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Konstantin Muranov
Nicolas Poliansky
Rudolf Winkler
Gebhard Rieger
Otto Schmut
Jutta Horwath-Winter

Protection by iodide of lens from selenite-induced cataract

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In memory of Prof. Otto Hockwin (1925–2002), honorary doctor of the Medical Faculty of the University of Halle, Germany

K. Muranov (✉) · N. Poliansky
Emanuel Institute
of Biochemical Physics RAS,
Kosygin Street 4, 117344 Moscow, Russia
e-mail: k.muranov@sky.chph.ras.ru
Tel.: +7-095-9397441
Fax: +7-095-1374101

R. Winkler · G. Rieger
Paracelsus Society for Balneology
and Iodine Research, Bad Hall, Austria

O. Schmut · J. Horwath-Winter
University Eye Hospital, Graz, Austria

Abstract *Background:* Iodide has been used empirically against different age-related eye diseases, including cataract. The purpose of the present study was to investigate the effect of iodide on selenite-induced cataract in rat lens. *Methods:* Young white rats received subcutaneously sodium selenite (20 and 30 nmol/g b.w.) on day 13 post partum (p.p.). Cataract development was measured by expert estimation and image data analysis. Potassium iodide (1.5 nmol/g b.w.) was given (1–5 times) i.p. at different times with respect to the selenite administration. Lens opacification was analyzed in selenite, selenite–iodide, iodide and control groups on day 7 after selenite administration. *Results:* Iodide showed a significant protective effect against selenite cataract when injected 2 days (2 times) before selenite injection, i.e., on days 11 and 12 p.p. No significant effects on lens opacity were found: (1) after only one iodide injection (on day 12 p.p.), (2) after an initial iodide administration 1 h before selenite and (3) after injections of iodide once a day for 5 consecutive days. The protective effect of iodide was the same (about 50%) for both selenite doses used. *Conclusions:* There is a time-dependent protective influence of iodide against selenite cataract development. It is supposed that the anti-cataract effect of iodide could be based on direct or indirect antioxidant mechanisms.

Introduction

Iodine-containing waters have been used empirically in Bad Hall (Austria) against different eye diseases, including incipient cataracts, for a long time [4]. Cataractogenesis is a multifactorial pathological process in which many risk factors and different causes are involved [5]. Nevertheless, it has been shown that free radicals and, in consequence, oxidation damage are increasingly responsible for the opacification of the lens [7, 8, 9, 12, 16, 17]. Iodide displays some kind of protective effects against reactive oxygen species, especially in the lens, as demonstrated by Elstner et al. in model experiments [3]. Moreover, increased glutathione peroxidase activity has been found in the livers of rats after administration of iodide at doses which are relevant in balneotherapeutic treatment [18]. It was also found that iodide delayed the onset and development of Emory mouse cataracts—whose development is associated mainly with a weakening in the antioxidative defenses of the lens—by administration of iodide via the drinking water during the ca. 50 weeks of the experiment [1]. However, the molecular mechanisms of iodide effect on cataractogenesis are still not understood. As the selenite-induced cataract is a cataract model mainly dependent on oxidative stress, in which oxidation of the critical sulfhydryl groups is essential for the initiation of cataractogenesis [14, 15], this model seems to be appropriate to study the iodide effect on oxidative damages of lens structures.

We used two main schemes of iodide administration: Preceding injections (before selenite administration) were used for the creation of an essential iodide concentration in the lens, perhaps causing a preventive effect. Injections after selenite were used for study of the influence of iodide on the process of cataract formation. The results showed that only preceding injections were effective against cataract. Iodide dose (1.5 nmol KI/g body weight) was deduced primarily from a therapeutically applied iodine drinking cure as used in Bad Hall, corresponding to about 200 µg/kg body weight.

