BULLETIN OF TAIPEI MEDICAL COLLEGE, VOL. II, DEC. 1980
p.25-31

Dietary Effect of Abrine on Mimosine Cataract in Rat

SHU-JEN LIN, SHIOW-SHONG YANG, AND YIH-CHIH TUNG

Cataracts may be caused by many different agents. Of dietary cataracts, protein deficient diets or diets omitting certain amino acids, such as tryptophan, can induce cataracts in experimental animals. Diet deficiency of phenylalanine, valine and histidine has likewise been found to be cataractogenic [1]. Because of the unusually high protein content of the lens, the disturbance of the metabolism of lens protein may be cataractogenic. Mimosine (3-hydroxy-4-pyridone-α-amino propionic acid), a toxic amino acid isolated from the seeds of Leucaena glauca Benth, was shown to be cataractogenic to experimental rats [2,3]. It was found that by adding 1% DL-phenylalanine or 1% L-tyrosine to a diet containing mimosine aggravated or alleviated the cataract formation in rats, respectively [4]. Rats, fed by 3% tyrosine (in 6% casein basal diet) showed the formation of cataract. The supplement of 0.2% tryptophan to this high tyrosine diet can alleviate the occurrence of cataract [5]. Since the mechanism of cataract formation produced by mimosine is still in question, and our laboratory is especially interested in studying naturally occurring indole compounds, we have investigated the effect of abrine, an N-monomethyl derivative of tryptophan, on the cataractogenesis of mimosine.

MATERIALS AND METHODS

Mimosine was prepared from the seeds of Leucaena glauca Benth [6], and abrine from the seeds of Abrus pricatorius L. [7], according to the previous reports.

Twenty-five male weaning rats of Long Evan strain were used in this experiment. Their body weights and groupings are presented in Table 1. Rats of each group were individually housed in a raised screen bottom cage. Food and tap water were supplied ad libitum. The food was in dry-powdered form. The composition of the casein basal diet is shown in Table 2. Mimosine and abrine were substituted at the expense of carbohydrate.

Rats were examined with a Goldmann slitlamp weekly for their ocular changes; 1% of homatropine solution was applied for the preparation of mydriasis. Before the experiments, rats were first screened: they were identified with clear lenses but with various degrees of keratitis (see Table 3); nevertheless, all rats were considered to be qualified for the experiments. After feeding experiments, an arbitrary score of one "+" to three

Department of Biochemistry, Taipei Medical College, Taipei, Taiwan, Republic of China.
Received for Publication: September 1980

Table 1. Grouping of Rats

Twenty-five male weaning rats of Long Evans Strain, Ranged from 40 to 60 gm. of body weight, were divided into four groups.

	Number of Rats	Initial Average Body Weight (gm)	Final Average Body Weight (gm)
I. Basal Diet	6	44.7	87.5
II. 0.5% Abrine	6	47.3	84.7
III. 0.5% Mimosine	6	48	59.5
IV. 0.5% Mimosine plus 0.5% Abrine	7	48.7	54.3

Table 2. Composition of the Casein Basal Diet: (in per cent)

Vitamin free casein	6.0
DL-Methionine	0.2
Wesson salt mixture	4.0
Vitamin mixture	0.5
Choline chloride	0.2
Peanut oil	5.0
Corn starch	84.1*

* Experimental amino acids were substituted at the expense of corn starch [5].

Table 3. Screen Test for Eye Lesions of Rats by Clinical Examination before the Experiment

	Number of Rats Identified	
	Left Eye	Right Eye
Cataract	0	0
Keratitis (+)	9	5
Keratitis (++)	16	17
Keratitis (+++)	0	3

"+" was used to describe the severity of cataract formation.

At the end of the sixth week after the feeding experiments, all rats were killed. Their eye balls were enucleated and prepared for histological examination for ocular changes. The degree of eye lesion under microscopic examination was described by a score of "—" to three"+". Since the histological sections of the eye balls were cut through the anterior and posterior poles in arbitrary planes, the results are not necessarily comparable to clinical observation because of the subjective nature of the scoring procedure.

