ACTA MEDICA (HRADEC KRÁLOVÉ)

1997, Vol. **40**, No. **2**

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ORIGINAL ARTICLES

ISSN 1211 - 4286

ORIGINAL ARTICLE

EFFECT OF DEHYDROEPIANDROSTERONE ON LIPEMIA, GLUCOSE TOLERANCE, INSULINEMIA, INSULIN BINDING TO ERYTHROCYTES IN SHR/N-CP LEAN RATS OF KOLETSKY TYPE

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Summary: Experiments were performed in the genetically hypertensive lean males of Koletsky type.It was monitored the effect of dehydroepiandrosterone (DHEA) treatment on lipemia, glucose tolerance, insulinemia, insulin binding to erythrocytes, fat pads, body weight and pellet intake. DHEA was applied in two doses: 10 and 20 mg per kg b.w.,i.p.,for ll days when glucose tolerance was monitored and for 21 days when the remaining parameters were analyzed. DHEA shows dose dependent decrease in changes of body weight over injection period, in plasma triglycerides and total plasma cholesterol, decrease being most expressed under the higher dose. High as well low of DHEA decreases the sum of glycaemia obtained 30, 60, 120 and 180 min after glucose loading (area under the curve) i.e., DHEA alleviates genetically based glucose intolerance. DHEA induced hypophagia under the higher dose treatment. Insulin binding to erythrocytes was not influenced by DHEA.

Key words: *SHR of Koletsky type; Triglycerides; Cholesterol; Glucose tolerance; Insulinemia; Insulin binding to erythrocytes; Pellet intake; Dehydroepiandrosterone*

Introduction

In our previous study (10) we documented that the alleviation of glucose intolerance in SHR-cp lean males by the long lasting terguride treament is accompanied not only by decrease of insulinemia, but by the increase of insulin binding to erythrocytes as well. Thus the possible causal relationship between increase of insulin binding, the decrease of insulinemia and alleviation of glucose intolerance in the SHR-cp lean males can be taken into consideration. But the findings of Nestler et al (17) do not exclude that the above mentioned changes in insulin binding,insulinemia and in glucose tolerance can be considered only as coincidence without causal relationship or that there are more regulative mechanism of glucose tolerance. The mentioned authors claimed that in neither their study of young nonobese (16) nor obese men (17,21) did DHEA administration affect either fasting serum insulin or glucose levels or tissue sensitivity to insulin (as determined by either the hyperinsulinemic-euglycemic clamp technique (5)or Bergman's modified minimal model technique (1), respectively). Nevertheless, they found DHEA's beneficial action on glucose tolerance. Coleman et al.(7) found that DHEA is potent to prevent the development of diabetes in genetically diabetic (db/db) mice. Moreover, they found (7) that DHEA improved glucose tolerance and reduced plasma insulin in old normal BL/6 female mice. The mentined DHEA effect the authors (7) explained by increase of sensitivity to insulin.

Thus it is well founded to verify the effect of DHEA long lasting treatment in the SHR/N-cp lean rats.

Material and methods

Animals

Experiments were carried out in lean genetically hypertensive males of Koletsky type(controls:n=7,low dose of DHEA:n=9, high dose of DHEA:n=11). Lean SHR/N-cp rats represents dominant non-obese homozygotes and heterozygotes whereas their obese siblings are recessive homozygotes (cp-cp). The abnormal animals were obtained by Koletsky(12) when mating a female spontaneiously hypertensive rat (Okamoto -Aoki strain) with normotensive Sprague-Dawley male rat. The genetically obese animals appeared after several generations of selective inbreeding of hypertensive offsprings of the original cross.

After weaning at the age of 30 days the animals were kept in group of four and supplied with water and DOS-2b pelleted diet ad libitum. During the experiment the animals were kept in group of two in PVC boxes (humidity:

55+10%, room temperature:22+1°C, natural lighting). Body weight,water and pellet intake was daily controlled (except weekends).

Dehydoepiandrosterone (DHEA) treatment

The drug was appliedd i.p. at the dose l0 and 20 mg per kg b.w. for 21 days (when lipemia, insulinemia, insulin binding to erythrocytes , body fat pads and percentage changes of body weight over injection period was investigated) or for ll days (when glucose tolerance was monitored).DHEA was dissolved in aqua pro inj. (l0 or 20 mg in l ml),solution was applied 0.1 ml/l00 g b.w.In control animals aqua pro inj. 0.1ml/100 g b.w. was applied i.p. DHEA was obtained from Web Advanced Products, Inc., Woodd Cross, UT, USA.