Materials and methods

Animal care and cataract induction

White rat mothers and their litter were kept in individual cages. They were fed a laboratory chow rodent diet (Agriculture Technologies, Moscow, Russia) and water, ad libitum. Temperature was maintained at 20°C and light was turned on and off at 12-h intervals. To initiate cataract, the rat pups were injected subcutaneously on day 13 post partum with a solution of sodium selenite, Na_2SeO_3 (Sigma Chemicals, St. Louis, MO, USA), dissolved in 0.9% NaCl to give doses of 20 and 30 nmol/g body weight of selenite. Following selenite injection, opacification progressed rapidly to maturity by day 4 or 5 post injection. Observations were made on day 7 after selenite administration under Nembutal narcosis. Lens opacification was observed by photo slit-lamp microscope (SL-2, Russia) and photographed (Tasma, Russia, ISO 400). The angle of the slit lamp was 35°. The pupils were dilated with a drop of 1% atropine and 10% phenylephrine hydrochloride (Sigma) 1/1 v/v mixture.

Generally, lens opacities in the two eyes of one animal were identical, but lateral variations in lens cataract were found in around 10% of cases. The frequency of such cases was not depending on the experimental group. Therefore every lens cataract was measured individually, the contralateral eye lenses were used for statistical sample form.

All experiments were done in accordance with the ARVO Resolution on the Use of Animals in Research.

Subjective classification of cataract

Two researchers (K.M., N.P.) individually graded the lens photographs (masked, and projected in random order) on a cataract grade scale represented in Fig. 1. Clear lens was classed as grade 0. Cataract was classed as grade 1 if swollen fibers and subcapsular opacities were observed in the lens; grade 2 if nuclear cataract was observed in the lens, but swollen fibers were still visible in the lens cortex; grade 3 in the case of strong nuclear cataract with perinuclear area opacity in the lens; and grade 4 in the case of total opacity of the lens (not shown).

Instrumental measurement of cataract

The digital images from photograph scan (256 gray-scale gradations, 360 pixels/in.) were adjusted to standard brightness/contrast ratio and analyzed with Scion Image software (Scion Image, Release Beta 3b, Scion Corporation 1998). To avoid bias during this procedure, all image files were randomly renamed before the analysis. Image analysis was performed as follows: the operator defined area of lens and area of lens nuclear opacity on the digital

