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OCULAR LESIONS AND EXPERIMENTAL CHOLINE DEFICIENCY

GEORGINA P. OSSANI, DAVID PELAYES[1], MARIA L. DIAZ[2], NESTOR R. LAGO[1], SILVIA L. FARIÑA[1], ALBERTO J. MONSERRAT[1], JORGE O. ZARATE[1]

[1]*Laboratorio de Investigaciones Oftalmológicas y Visuales, Centro de Patología Experimental, Departamento de Patología, Facultad de Medicina, Universidad de Buenos Aires*;
[2]*Servicio de Medicina Nuclear, Hospital Británico, Buenos Aires*

Abstract Previous studies have shown ocular haemorrhages in choline-deficient rats. The aim of this paper is to study further the relationship between ocular and renal lesions and biochemical alterations in rats fed a choline-deficient diet. Fifty one weanling male Wistar rats, were divided into two groups. Thirty one of them were fed a choline-deficient diet and the rest was fed a choline-supplemented diet *ad libitum*. Animals from both groups were killed between the fifth and the eighth day. Urea, creatinine and homocysteine concentrations in blood were determined. Eyes were used for light microscopy study; high resolution light microscopy and the study of the retina as "rétine a plat". Kidneys were studied by light microscopy. Choline-supplemented rats did not show ocular or renal lesion. Choline-deficient rats that showed renal lesions, tubular or cortical necrosis, did not always have ocular changes. There were no ocular changes in the only choline-deficient rat without renal lesion. The ocular changes consisted mainly in haemorrhage in both cameras and ciliary and vitreous bodies. Correlations between ocular and renal lesion ($r=0.72$, $p<0.0001$, CI 95%: 0.48-0.86); ocular lesion and creatinine ($r=0.86$, $p<0.0001$, CI 95%: 0.72-0.93) and ocular lesion and urea ($r=0.70$, $p<0.0001$, CI 95%: 0.44-0.85) were positive. Choline-deficiency induces ocular haemorrhagic lesions after the development of renal necrosis. The ocular pathology could be due to the immaturity of the ocular vasculature at this age. The hyaloid, choroid and retinal system are involved.

Key words: choline-deficiency, eye, kidney, acute renal failure

Resumen ***Lesiones oculares y deficiencia experimental de colina.*** Estudios previos han demostrado hemorragia ocular en ratas deficientes en colina. El objetivo de este trabajo es profundizar en la relación entre las alteraciones oculares, renales y bioquímicas en ratas deficientes en colina. Cincuenta y una ratas Wistar macho recién destetadas fueron divididas en dos grupos: treinta y una fueron alimentadas con una dieta colino deficiente y el resto con colina suplementada *ad-libitum.* Los animales de ambos grupos fueron sacrificados entre el quinto y el octavo día. Se midió la concentración de urea, creatinina y homocisteína en sangre. Los ojos fueron estudiados por microscopía de luz, microscopía óptica de alta resolución y para el estudio de la retina como retina plana. Los riñones fueron estudiados por microscopía de luz. Las ratas suplementadas con colina no mostraron lesiones oculares o renales. Las colino deficientes que mostraron lesiones renales, necrosis tubular o cortical, no siempre tuvieron cambios oculares. No se encontraron cambios oculares en la única rata deficiente en colina sin lesión renal. Los cambios oculares consistieron principalmente en hemorragia en ambas cámaras, cuerpo ciliar y vítreo. La correlación entre la lesión ocular y renal ($r=0.72$, $p<0.0001$, CI 95%: 0.48-0.86), lesión ocular y creatinina ($r=0.86$, $p<0.0001$, CI 95%: 0.72-0.93) y lesión ocular y urea ($r=0.70$, $p<0.0001$, CI 95%: 0.44-0.85) fue positiva. La deficiencia de colina induce lesiones oculares luego del desarrollo de la necrosis renal. La patología ocular podría ser debida a la inmadurez de los vasos oculares. El sistema hialoide, coroideo y retinal están involucrados.