Insulin binding to rat erythrocytes

Plasma was separated from approximately 3 ml of heparinized blood drawn by cardiac puncture(under ether anaesthesia by open chest). Erythrocytes were obtained in the presence of constant amount of ^{125}I(A-14) insulin (33pM) at l5°C 3 hours.Results were corrected for nonspecific binding. The details of the method are published previously (Hilgertová et al.(l990).

Plasma lipids and insulinemia.

Blood sampled by cardiac puncture (under light ether anaesthesia at 07.00 a.m. after l4 h starvation) was centrifuged and the serum stored in plastic tubes at -2O° C.Total plasma cholesterol and plasma triglycerides were estimated enzymatically by Hitachi analyzer,plasma insulin was estimeted by RIA.

Glucose tolerance

Blood was sampled to heparinized capillaries (from the retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycaemia) as well as 30,60,l20 and l80 min after glucose loading. Glucose (3g/kg b.w., 30% solution) was applied intragastrically after 14 h starvation. Glycaemia was estimated enzymatically.

Fad pads

Immediately after finishing the cardial puncture the animal was decapitated, epididymal and retroperitoneal fat pads were weighted and their weight expressed in g/100 g b.w.

Statistics

The data were analyzed by program SOLO. Statistical significance of intergroup differences was evaluated by t-test.

Results

Effect of DHEA on % of changes of body weight (Table l)

When compared to controls, low dose shows no effect, high dose shows profound decrease. Effect of low dose and high dose differs significantly.

Effect of DHEA on fat pads (Table l)

DHEA shows no effect on epididymal and/or retroperitoneal fat pads.

Table l:

Group	Initial body weight (g)	% changes of body over injection period	Epididymal fat pad (g/l00g b.w.) b.w.)	Retroperitoneal fat pad(g/100g
Controls(7)	289+16	+7.00+2.89	1.29+0.31	1.25+0.28
DHEA-10(9)	268+21^{bB}	+7.00+3.88	1.13+0.39	1.05+0.39
DHEA-20(11)	288+26	+1.91+2.59^{dD}	1.29+0.17	1.33+0.33

Means + SEM are presented. The number of animals per group is in the brackets. The significance of values (by two tailed t-test) refers to the comparison between control and dehydroepiadrosteron treated animals. Abbreviations: DHEA-10: 10 mg/kg b.w., DHEA-20: 20 mg/kg b.w., a - P<0.l0,b - P<0.05, c - P<0.02, d - P<0.01(significance versus controls). Capital:significance DHEA-10 versus DHEA-20.

Effect of DHEA on plasma triglycerides and total plasma cholesterol (Table 2)

DHEA shows dose dependent effect (i.e. decrease) on plasma triglycerides as well as on total plasma cholesterol.

In both cases high dose shows significantly greater effect.

Low dose shows effect only on plasma triglycerides.

Pellet intake (Table 2)

Hypophagia was induced by the higher dose. Low dose remained without effect.

Table 2:

Group	Plasma triglycerides (mmol/l)	Total plasma cholesterol (mmol/l)	Pellet intake (g/100g b.w./day
Controls(7)	0.90+0.15	1.80+0.16	7.77+0.78
DHEA-10(9)	0.67+0.08^{d}	1.78+0.10	7.19+0.65
DHEA-20(11)	0.53+0.09^{dD}	1.56+0.12^{D}	6.93+0.58^{d}

Means + SEM are presented. The abbreviations are the same as in Table l.

Basal glycaemia (Table 3)

Under the high as well as low dose there is a decrease.

Glucose tolerance (Table 3)

High as well as low dose decrease the sum of glycaemia obtained 30,60,l20 and l80 min after glucose loading (area under the curve).

Insulinemia (Table 3)

Insulinemia shows no changes after long lasting DHEA treatment.

Percentage of insulin binding to erythrocytes (Table 3)

Insulin binding was not influenced by long lasting DHEA treatment.

Table 3:

Group	Basal glycaemia (mmol/l)	Glucose tolerance -" area under the curve" (mmol/l)	IRI (pmol/l)	% insulin binding to erythrocytes
Controls(7)	5.45+0.61	29.32+2.13	243+77	2.05+0.92
DHEA-10(9)	4.46+0.44[d]	24.04+2.71[d]	222+38	2.24+0.88
DHEA-20(11)	4.18+0.44[d]	25.85+3.37[c]	206+103	1.64+0.67(9)

Mean + SEM are presented. The abbreviations are the same as in Table l."Area under the curve" represents the sum of glycaemia 30,60,120 and 180 min after glucose loading.