RESULTS AND DISCUSSION

At the third week of the feeding experiment, rats fed with 0.5% mimosine diet all produced cataracts with various degrees of opacity in the anterior and/or posterior part of the cortex. At the fourth week, these cataracts developed into a mature form (see Table 4). This result was generally similar to that reported previously [4]

Table 4. Incidence of Cataract Formation (Slitlamp)

	Basal Diet	0.5% Abrine	0.5% Mimosine			0.5% Mimosine + 0.5% Abrine		
	Incidence	Incidence	Incidence	Severity		Incidence	Severity	
				L	R		L	R
1st week	0/6	0/6	1/6	0.2	0	1/7	0.1	0.1
2nd week	0/6	0/6	4/6	0.7	0.7	3/7	0.1	0.1
3rd week	0/6	0/6	6/6	1.9	1.6	4/7	0.3	0.9
4th week	0/6	0/6	6/6	2.7	2.5	4/7	0.9	0.9
5th week	0/6	0/6	6/6	2.7	2.5	4/7	0.9	0.9
6th week	0/6	0/6	6/6	2.7	2.7	4/7	1.1	1.1

Interpretation:

Number in each denominator represents the number of rats examined, and in each numerator, the number of rats produced cataract.

Severity was judged by a score of 0 to "+". These data are expressed with the total number of "+" divided by the number of eyes examined.

L stands for left eyes and R for right eyes.

When 0.5% abrine was added in the diet containing 6% casein and 0.5% mimosine at the third week of the feeding experiment, only 4 out of 7 rats developed a mild form of cataract. The other 3 rats of this group showed clear lenses throughout the experiment (see Table 4). Adding 0.5% abrine to a diet containing 0.5% mimosine showed a decreased incidence and alleviation of the severity of cataract induced by mimosine, although it could not completely prevent the occurrence of cataracts.

No change in the lens were found in rats fed on basal diets alone and those fed diets containing 0.5% abrine throughout the experiment.

At the end of feeding experiment (after six weeks), all rats were killed, and both of their eye balls were enucleated and fixed in formalin. Microscopic examination was made on a routine paraffin section stained by hematoxylin and eosin.

All of the six rats fed on 0.5% mimosine containing diet developed cataract, mostly bilateral and rarely unilateral. The types of lenticular change and estimated degrees of cataract are presented in Table 5. The cataracts developed were all cortical cataract - mostly mature of Morgagnian catarac Only a few lenses showed stratification of the subcapsular epithelium. This finding, together with the absence of phycolytic glaucoma, absence of macrophage or lens protein in the posterior chamber, no pathological finding in the other intraocular structures rupture, or degeneration of lens capsule is not the important factor in mimosine cataract.

Swelling of the cortical cells was universal. The swelling was often tremendous and balloon-like (so called bladder cells). The

Table 5. Histological Observation of Cataract

Group	Animal Number	Epithel. Proli.	Bladder Cell	Liquef.	Morgan. globule	Conclusion
Basal diet	1. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	2. Lt.	0	0	0	0	?
	Rt.	–	–	–	–	–
	3. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	4. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	5. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	6. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
0.5% Abrine diet	1. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	2. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	3. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	4. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	5. Lt.	–	–	–	–	–
	Rt.	0	0	0	0	?
	6. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
0.5% Mimosine diet	1. Lt.	–	++	+++	+++	+++
	Rt.	++	++	+++	++	+++
	2. Lt.	–	+++	++	+++	+++
	Rt.	+	+++	+++	+++	+++
	3. Lt.	++	+++	+++	+++	+++
	Rt.	++	+++	+++	+++	+++
	4. Lt.	–	+++	+++	++++	+++
	Rt.	–	++	+++	+++	+++
	5. Lt.	–	++	+++	+++	+++
	Rt.	–	++	–	+	+
	6. Lt.	–	+++	+++	+++	+++
	Rt.	–	+	–	–	±
0.5% Mimosine plus 0.5% Abrine diet	1. Lt.	–	++	++	+	++
	Rt.	–	++	+++	+	++
	2. Lt.	–	++	–	+	+
	Rt.	–	+	–	–	±
	3. Lt.	–	–	–	–	–
	Rt.	–	–	–	–	–
	4. Lt.	0	+	–	+	+
	Rt.	–	+	–	–	±
	5. Lt.	–	++	+++	+++	+++
	Rt.	++	–	+++	++	+++
	6. Lt.	–	+	–	–	±
	Rt.	–	–	–	–	–
	7. Lt.	–	+	–	–	±
	Rt.	–	+	–	–	±

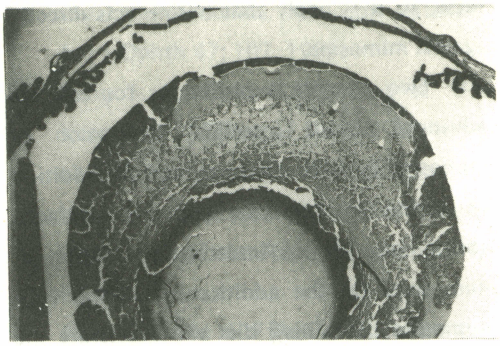

Fig. 1. Severe cataract. Marked liquefaction and globular decomposition of cortical fibers but rather intact nucleus.