Palabras clave: colino-deficiencia, ojo, riñón, insuficiencia renal aguda

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Postal address: Dra. Georgina P. Ossani. Laboratorio de Investigaciones Oftalmológicas y Visuales, Centro de Patología Experimental Departamento de Patología, Facultad de Medicina, Universidad de Buenos Aires, J. E. Uriburu 950 5to piso, 1114 Buenos Aires, Argentina
Fax: (54-11) 4508-3602 e-mail: georginaossani@yahoo.com.ar.

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Choline is a precursor for the synthesis of acetylcholine, it is required for the synthesis of phospholipids that constitute cell membranes and, through betaine it is a source of labile methyl groups[1, 2].

Weanling rats fed a choline-deficient diet show a variety of biochemical, functional and morphologic alterations that involve different organs such as the kidneys, liver, heart, or eyes[1, 2]. Renal pathology is characterized by tubular or cortical necrosis[3], liver alterations by fatty changes, cirrhosis and eventually cancer[2, 4], and heart changes by necrosis[5, 6].

As far as we know, there are only two previous papers that refer to the ocular changes as the primary interest in choline-defficient (CD) rats. Both describe the presence of haemorrhages; almost all of them situated in parts of the eye supplied by the hyaloid arterial system[7,8].

The present paper deals with the ocular alterations due to choline nutritional deficiency in weanling male Wistar rats, and its correlation with renal lesions and biochemical alterations.

Materials and Methods

Fifty one weanling male Wistar rats, from the Bioterium of the Department of Pathology, School of Medicine, University of Buenos Aires, were divided into two groups. One of them (31 animals) was fed a CD *ad libitum* (diet 1, Table 1); the other (20 animals) was fed a choline supplemented diet (CS) *ad libitum* (diet 2, Table 1).

Food intake and body weight were recorded daily. Ophthalmoscopic examination was carried out from the third day until the sacrifice. All experimental procedures conformed to The Association for Research in Vision and Ophtalmology (ARVO) statement for the use of animals in ophthalmic and vision research[9].

Animals from both groups were killed between the fifth and the eighth day.

Urea (*Wiener Laboratory*, Buenos Aires), creatinine (*Wiener Laboratory*, Buenos Aires) and homocysteine[10] concentrations were determined in blood.

Both eyes were labelled with threads of different colours in the superior part. Then, they were removed and fixed in buffered formalin for at least seven days. Afterwards, eyes were cut horizontally. The superior right half and the inferior left half were embedded in paraffin and sections were stained with hematoxylin and eosin following standard methods for histopathological study. The inferior right half and the superior left half were embedded in *Polibed* and sections were stained with toluidine blue and fuchsin for high resolution light microscopy (HRLM)[11] or they were used to study the retina as "rétine a plat"[12] by surface light microscopy[13, 14].

By the different methods previously mentioned, it was analysed the presence or absence of haemorrhage in the different parts of the eye.

For classification of ocular lesions a macroscopic score was made: 0 (normal), 1 (punctiform haemorrhage), 2 (haemorrhage in anterior chamber), 3 (haemorrhage in vitreous), 4 (2 plus 3). Data from both eyes were employed.

Both kidneys were removed, weighted, fixed in buffered formalin and embedded in paraffin. Then, sections were cut and stained with hematoxylin and eosin in order to analyse the histopathological alterations.

TABLE 1.– *Composition of diets (g/100g)*

Components	Diet 1	Diet 2
Soybean protein[1]	20.00	20.00
Hydrogenated vegetable oil[2]	10.00	10.00
Corn oil[3]	4.0	4.0
Sucrose	55.50	55.15
Cellulose[4]	4.00	4.00
Vitamin mixture (without choline)[5]	4.00	4.00
Salts[6]	2.00	2.00
L-cystine[7]	0.50	0.50
Choline chloride	0.00	0.35