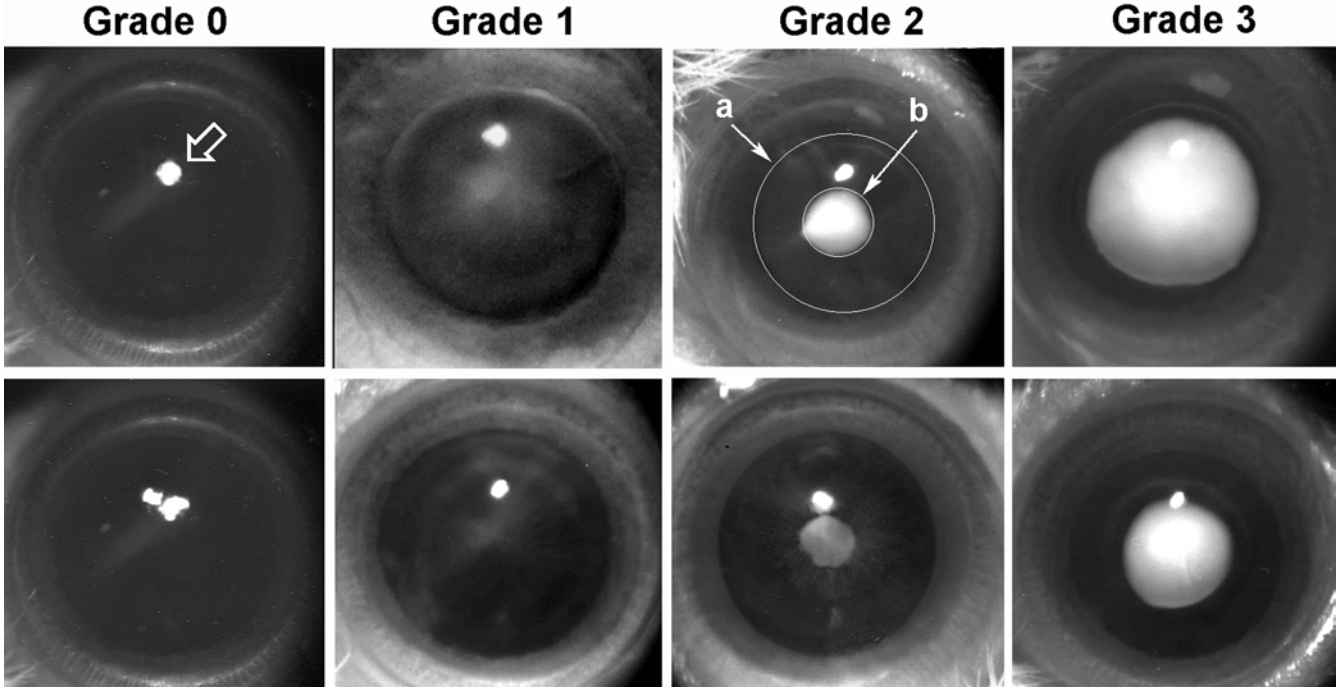

Fig. 1 Cataract grade scale and marking of lens area for image analysis. Columns demonstrate cataract grades, rows show upper and lower border of every grade. The *open arrow* shows a slit-lamp flash. Marking of photograph for image analysis: *a* border of lens area; *b* nuclear opacity border. *Grade 0*: clear lens; *grade 1*: swollen fibers and subcapsular opacities observed; *grade 2*: nuclear cataract in lens and swollen fibers in lens cortex; *grade 3*: strong nuclear cataract with perinuclear area opacity in lens; *grade 4* (not shown): total opacity of lens

Table 1 Schemes of iodide application (*Se* sodium selenite injection, 30 nmol/g b.w. or 20 nmol/g b.w.; *KI* potassium iodide injection, 1.5 nmol/g b.w.). Every scheme consisted of the following four experimen-tal groups: control (no injection), iodide control (iodide injections only) selenite (selenite injections only) and iodide + selenite (iodide and selenite injections)

Application scheme	Postnatal day							
	11	12	13	14	15	16	17	18
I2Se (30 nmol/g)	KI	KI	Se					
I1Se (30 nmol/g)		KI	Se					
ISeI5 (30 nmol/g)			KI + Se	KI	KI	KI	KI	KI
I2Se (20 nmol/g)	KI	KI	Se					

images (TIFF files, Fig. 1), then a computer program counted the number and intensity of pixels (on a scale of 0 to 255). Thus, we measured the following lens parameters:

1. Lens area (pixel number)
2. Nuclear opacity area (pixel number)
3. Pixel intensity set of lens area
4. Pixel intensity set of nuclear opacity area

In general, selenite-induced cataract is characterized both by nuclear and cortical opacity formation. Therefore, these cataract types should be measured. Using the above data, the following parameters were calculated:

1. Mean pixel intensity distribution of the lens area
2. Mean pixel intensity distribution of the nuclear cataract area
3. Mean pixel intensity distribution of the cortical cataract area
4. The "volume" of the lens nuclear opacity, i.e., the sum pixel intensity of the nuclear cataract area. For easier comprehension the intensity was expressed as the sum of pixels divided by 10,000.
5. Mean pixel intensity distribution of the cortical cataract area

We favored the "volume" of lens nuclear opacity, not only because it showed the best correlation with subjective classification (R=0.85) but also because this parameter is directly connected with oxidative stress. The mean pixel intensity of the whole lens area includes the nuclear opacity data, but this parameter may produce biased data in the case of low nuclear cataract. This can be seen from Fig. 1: the lower end of the range of cataract grade 2 is more than the upper end of the range of grade 1, but, in this case, the means ± standard deviation of pixel intensity of the lens area are the same.

Iodide administration

Rat pups received daily a single intraperitoneal injection (1.5 nmol/g b.w.) of potassium iodide (KI; Sigma) according to the schemes shown in Table 1. In the case of the ISeI5 scheme, for example, after an initial dose of iodide 1 h before selenite, iodide was administered daily for 5 days.

Every scheme consisted of the following four experimental groups: control (no injection); iodide control (iodide injections only); selenite (selenite injection only); iodide + selenite (iodide and selenite injections).

Statistical analysis

Statistical analysis of the data was carried out using descriptive statistics, the Mann–Whitney U-test, and nonparametric Spearman rank correlation.