Discussion

As mentioned in the introduction in our previous paper (10) we documented in SHR/N-cp lean males that long lasting terguride treatment shows alleviation of glucose intolerance. This is accompanied by decrease of insulinemia and by increase of percentage of specific insulin binding to erythrocytes. Our recent data documented that the changes in glucose tolerance is not in all cases accompanied by parallel changes in insulinemia and in insulin binding.

At this place it would be suitable to mention the data obtained by Škrha et al. (20). They monitored the effect of short term fasting in obese type 2 diabetes mellitus. Short term fasting reduced slightly but significantly body weight, which was accompanied by reduction of fasting plasma glucose, by increased glucose disposal rate and by increase of metabolic clearance rate of glucose. No changes of insulin receptors on erythrocytes were observed.

There are two common features with our results, i.e., the beneficial effect of treatment of the abnormalities in glucose metabolism in the mentioned type of patients is not accompanied by the changes in insuline receptors on erythrocytes but there is decrease of fasting plasma glucose. The mentioned results(20) suggest that elevated insulin sensitivity has not to be accompanied by changes in insulin receptors on erythrocytes.

Svačina et al. (18) nowdays when studying the relationship between the the basal level of DHEA and insulin sensitivity they found that there is significant positive correlation between the mentioned parameters. They studied the changes of plasmatic DHEA during IVGTT in health subject and in patients suffering from diabetes mellitus. The increase of insulin was followed by elevation of DHEA. They estimated tissue sensitivity to insulin by hyperinsulinemic-euglycaemic clamp technique.These data suggest a close relationship between insulinemia induced by IVGTT and DHEA. Direct pendant to our measurement is presented by Nestler at al.(16,17)and by Coleman et al (6,7).Nestler et al. (16,17) found that in young obese as well as nonobese men DHEA shows no changes in tissue sensitivity to insulin (as determined by hyperinsulinemic-euclycaemic clamp technique),but that DHEA shows beneficial effect on glucose tolerance. Coleman et al. (6) demonstrated that DHEA addministration prevented the development of diabetes mellitus in genetically diabetic (db/db) or obese (ob/ob) mice. In the other paper Coleman et al. (7) showed that DHEA increases tissue sensitivity to insulin in aged normal mice. As the markers of the elevation of tissue sensitivity to insulin they judged the improved glucose tolerance and reduced plasma insulin The mentioned beneficial effect of DHEA can be in our experiments expressed by decrease of basal glycaemia and by the decrease of „area under the curve", i.e. by the decrease of sum of glycaemia 30,60,120 and l80 min after glucose loading. Both changes can be viewed as an expression of elevation of insulin sensitivity. But it remains to be solved why this assumed change in insulin sensitivity is not accompanied by the changes in insulin binding to erythrocytes and by some changes in insulinemia.

This question arises when we consider the effect of terguride on the glucose tolerance in the animals of the same strain and sex, i.e., SHR/N-cp lean. We found that the decrease of glucose tolerance was accompanied by elevation of insulin binding to erythrocytes and by decrease by insulinemia (10).

It cannot be a priori excluded that the same changes in the glucose tolerance induced by two different substances (terguride versus DHEA) are based on the changes of insulin sensitivity (i.e., its elevation), but this assumed increase of insulin sensitivity is not accompanied in the same manner by changes in insulin binding to erythrocytes and by the changes in insulinemia. Similarly, while the glucose intolerance which we found in SHR/N-cp obese rats as well as in their lean siblings (9) is accompanied by profoundly reduced specific insulin binding on erythrocytes (11), then glucose intolerance induced by brain oligemic hypoxia shows no changes in insulin binding to erythrocytes (in preparation).

On the other hand, glucose intolerance induced by oligemic brain hypoxia is accompanied by hyperinsulinemia. All the mentioned data suggest that glucose intolerance is conditioned by decrease of insulin sensitivity , but its expression in the changes of insulin binding to erythrocytes and the changes in insulinemia can be dependent on the factor which evoked the glucose intolerance (genetic factor versus brain oligemic hypoxia) and/or on factor alleviating glucose intolerance (terguride versus DHEA).

It is known (19) that under the conditions of insuficiency of insulin secretion (diabetes I) or when insulin resistence was developed (diabtes II), triglycerides are elevated by the intensified lipolysis and by increase input of nonestirified acids to the liver. DHEA in our series of experiments decreases the basal glycaemia and increase glucose tolerance (Table 3).Considering the last mentioned DHEA effect as an expression of increase of insulin sensitivity, then decrease of triglycerides induced by DHEA (Table 2) can be judged as a consequence of the changes of the mentioned insulin sensitivity (i.e., its elevation). As mentioned above, the effect of DHEA is not limited only to changes in glucose tolerance. We documented dose dependent effect on plasma triglycerides and total plasma cholesterol.