[1]*Soybean protein. Grade II. US Biochem. Corp. USA.*
[2]*Mazola. Refinerías de Maíz, Buenos Aires, Argentina.*
[3]*Flora Dánica, Buenos Aires, Argentina.*
[4]*Celufil. Non-nutritive Bulk, U.S. Biochem. Corp., USA.*
[5]*Vitamine mixture (without choline). Test diet 234312 57MV*
[6]*Salt Mixture, Wesson modification, U.S. Biochem. Corp. USA.*
[7]*U.S. Biochem. Corp., USA.*

TABLE 2.– *Histologic classification of renal necrosis*

A: Kidney without necrosis (grade 0)
B: Acute tubular necrosis
  Grade 1: isolated foci of celular necrosis in some tubules.
  Grade 2: small groups of tubules with necrosis.
  Grade 3: zones of tubular necrosis.
  Grade 4: confluent zones of tubular necrosis.
C: Cortical necrosis
  Grade 5: grade 4 plus isolated foci of cortical necrosis.
  Grade 6: grade 4 plus multiple foci of cortical necrosis.
  Grade 7: grade 4 plus confluent foci of cortical necrosis.
  Grade 8: massive cortical necrosis.
D: Repair
  Repair is characterized by different degrees of intertitial fibrosis, tubular atrophy, tubular regeneration, glomerular fibrosis, etc. According to its extention it is divided into four grades.

Monserrat et al[15].

Histologic classification of renal necrosis was taken from Monserrat et al[15] (Table 2).

Levels of urea, creatinine and homocysteine in serum were compared by one way analysis of variance (ANOVA). In the case of significant differences ANOVA was followed by the Tukey-Kramer test. Differences which resulted in p values lower than 0.05 were considered significant (S). Correlations between ocular lesion and renal lesion, creatinine and urea were made by Spearman test. The statistical evaluation was made using the program GraphPad Instat version 3.05, GraphPad Software, San Diego, California.

Results

Rats fed a CS diet did not show any ocular or renal lesions.

All rats, except for one, fed a CD diet showed renal necrosis (Table 3). The only CD rat that did not show renal lesion, did not have any ocular alteration.

Twelve out of 31 CD rats showed renal necrosis but did not have ocular changes (Tables 3 and 4).

The acute renal changes were grossly characterized by an increase in size and weight and purplish red discoloration. Necrosis involved mainly proximal convoluted tubules and was characterized by increased eosinophilia, pyknosis and mainly kariolysis; in more advanced stages glomerular and vascular necrosis were also observed.

Those CD rats that showed ocular lesions developed haemorrhages, most frequently in anterior chamber, posterior chamber, ciliary region and vitreous. Macroscopic appearance is shown in Figure 1.

Histopathological changes are observed in Figure 2 (panoramic view).

There were no ocular changes in the rat without renal lesion. However, there were twelve CD rats that did not have ocular haemorrhage but did have renal necrosis (Tables 3 and 4).

In most of the animals, the grade of renal necrosis increased in relation with the day of sacrifice, the later the day the greater the lesion.

Urea and creatinine levels increased in relation with the grade of renal lesion (Table 5). Homocysteine levels were increased in CD rats despite of the grade of renal alteration; it was also increased in the rat without renal necrosis (Table 5).

Rats grew adequately well during the first five days. Afterwards, CD rats lost weight, due to renal damage[16].

HRLM (Figure 3) and the “rétine a plat” (Figure 4) allowed a more precise study of the ocular damage.

There was a good relationship between ophthalmoscopic examination and ocular lesions.

Correlations between grade of ocular and renal lesions ($r=0.72$, $p<0.0001$, CI 95%: 0.48-0.86); grade of ocular lesion and day of sacrifice ($r=0.87$, $p<0.0001$, CI 95%: 0.74-0.93) grade of ocular lesion and creatinine ($r=0.86$, $p<0.0001$, CI 95%: 0.72-0.93) and grade of ocular lesion and urea ($r=0.70$, $p<0.0001$, CI 95%: 0.44-0.85) were high and positive. From the four rats killed at the fifth day, three of them showed renal damage; however, none of them displayed ocular lesion.

