content in seed (m moles/kg) ± SEM (n)	Mean concentration (mM) in 5% v/v dilution of seed extract in Ringer solution
Na^+	< 0.08 (3) (below detection)	115
K^+	92.0 ± 0.07 (3)	57
Mg^{2+}	9.70 ± 0.01 (3)	6.0
Ca^{2+}	1.00 ± 0.01 (3)	2.5
Mn^{2+}	0.13 (1)	0.08
β-ODAP	20.0 ± 0.20 (4)	12.5
α-ODAP	6.80 ± 1.80 (4)	4.2

Table 2. Effect of diluted seed extract, or solutions simulating its composition, on the action potential in frog sciatic nerve in vitro

Component in Ringer solution	Effect on the action potential after 30 min at 20 °C
1. Seed extract (0.625 g seed/ml)	Reversibly abolished
2. Seed extract (ashed)	Reversibly abolished
3. Seed extract (sodium cobaltinitrite treated)	None
4. KCl (57 mM)	Reversibly abolished
5. KCl (57 mM) (sodium cobaltinitrite treated)	None
6. α-ODAP (4.2 mM)	None
7. β-ODAP (12.5 mM)	None
8. α-ODAP (4.2 mM) plus β-ODAP (12.5 mM)	None
9. $MgCl_2$ (6.0 mM) plus $MnCl_2$ (0.08 mM)	None