While decrease of cholesterol was found only after higher dose, then decrease of plasma triglycerides was found under low as well as high dose (Table 2). Moreover, the decrease after high dose was significantly higher than after low dose.

As to the effect of DHEA on plasma triglycerides our data are in accordance with Mohan et al. (14). They found in rat that treatment with DHEA lowered triglyceride levels regardless of whether it was elevated by diet or not. On the other hand, total plasma cholesterol levels were lowered by DHEA only when it was higher than normal due to feeding the condensed milk diet (14). Kurzman et al. (13) found that DHEA is potent to lower total plasma cholesterol in both lean and obese dogs. Moreover, studies (8,16) using human subjects have shown decrease in low density lipoprotein cholesterol levels following DHEA treatment.

At this place the nutrition parameters of our rats must be mentioned. We demonstrated (Table 2) dose dependent effect of DHEA on pellet intake. While the low dose remained without effect, then the higher dose induced hypophagia.

It cannot be excluded that the decrease of triglycerides as well as cholesterol are done to a certain degree by hypophagia.It does not hold good for triglycerides under the lower dose of DHEA. There was not proved statistically significant decrease of pellet intake.

With the same precaution we must take the lowering effect of DHEA in basal glycaemia and in percentage of changes of body weight over injection period. Especially under the higher dose the effect of DHEA induced hypophagia cannot be overlooked.

As to the effect of DHEA on food intake per se there is no uniformity in the published data. On one side, Cleary et al (2,3) when studying the effect of DHEA in obese Zucker rat, they found that cumulative food intakes tended to be lower in obese-treated compared with obese-nontreated rats. On the other hand, Cleary (4) documented that DHEA in Sprague-Dawley rats DHEA does not show any effect on nutrition.

It is obvious that when we want to study hypolipidemic effect of DHEA then species, genetic, and nutritional factors must be taken into consideration.

Conclusions

Data presented by Nestler et al. (16,17), Svačina et al. (18), Škrha et al. (20) as well as our previous data (10) and recent findings suggest that DHEA can be potent to influence insulin sensitivity but that this influence is not expressed in all cases by change in insulin binding to erythrocytes and by the changes in insulinemia.Moreover, DHEA can show species dependent effect in the influence on insulin sensitivity.

Acknowledgement

This paper was supported by Internal Grant Agency of Ministry of Health of the Czech Republic No 3684-3. The authors wish to thank Carl T. Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA,for providing the genetically hypertensive rats of Koletsky type.

References

1. Berdanier CD, McIntosh MK. Further studies on the effects of dehydroepiandrosterone on hepatic metabolism in BHE rats. Proc Soc Exp Biol Med l989;l92:242-7.
2. Cleary MP, Shepherd A, Jenks B. Effect of dehydroepiandrosteron in lean and obese Zucker rats. J Nutr l984;ll4:1242-51.
3. Cleary MP, Fox N, Lazin B, Billheimer JT. A comparison of the effects of dehydroepiandrosterone treatment to ad libitum and pair-feeding in the obese Zucker rat. Nutr Res l985;5:1247-57.
4. Cleary MP, Shepherd A, Zisk J., Schwartz AG. Effect of dehydroepiandrosterone on body weight and food intake in rats. Nutr Behav l983;1:127-36.
5. Cleary MP, Hood SS, Chando C, Hanson CT, Billheimer JT. Response of sucrose-fed BHE-rats to dehydroepiandrosterone. Nutr Res l984;4:485-94.
6. Coleman DL, Leiter EH, Schwitzer RW. Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice.Diabetes l982;31:830-3.
7. Coleman DL, Schwitzer RW, Leiter EH. Effect of genetic background on the threrapeutic effects of dehydroepiandrosterone (DHEA) in diabetes-obesity mutans and in aged normal mice. Diabetes l984;33:26-32.
8. Fassati P, Fessati M, Šonka J, Josifko M. Treatment of stabilized liver cirrhosis by dehydroepiandrosterone sulphate. Agressologie l973;14:259-68.
9. Golda V, Cvak L. Terguride but not bromocriptine alleviatted glucose tolerance abnormalities and hyperlipidemia in obese and lean genetically hypertensive Koletsky rats. Physiol Res l994;43:299-305.
10. Golda V, Hilgertová J. Effect of tran-dihydro-lisuride on insulin binding, insulinemia, glucose tolerance and cholesterolemia in SHR Koletsky rats. Physiol Res (in press).
11. Hilgertová J, Kummel L, Hovorka R, Golda V. Decreased insulin binding and internalization in hepatocy-