TABLE 3.– *Day of killing, ocular and renal lesions in CD rats*

Day	Rats (n)	Renal lesión (n)	Ocular lesión (n)*
5	4	3	0
6	9	9	1
7	9	9	8
8	9	9	9
Total	31	30	18

**In at least one eye.*

TABLE 4.– *Grade of renal and ocular damage*

Grade of renal lesión	Rats (n)	Ocular lesión (n)	Grade of ocular damage* 0	1	2	3	4
0	1		1				
1	5		5				
3	2		2				
4	5	2	3	1			1
5 18	16	2	2	2	6		6
Total	31	18	13	3	2	6	7

**According to the eye with the higher grade of damage.*
Grade of ocular damage:
0: normal
1: punctiform haemorrhage
2: haemorrhage in anterior chamber
3: haemorrhage in vitreous
4: 2 plus 3

TABLE 5.– *Urea, creatinine and homocysteine*

Rats	Urea (g/l) $\bar{X}$±SD	Creatinine (mg/dl) $\bar{X}$±SD	Homocysteine (µmols/l) $\bar{X}$±SD
CS	0.64±0.12	0.25±0.07	4.34±1
CD with KWN	0.2	0.2	15.35*
CD with ITN	0.70±0.23	0.36±0.07	14.12±3.7*
CD with MTN	1.92±0.98*	1.18±0.5*	12.3±4.9*
CD with CN	2.55±0.67*	2.90±0.8*	11.06±3.07*

**p< 0.05 vs CS.*
In the present Table, grades 1 and 2 were considered as initial tubular necrosis; grades 3 and 4 were considered as massive tubular necrosis and grade 5 as massive tubular necrosis plus cortical necrosis.
KWN: kidney without necrosis (grade 0)
ITN: initial tubular necrosis (grade 1 and 2)
MTN: massive tubular necrosis (grade 3 and 4)
CN: cortical necrosis (grade 5-8)

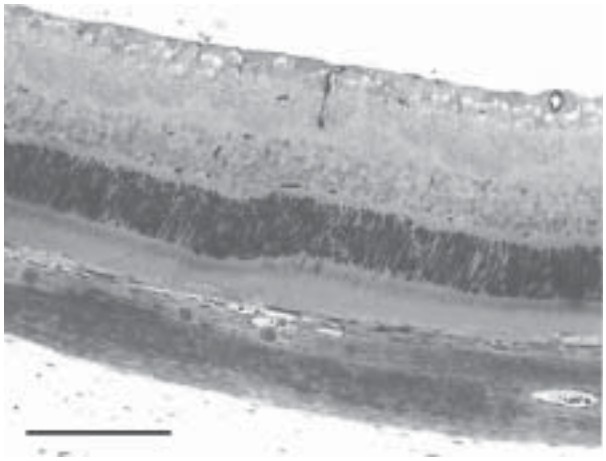

Fig. 3.– HRLM. Retina in CD rat with vascular proliferation, microhaemorrhage and congestion. Scale bar, 150µm.

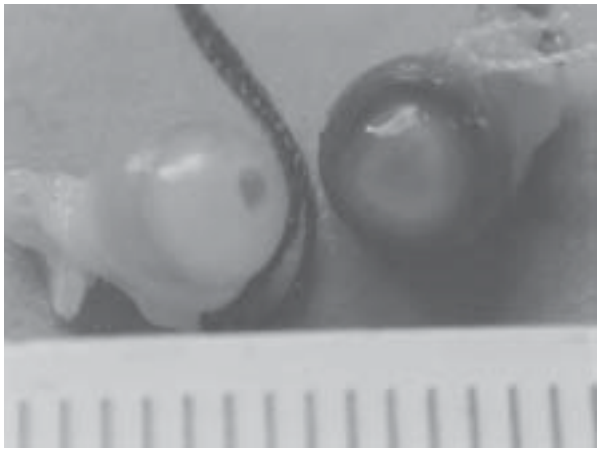

Fig. 1.– Eyes from rat killed the eighth day. One of them with severe haemorrhage and the other with punctiform haemorrhage.