Table 2 Influence of iodide on cataract formation (subjective classification grade) with 30 nmol/g b.w. and 20 nmol/g b.w. of sodium selenite

Application scheme	Valid *N*	Grade (mean ± SD)	*P*[a]
(2.1) Control	62	0.06±0.25	
(2.2) Iodide only	50	0.12±0.33	
(2.3) Se (30 nmol/g)	22	2.36±1.10	
(2.4.) I2Se (30 nmol/g)	22	1.50±1.01**	0.01
(2.5) I1Se (30 nmol/g)	22	2.22±0.81	0.17
(2.6) ISeI5 (30 nmol/g)	17	2.94±0.42	0.82
(2.7) Se (20 nmol/g)	22	1.50±1.18	
(2.8) I2Se (20 nmol/g)	18	1.00±0.00	0.09

[a] *P*: Mann–Whitney U-test, two-tailed probability of equaling and exceeding, comparison with the corresponding selenite group
** Significant difference (P=0.01) vs Se (30 nmol/g b.w.)

Results

Subjective classification data

Table 2 shows the cataract grades in rats that received Na_2SeO_3 (30 and 20 nmol/g of body weight) and KI according to the schemes shown in Table 1. Such doses of Na_2SeO_3 cause a marked nuclear cataract.

A standard image of the eye (sample median) is represented in Fig. 2A. A single KI injection before selenite diminished cataract by 30%, but this reduction was not significant. Two iodide injections decreased lens opacity significantly (P=0.01), by about 50% relative to the selenite group. The standard image (sample median) of eyes in Na_2SeO_3-injected rats that received two injections of KI is shown in Fig. 2B.

The cataracts induced with the lower dose of Na_2SeO_3 (20 nmol/g b.w) are less marked (Fig. 2C, D), but the protective effect of KI is in principle the same (about 50%; see Table 2).

The lenses of normal control rats (n=62) did not show any opacity except a very slight haziness in some lenses (Table 2; 2.1). Two animals with low cortical cataract were found in this group. The lenses of iodide-treated rats (n=50) (Table 2; 2.2) showed a similar picture, although the slight haziness and low cortical cataract were