tes and erythrocytes of SHR Koletsky rats depend on the presence of „f“ gene and on sex. Metabolic characteristics of hepatocytes from obese rats. In: Klimeš I, Howard BV, Kahn CR, eds. Insulin and cell membrane. New York:Harwood Academic Publishers, 1990:81-92.
12. Koletsky S. Pathologic findings and laboratory data in a new strain of hypertensive rats. Am J Pathol 1975;80:129-40.
13. Kurzman ID, MacEven EG, Haffa ALM. Reduction in body weight and cholesterol in spontaneously obese dogs by dehydroepiandrosterone. Int J Obes 1990;14:95-104.
14. Mohan PF, Ihnen JS, Levin BE, Cleary MP. Effects of dehydroepiandrosterone treatment in rats with diet-induced obesity. J Nutr 1990;120:1103-14.
15. Muller S, Cleary MP. Glucose metabolism in isolated fat cells from lean and obese Zucker rats following treatment with dehydroepiandrosterone. Metabolism 1985; 34:278-84.
16. Nestler JE, Barlascini CO, Clore JN, Blackard WG. Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. J Clin Endocrinol Metab 1988;66:57-61.
17. Nestler JE, John N, Blackard C, Blackard WG. Metabolism and actions of dehydroepiandrosteone in humans. J Steroid Biochem Molec Biol 1991;40:599-605.
18. Svačina Š, Kvapil M, Haas T, Šonka J. Insulin, steroids and steroidogenese. Česk Fyziol 1997;46:51-6.
19. Škrha J. Insulin resistance and non-insulin-dependent diabetes mellitus. Vnitr Lek 1994;41:105-9.
20.Škrha J, Šindelka G, Hilgertová J. The effect of dietary regimen and vitamin E on insulin action in obese type 2 diabetes mellitus. Abstract of 3rd International Smolenice Insulin Symposium on Lipids and Syndrome of Insulin Resistance, August 28-September 1996, p.74.
21. Usiskin KS, Butterworth S, Clore JN et al. Lack of effect of dehydroepiandrosterone in obese man. Int J Obesity 1990;14:457-63.

Submitted May 1997.
Accepted June 1997.

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ORIGINAL ARTICLE

CHANGES OF CHOLINESTERASE ACTIVITY IN THE ERYTHROCYTES, PLASMA, DIAPHRAGM, LIVER AND VARIOUS PARTS OF THE BRAIN IN THE RABBIT FOLLOWING TRANSFUSION OF ERYTHROCYTES WITH SOMAN INHIBITED ACETYLCHOLINESTERASE

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Summary: 1. The changes of cholinesterase activity in rabbit blood, peripheral tissues and the central nervous system following transfusion of erythrocytes with soman inhibited acetylcholinesterase we- were demonstrated. 2. After incubation with soman for 0.5 or 24h, erythrocytes without acetylcholinesterase activity were injected to intact rabbits and cholinesterase activity in the erythrocytes, plasma, diaphragm, liver and various parts of the brain were evaluated 24h following blood-transfusion. 3. When erythrocytes were incubated with soman for 24h, no changes of cholinesterase activity in the rabbit following blood-transfusion were observed with an exception of erythrocyte acetylcholinesterase. 4. When erythrocytes were incubated with soman for 0.5h, a significant decrease in cholinesterase activity in the erythrocytes, plasma, diaphragm and liver following blood-transfusion was found. These data show that soman is able to release from erythrocytes and inhibit cholinesterase activities not only in vitro but also in vivo although the significant inhibition of cholinesterase activities by soman was only observed in the peripheral compartment.

Key words: *Soman; Acetycholinesterase; Butyrylcholinesterase; Blood-transfusion; Rabbit*

Introduction

In spite of a good knowledge of the basic mechanism of nerve agent toxic effects, the treatment of acute intoxication with nerve agents has not been satisfactorily efficacious yet (3). Especially soman (pinacolyl methylphosphonofluoridate) is really resistant to antidotal treatment (7,8,10). Soman differs from many other organophosphates in the rate of aging and in the existence of a depot in the organism (2). The rapid process of aging, that means the monodealkylation of soman-inhibited acetylcholinesterase (AChE, EC 3.1.1.7), prevents both the spontaneous reactivation and the reactivation induced by oximes (2,5). The existence of a soman depot in the organism can also influence soman poisoning because soman can be released from the depot and cause a new attack of intoxication. This depot was described for the skin, muscles and lung (2,6).