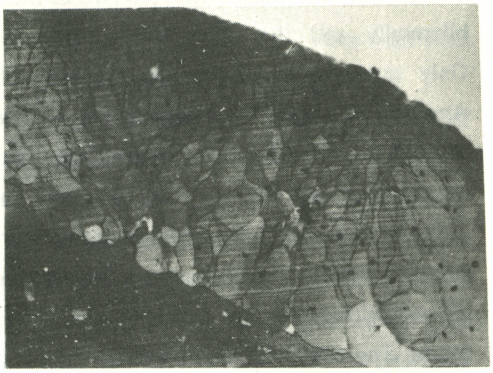

Fig. 2. Bladder cells. Marked swelling of cortical cells. They are mostly located in the equator.

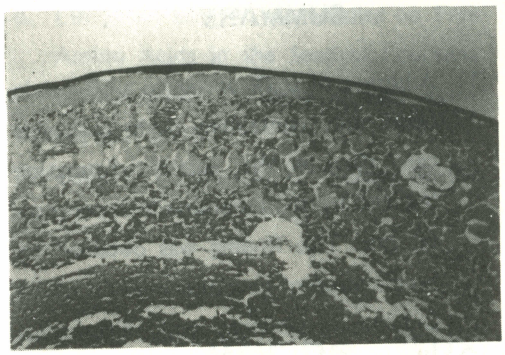

Fig. 3. Marked globular decomposition of cortical fibers and a space filled with homogeneous albuminous fluid beneath the intact epithelium at anterior pole.

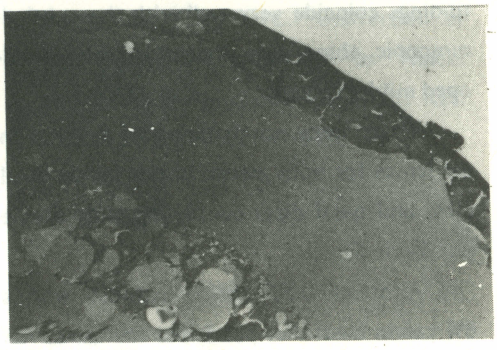

Fig. 4. A lake of albuminous fluid covered by a few lager of bladder cells. The left lower show globular particles mixed in swollen cortical fibers.

staining reaction of those cells vary considerably; many were markedly acidophilic, others pale pinkish. The swollen cells packed tightly together at first, but later the cell membrane disappeared, forming homogeneous eosinophilic fluid. The bladder cells were most numerous in the equator and a few cells extended to the anterior cortical surface, but none to the posterior pole. Liquefaction of the cortical fibers involved both the anterior hemisphere and the posterior hemisphere. It began from the subepithelial cortex or under the ballooning cell layer. For most of the mimosine fed animals, the liquefactive change of the cortical fibers involved the entire cortex; thus, a Morgagnian cataract resulted and the distance between the anterior pole and posterior pole increased. Coagulative decomposition of the lens fibers or Morgagnian globule formation was also a common finding. They were deeply situated, often at the junction of the liquefied cortical fibers and the periphery of the nuclei. Groups of these globular protein particles packed in the clefts of the disintegrated peripheral nuclear fibers were also commonly seen.

Of the group of rats fed on 0.5% mimosine with 0.5% abrine, four out of the seven

rats developed cortical cataract. Two of them bilaterally and the other two unilaterally. Only one showed severe bilateral cataract. Among the eight non-cataract lens, five showed a few bladder cells in the equator. But the bladder cells did not extend to the anterior or posterior hemispheres and no Morgagnian globule formation nor liquefactive decomposition of the cortical fibers was found. The actual disturbance to transmission of light was unknown, so the estimation of the degree of cataract to these lenses was given a "±". (Table 5). These histological findings coincide very well with the ophthalmoscopic observation that this group developed milder cataract.