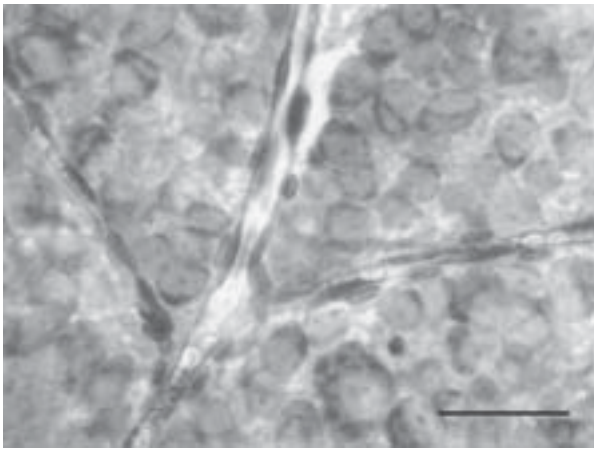

Fig. 4.– Rétine a plat in CD rat. Vascular network. Scale bar, 50µm.

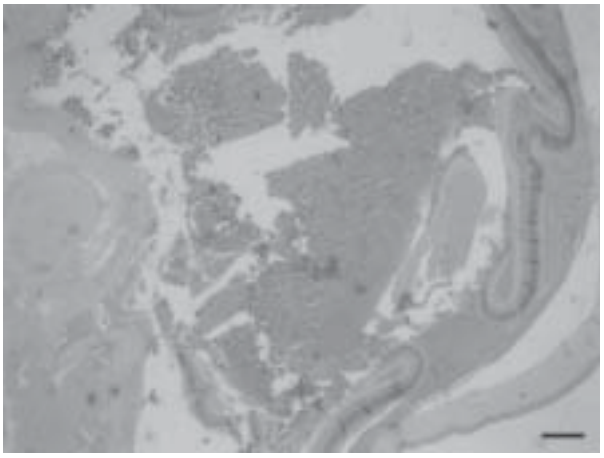

Fig. 2.– Microscopic panoramic view. Ocular haemorrhage lesions. (H-E). Scale bar, 150 µm.

Discussion

The relationship between ocular and renal pathology in human beings is well known in some type of alterations[17-19]. Ocular neovascularization and haemorrage with associated hiperpermeability are the most common etiologies of visual loss. Neovascularization is another major etiology of visual impairment in diverse conditions such as: radiation retinopathy, sickle cells retinopathy and probably in metabolic alterations secondary to nephropathies. Most disorders associated with ocular neovascularization and haemorrage have several characteristics in common, although each disease has unique etiologies; finally all of them lead to areas of nonperfusion and the development of haemorragic changes. The information is scanty in other situations, such as the relationship between acute renal failure and ocular damage[20].

The aim of this work was to study the ocular alterations occuring in the acute renal failure due to choline deficiency.

To the best of our knowledge only two papers have primary adressed the ocular alterations in choline-deficiency[7, 8]. Bellows and Chinn[7] concluded that: 1. "Free blood was most frequently found between the anterior limiting membrane of the vitreous and the cristalline lens; 2. The ciliary processes were swollen and frequentely hemorrhagic; 3. The presence of blood in the anterior chamber was not uncommon". Burns and Hartroft[8] described that the haemorrhage was located mainly between the vitreous and the crystalline lens, with extension in a few cases to the ciliary region and into the aqueous chambers. In addition they found ocular haemorrhages in 80% of weanling rats with bilateral nephrectomy but not in adult rats. On the other hand, choline-deficiency did not induce ocular haemorrhages in adult rats. The hyaloid arterial system would be the origin of the haemorrhages.