Another structure, where soman could be released from, is the erythrocyte. In experiments in vitro, the ability of soman to release from erythrocytes after inbubation with them was found. This ability was evaluated with the help of incubation of soman-intoxicated erythrocytes with horse plasma butyrylcholinesterase (BuChE, EC 3.1.1.8). The soman ability to release from erythrocytes depends on the time of soman incubation with them. The amount of released soman decreases if the time of erythrocyte incubation with soman increases (1).

The ability of soman to release from erythrocytes could be important for acute soman intoxication because a considerable part of soman could be transported to the site of its toxic effect after its binding on erythrocytes and could reinhibit oxime-reactivated AChE in the peripheral or central compartment.

The aim of this study was to demonstrate the changes of AChE or BuChE activity in the peripheral and central compartments following blood-transfusion of rabbits with soman-incubated erythrocytes.

Methods

Male rabbits (3.4 - 3.7kg) obtained from Konárovice were kept in the animal house at the Department of Toxicology of the Military Medical Academy. The animals were allowed free access to standard laboratory food and tap water. They were housed in an air-conditioned room (20-22°C) on 12-h light/12-h dark cycles. Handling of experimental animals was made under the supervision of the Ethics Commitee of Medical Faculty of Charles University and Military Medical Academy.

After exsanguination of the anaesthetized rabbit (urethan i.p. 1g/kg), the blood was centrifuged to obtain erythrocytes and plasma. Erythrocytes were incubated with soman (10^{-3}M) for 0.5 or 24h. Following the incubation and separation of erythrocytes from soman solution by

centrifugation, erythrocytes were washed with saline solution three times.

These erythrocytes were added into rabbit plasma and exchanged blood-transfusion to another rabbit was performed (30ml/kg). The number of animals was six in the control (blood-transfusion with erythrocytes without incubation with soman) as well as experimental groups. The experiments were terminated 24h after blood-transfusion. Each rabbit was killed by air embolism into the carotid artery; blood, diaphragm, liver and brain were removed and the brain was divided into parts. Hemispheres, cerebellum, medulla oblongata and pons Varoli were chosen for the experiments. The blood was centrifuged to obtain erythrocytes and plasma. The erythrocytes were hemolysed and the diaphragm, liver and brain parts were homogenized in distilled water. The AChE and BuChE activity were measured by a spectrophotometric assay using acetylthiocholine or butyrylthiocholine as the substrates (4).

The total AChE or BuChE activities were expressed as μmol substrate hydrolyzed/ml/min and presented as percents of controls. Statistical significance was determined by the use of Student t test and differences were considered significant when $p < 0.05$. Statistical evaluation was performed with relevant programmes using an ADT 4500 computer.

Results

The changes of AChE or BuChE activities in the erythrocytes, plasma, diaphragm, liver and various parts of the brain following blood-transfusion with erythrocytes incubated with soman for 24h are shown in Fig. 1. Only the erythrocyte AChE activity significantly decreased ($p < 0.05$) in comparison with control rabbits because the erythrocytes without AChE activity were injected into rabbits. The BuChE activity in the plasma and liver as well as the AChE activity in the diaphragm and brain parts tested were not significantly changed in comparison with controls.

The changes of AChE or BuChE activities in the erythrocytes, plasma, liver, diaphragm and various parts of the brain following blood-transfusion with erythrocytes incubated with soman for half an hour are shown in Fig. 2. Not only the erythrocyte AChE activity but also the diaphragm AChE activity as well as the plasma and liver BuChE activity were significantly decreased in comparison with control rabbits ($p < 0.05$). On the other hand, the AChE activity in various parts of the brain was not significantly different from the control AChE activity values.