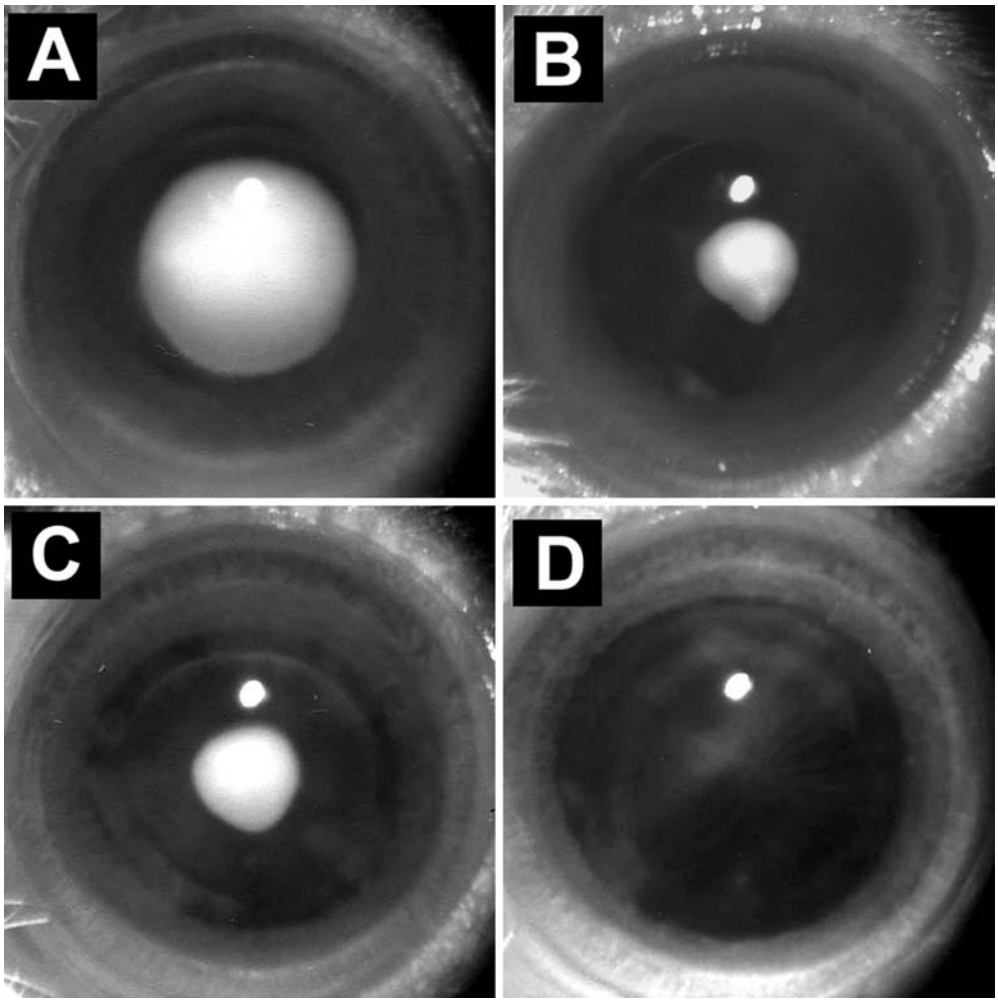

Fig. 2 **A** Standard cataract induced with sodium selenite injection (30 nmol/g body weight); **B** influence of potassium iodide (two daily injections, 1.5 nmol/g body weight). **C** Standard cataract induced with sodium selenite injection (20 nmol/g body weight); **D** influence of potassium iodide (two daily injections, 1.5 nmol/g body weight)

observed more often: four animals with low cortical cataract were found in this group. Selenite injection (30 nmol/g) caused formation of strong nuclear cataract as a rule, but cortical cataracts without nuclear opacity were found too (n=22) (Table 2; 2.3). As shown in Table 2 (2.4), iodide significantly protects rat lenses from selenite-induced cataract when injected on 2 days before selenite administration (n=22). One single dose of KI before (n=22) or five injections of KI after selenite injections (n=17) do not significantly inhibit cataractogenesis (Table 2; 2.5, 2.6). In Table 2 (2.8) the influence of iodide on 20 nmol/g selenite injections is presented.

Image data analysis

Choice of parameters

The lens opacities are described by five parameters (see Materials and methods). Spearman rank order correlation between image analysis data and subjective classification of cataract was used for the choice of the most adequate parameters. Lens nuclear opacity "volume" showed the best correlation with subjective classification (R=0.85). Mean pixel intensity distribution of the lens area displayed a smaller correlation (R=0.82); other parameters did not yield significant correlations.

As selenite-induced cataract is a complex of nuclear and cortical opacity, it can be assumed that two parameters ("volume" of the lens nuclear opacity and pixel intensity mean of the cortical cataract area) could be used to measure selenite-induced cataract.

Image analysis results

Figure 3 shows the KI influence on lens nuclear opacity ("volume"). Cataract was induced with injection of 30 nmol/g of Na_2SeO_3. Both a single injection of KI and two KI injections before selenite administration decreased the selenite-induced cataract. However, only the

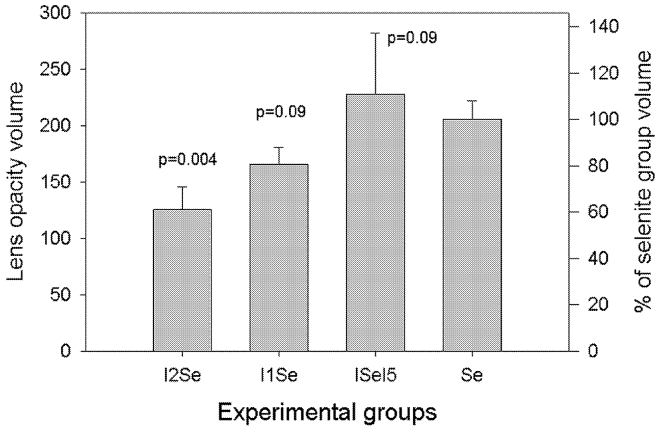

Fig. 3 Effect of iodide on cataract ("volume") induced with 30 nmol/g body weight of sodium selenite. Mean of nuclear opacity volume ± standard error, Mann–Whitney nonparametric test compared with selenite group (*Se*), two-tailed probability of equaling and exceeding. The right axis shows percentage of selenite group volume. ***P*=0.004 vs Se

effect of two preceding injections of iodide was significant (*P*=0.004). Prolonged KI administration (five daily injections) after selenite did not protect the lens. The findings confirm the results of the subjective classification method.

We did not find any difference in cortical opacities among lenses of all studied experimental groups (data not shown). Therefore, elimination of cortical data does not bias the analysis result.