Ocular blood supply has some distinctive characteristics: 1. Retina constitutes the tissue with the greatest oxygen consumption by unit of weight; 2. Ocular blood supply is based on a triple vascular system: a: hyaloid system; b: choroid system; c: retinal system. The hyaloid artery originates from the primitive dorsal ophthalmic artery; arborization of the hyaloid artery originates the tunica vasculosa lentis. After atrophy of the vasa hyaloidea, the eye has two separate circulatory systems that have different anatomical and physiological characteristics. Outer third of the retina is nourished by the chorideal circulation and the inner two thirds receive nutrition from the retinal circulation. These facts make the eye a vulnerable tissue to the biochemical alterations that follow renal necrosis in this experimental model such a increased levels of urea and creatinine[3, 21] and consumption coagulopathy[15].

Data showed that ocular lesion does not occur without a previous renal lesion (Tables 3 and 4). As the renal lesion increase with time[15, 22], so does the ocular lesion. This fact could explain the higher correlation between day of killing and ocular lesions than between grade of ocular and renal lesions. This indicates that some time is required to the development of the ocular lesion after the renal damage has appeared.

According to what it has been mentioned above, it is probably that through an unknown mechanism, the uremia induced by the renal lesion produces microhaemorrhages and massive haemorrhages in a place of low resistance like the eye. In this regard it is interesting to know that Burns and Hartroft[8] found ocular haemorrhages in weanling rats made uremic by bilateral nephrectomy.

It is probably that consumption coagulopathy that occurs in acute renal failure due to a choline-deficient diet may contribute to the development of ocular haemorrhages[15].

Another factor that could be involved in the ocular haemorrhage is arterial hypertension. Arterial hypertension has been described in adult rats deprived of dietary choline for 5 to 6 days when they were weanling and then transfered to a normal diet for a period of 4 to 7 months[23]. In addition, young male choline-deficient rats (65-85 g body weight) developed progresive hypertension after at least 2 weeks of the deficient diet[24]. However, neither Sobin and Landis[25] nor Hartroft[26] found arterial hypertension in wealing rats fed a choline-deficient diet; so this factor can be discarded in our study.

As shown in our study, the most frequently sites where ocular haemorrhages occurred were: anterior chamber, posterior chamber, ciliary region and vitreous. Taking into account the ocular blood supply we can state that all vascular systems are involved. This conclusion differs from those of the previous papers that assert that almost all the ocular haemorrhages where situated in sites supplied by the hyaloid system[7, 8].

The hyaloid circulation is a transitory network of blood vessels responsible for maturing the embryonic and fetal lens and possible post-vitreo retinal regression of this circulation is complete by approximately the third to fourth weeks after birth in the rat. In this experiment all rats were killed between the fifth and eight day after weaning.

Homocysteine was increased in all CD rats, even in the only one without renal damage, no relationship was found between the degree of renal damage and the increase of homocysteine. Since the only CD rat without renal damage had also similar increased levels of homocysteine, it is likely that the acute renal failure was not the cause of it. It is possible that this increase was due to the choline deficiency, as this metabolite, through betaine, is one of the methyl donors for the synthesis of methionine from homocysteine[2].

In summary, our results extent previous results of Bellows and Chinn[7] and Burns and Hartroft[8]. The main differences are: the significative correlation between morphological ocular and renal lesions and concentrations of urea and creatinine in serum and ocular damage and that we have found that the hyaloid system is not the only vascular system involved, although most of the haemorrhages occurs in its territory.

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GALILEI: El padre de la verdad es el tiempo y no la autoridad. ¡Nuestra ignorancia es infinita, disminuyamos de ella tan siquiera un milímetro cúbico! ¿Por qué ahora ese afán de aparecer sabios cuando podríamos ser un poco menos tontos? He tenido la inconcebible felicidad de recibir un instrumento [telescopio] con el cual se puede observar una puntita del universo, algo, no mucho. ¡Utilícenlo!

Bertoldt Brecht (1898-1956)

Galileo Galilei (Leben des Galilei) versión de 1955; con música de Hanns Eisler (1898-1962).
Traducción castellana de Oswald Bayer. Buenos Aires: Nueva Visión, 1984, p 132