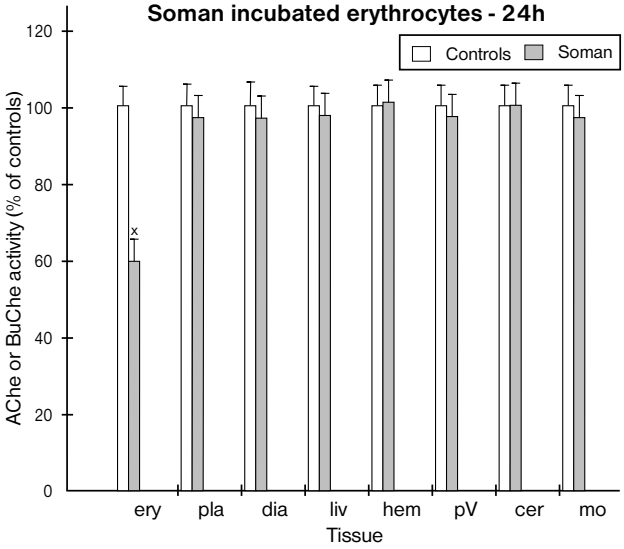

Fig. 1: Changes of AChE activity in the erythrocytes (ery), diaphragm (dia) and various parts of the brain (hem, pV, cer, mo) and the BuChE activity in the plasma (pla) and liver (liv) following blood-transfusion with erythrocytes incubated with soman for 24h in rabbits.

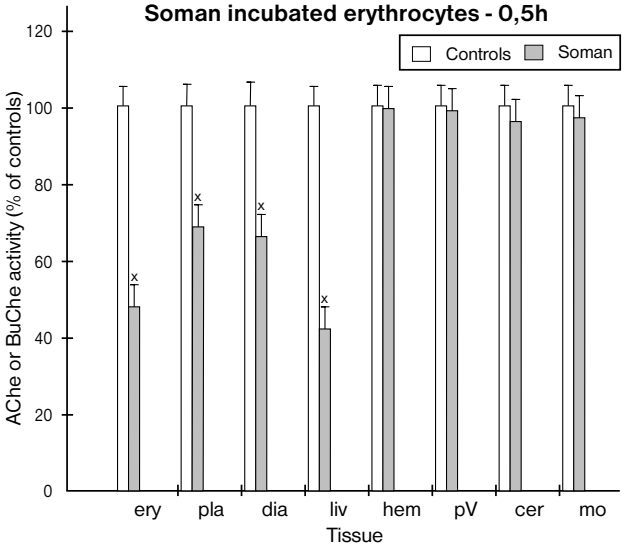

Fig. 2: Changes of AChE activity in the erythrocytes (ery), diaphragm (dia) and various parts of the brain (hem, pV, cer, mo) and the BuChE activity in the plasma (pla) and liver (liv) following blood-transfusion with erythrocytes incubated with soman for 0.5h in rabbits.

Discussion

Antidotal treatment that consists of anticholinergic drugs to counteract the accumulation of acetylcholine and oximes to reactivate the nerve agent inhibited AChE (3,9) is not sufficiently effective against acute soman intoxication because of rapid aging and depot existence (2,7,8,10). To improve the efficacy of antidotal treatment of acute intoxication with soman, it is necessary to better understand the toxikinetics as well as toxidynamics of soman intoxication (2).

Our results confirm that soman is able to release from erythrocytes after binding on them. The rate of soman releasing depends on the time of the incubation of erythrocy-

tes with soman. Following 24h incubation, soman released in a concentration of 2.62 . 10^{-10}M (1) and it was not able to inhibit the AChE activity in vitro and in vivo after blood-transfusion of the rabbits with soman-incubated erythrocytes. Following 0.5h incubation, soman released in a concentration of 4.64 . 10^{-8}M (1) and it was able to inhibit the AChE or BuChE activities not only in vitro but also in vivo after blood-transfusion of the rabbits with soman-incubated erythrocytes.

The significant decrease in the cholinesterase activities was demonstrated in the peripheral compartment (plasma, diaphragm, liver) but not in the central compartment (various parts of brain). It is very difficult to explain this fact. We suppose that the features of soman can be changed following its releasing from erythrocytes and that is why it is not able to across the blood-brain barrier. The further research with labeled soman could be useful for the confirmation of our hypothesis.

In conclusion, not only the skin, muscles or lung but also erythrocytes could be depot for soman and soman releasing from erythrocytes could contribute to the development of acute soman intoxication.

Acknowledgement

The authors express their appreciation to Mrs. R. Věříšová and M. Zechovská for their technical assistance and to Mr. V. Bláha for his help with statistical evaluation.

References

1. Bajgar J, Kassa J, Krs O, Fusek J. Inhibition of rabbit erythrocyte acetylcholinesterase by soman (lecture). The Fifth International Symposium on Protection against Chemical and Biological Warfare Agents, June 11-16, 1995, Stockholm Sweden.