Discussion

The data of the present study demonstrate that iodide can protect against selenite-induced cataract, the I2Se scheme of iodide administration being most effective. The pathogenesis of selenite-induced cataract is strongly connected with an oxidative damage: Selenite causes an oxidation of protein and non-protein sulfhydryl groups; this leads to ion pump damage and to disturbance of the electrolytic balance. The intracellular calcium level increases, which activates the calcium-dependent protease calpain. Calpain partly hydrolyzes intracellular proteins, especially β-crystallin. Protein aggregates scatter light, and lens opacity increases [14, 15]. *Low* oxidative stress induced by a low selenite dose (<15 nmol/kg) causes biochemical processes before calpain activation, such as disturbance of the lens membranes' ion permeability, water influx, and swelling of the fiber cells. Cortical opacity is a marker of these events. *Moderate* oxidative stress (20–30 nmol/kg of selenite) causes nuclear opacity through the calpain proteolysis of lens proteins. *High* oxidative stress (>30 nmol/kg) causes intensified injury, i.e. damage of both nucleus and perinuclear area and, eventually, of the whole lens. In our study, iodide protected the lens against the selenite-induced nuclear opacity (see Figs. 2 and 3, Table 2), whereas cortical opacity was not diminished. We assume that the molecular mechanism of the iodide effect is connected with protection against mild or high oxidative stress induced by selenite.

We propose that the protective effect of iodide could be connected with protection against oxidative stress induced by selenite.

Iodide as a reducing agent and electron donor has demonstrated antioxidant activity in vitro [18] and in vivo [10], but the exact molecular mechanism is unknown. Two mechanisms can be suggested: (1) direct influence on free radical oxidation in the lens; (2) an indirect effect through activation of a system that increases the antioxidant potential in the lens.

Therefore, replenishment or augmentation of antioxidants is necessary to maintain a constant protective effect. For instance, several daily injections of water-soluble ascorbic acid after selenite exposure were effective against selenite-induced cataract [2], as well as other antioxidants effectively prevent the selenite-induced cataract [11].

In addition, iodide could also initiate the activation of antioxidant enzymes to protect the lens from cataract formation by selenite. Winkler et al. [18] found an activation of protective enzymes, glutathione peroxidase and catalase during balneotherapeutic treatment with iodide in vivo. These authors supposed that iodide could be involved as a cofactor of peroxidases, which catalytically degrade hydrogen peroxide. As H_2O_2 is the major oxidant contributing to cataract formation [16], it is possible that the protective effect of iodide is connected with lens peroxidase activation.

Lowering of the stress activator, i.e., the selenite concentration in the eye, could be another reason for the activity of iodide against the induction of cataract by selenite. For instance, disulfiram inhibits cataract formation through the decrease of selenite level in the eye [6] and directly or indirectly influences the oxidative processes in the lens. We imagine, however, that the protection against oxidative stress through a lowering of the selenite level in the eye is improbable for the following reasons: iodide (I^-) and selenite (SeO_3^{2-}) are both anions and could not form a complex. A redox interaction of these substances is impossible upon either thermodynamics or medium pH.

A certain level of iodine (or iodide) must be reached in the organism to protect against oxidative reactions. Literature data describing iodide penetration into the lens are sparse. In previous studies [13] it has been shown that after administration of balneotherapeutically relevant doses, the lens is one of the organs with very little iodine uptake. In pilot studies we have found a detectable, age-dependent radioactive iodine-125 penetration

in rats, decreasing with the age of the animals. Therefore, a sufficient iodide supplementation of the whole organism by intraperitoneal injections seems to be necessary for the protection of the lens against selenite-induced cataract formation. The exact relationship between the amount of iodine obtained by injection and the amount (concentration) obtained by ingestion is not known. However, the iodine dose used in our experiments (1.5 nmol KI/g b.w.) is realistic insofar as it approximates the daily iodine amount (about 14 mg for a 70-kg person) obtained by a combined iodine cure as used in Bad Hall.

In summary, a time-dependent protective influence of iodide against selenite cataract development can be assumed, based probably on direct or indirect antioxidant mechanisms.

Further studies of the biochemical changes accompanying the formation of the selenite-induced cataract after iodide exposure, together with pharmacokinetic data on iodine distribution, are necessary to find out the real mechanism of iodide influence.

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