There were no pathological findings in the lenses for both groups of rats fed on basal diets and basal diets containing 0.5% abrine (see Table 5).

Although 0.5% abrine included with a 6% casein basal diet promoted the growth of rats considerably and this observation confirms the finding in an earlier report [8], the addition of 0.5% abrine to a 0.5% mimosine diet could not improve the growth retardation caused by the toxicity of mimosine (see Table 1). This might be due to the different metabolic rate of these two amino acids. Abrine might be metabolized faster than mimosine, since a considerable portion of consumed mimosine was found in the urine of rats without any change [9].

Adding niacin to a mimosine containing diet can not prevent the formation of cataracts [3]. Since abrine may be metabolized to tryptophan by N-methyl-amino-acid oxidase, and metabolized further to niacin [10], it should follow that abrine can not prevent cataract formation either. But the addition of abrine reduces the incidence of cataracts, so it is likely that abrine acts directly against mimosine.

Two possible explanations for this are advanced. Since intestinal absorption of mimosine leads to cataracts, and the addition of abrine to mimosine prevents cataracts, abrine may reduce intestinal absorption of mimosine. Or the addition of abrine to a mimosine containing diet can accelerate the metabolism of mimosine, thus increasing the rate of catabolism by the organism.

SUMMARY

(1) Addition of 0.5% abrine to a 6% casein basal diet containing 0.5% mimosine can alleviate the cataract formation induced by the mimosine in experimental rats. This finding was obtained by examination of enucleate eyeballs with a Goldmann slitlamp and histological observation.

(2) Growth retardation, one of the toxic effects of mimosine, is not prevented by the presence of abrine.

ACKNOWLEDGEMENT

The authors wish to express their sincere thank to Prof. T. S. Huang, Department of Pathology, Chung-San Medical College for the pathological confirmation.

REFERENCE

1. Adler F. H.: Physiology of the Eye, Clinical application, 4th ed. St. Louis, Mosby 1957.
2. Yoshida R.K.: Doctoral dissertation University of Minnesota 1944.

3. Ludwig Von Sallmann, Patrica Grimes, and Eleanor Collins: Mimosine Cataract Am J Ophth 47; 107, 1959.
4. Yang S. S., Chin C. C., Ling K. H., Yang Y. F.: Effect of Dietary Addition of L-Tyrosine and DL-Phenylalanine on Mimosine Cataract. Transaction of the Ophth Soc of the Republic of China 7; 12-27, 1968.
5. Alam S. Q., et al: Effect of threonine on the Toxicity of Excess Tyrosine and Cataract Formation in the Rat. J Nutrition 89; 91-96, 1966.
6. Lin J. Y., Ling K. H.: Studies on Free Amino Acids in the Seed of Leucaena Glauca Benth. J Formosan Med Assoc 60; 651, 1961.
7. Tung Y. C., Liau M. C.: Studies on the Chemical Components of the Seed of Abrus Precatorius L J Formosan Med Assoc 59; 868, 1960.
8. Huang P. C., et al: The Metabolism of Alpha-N-Monomethyl-L-Tryptophan (L-Abrine) in Man and in Normal and Pyridoxine Deficient Rats. J Formosan Med Assoc 63; 285-293, 1964.
9. Lin J. Y., Ling K. H.: Studies on Free Amino Acids in the Seeds of Leucaena Glauca Benth, III. Biological Study on Mimosine. J Formosan Med Assoc 60; 657-665, 1961.
10. Sung S.C., Tung T. C.: Conversion of Monomethyl-L-Tryptophan to Niacin and N^1-Methylnicotinamide by the Rat. J Biolog Chem 186; 637-640, 1950.

Abrine 對因含羞酸所引起白內障的影響

林淑貞　楊秀雄　董一致

許多種不同的因素都能誘使眼睛發生白內障，其中之一爲胺基酸的不平衡。

含羞酸是一種具有毒性的胺基酸，加入飼料中可引起老鼠發生白內障。本研究是以 Goldmann Slitlamp 觀察老鼠眼睛的變化情形，及將眼球作組織學上的觀察。獲得以下兩個結論：

1.於含 0.5 %含羞酸的 6 % casein 基礎食物中（ basal diet ）加入 0.5 % 的 abrine 可減輕因含羞酸所引起的白內障症狀。

2.Abrine 不能阻止因含羞酸中毒所引起的生長抑制情況。

台北醫學院生化學科
民國六十九年九月受理