2. Bajgar J. Present views on toxidynamics of soman poisoning. Acta Med (Hradec Králové), 1996;39:101-5.

3. Dawson RM. Review of oximes available for treatment of nerve agent poisoning. J Appl Toxicol 1994;14:317-31.

4. Ellman GL, Courtney DK, Andres JrV, Featherstone RN. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.

5. Fleisher JH, Harris LW. Dealkylation as a mechanism for aging of cholinesterase after poisoning with pinacolyl methylphosphonofluoridate. Biochem Pharmacol 1965;14:641-8.

6. Kadar T, Raveh L, Cohen G et al. Distribution of H^3H-soman in mice. Arch Toxicol 1985;58:45-9.

7. Kassa J. Comparison of efficacy of two oximes (HI-6 and obidoxime) in soman poisoning in rats. Toxicology 1995;101:167-74.

8. Koplovitz I, Stewart JR. A comparison of the efficacy of HI-6 and 2-PAM against soman, tabun, sarin and VX in the rabbit. Toxicol Lett 1994;70:269-79.

9. Marrs TC. Organophoshate poisoning. Pharmacol Ther 1993;58:51- 66.

10. Shih T-M. Comparison of several oximes on reactivation of soman - inhibited blood, brain and tissue cholinesterase activity in rats. Arch Toxicol 1993;67:637-46.

This study was supported by the grant of IGA MZ No 4074-2.

Submitted May 1997.
Accepted June 1997.

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ORIGINAL ARTICLE

LONG-TERM RESULTS IN HAIRY CELL LEUKEMIA TREATED WITH 2-CHLORODEOXYADENOSINE

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Supported by Grant No 3690-2 IGA from the Ministry of Health of the Czech Republic.

Summary: We treated 19 patients with hairy cell leukemia (HCL) with 2-chlorodeoxyadenosine. 15 patients followed up at least 6 months were evaluated. The follow up period varied between 6 months and 37 months (median, 19 months). 8 patients were previously treated. The overall response in 15 evaluable HCL patients was 100 %, with 87 % complete hematological remissions including three patients with retroperitoneal and mediastinal lymphadenopathy and one patient with leukemic infiltrates of the cornea; 13 % of patients achieved partial hematological remission. Soluble interleukin - 2 receptor (sIL-2R) considered as a reliable non-invasive marker of HCL tumor burden dropped from the median of 1350 pM/ml (range 188 to 9000 pM/ml) to the median of 84,3 pM/ml (range 37 to 382 pM/ml) RdW which reflects the anisocytosis of red cells decreased after therapy from the median of 20,6 % (range 13,1 - 25,0 %) to the median of 13,7 % (range 12,4 - 16,3 %).

Key words: *HCL; Therapy with chlorodeoxyadenosine; Long-term results; sIL-2R; RdW*

Hairy cell leukemia is a chronic lymphoproliferative disorder characterized by abnormal mononuclear cells of B lymphocyte origin infiltrating bone marrow and spleen. Patients, usually middle-aged men, often present with some combination of anemia, neutropenia, thrombocytopenia and splenomegoly. There is currently no single antibody which identifies an antigen unique to the hairy cells. These cells mostly demonstrate the pan-B-cells antigens Ig+, CD 19+, CD 20+, CD 22+, and usually lack surface CD 5- and CD 21-. Recently DBA 44, the hairy cell associated monoclonal antibody, has been used for demonstration of hairy cell in biopsy sections. Hairy cells characteristically express the receptor for interleukin-2 (IL-2R) on their membrane and although sIL-2R production is not unique only to hairy cells, the serum levels can be used as a marker of leukemic cell burden at diagnosis and for monitoring therapeutic efficiency, and for the detection of minimal residual disease (1,4,17).

Although the disease is relatively indolent, the majority of patients require treatment for life-treatening pancytopenia or symptomatic splenomegaly. Slenectomy has been used for over three decades as the initial treatment option for HCL. Splenectomy has been certainly beneficial for some patients (3) resulting in a significant improvement of their pancytopenia but splenectomy has no effect on bone marrow infiltration by leukemic cells. As a result, approximately 50 % of splenectomized patients have recurrent cytopenias that require systemic therapy. Interferon-alpha was the first drug in which de possibility to cure HCL was originally considered. This expectance was not fulfilled. Interferon-alpha was highly effective in the management of HCL but it did not have a curative potential. Relapses were observed wihin the 6^{th} and 28^{th} month after the with drawal of the therapy with interferon alpha. The introduction of two new purine analogues, 2-deoxycoformycin (DCF) and 2-chlorodeoxyadenosine (2-CdA) has dramatically improved treatment option in the last years (16). 2-CdA has been shown to induce complete remission (CR) in the majority of patients, with only a single cycle and a paucity of toxicities (7,16). However, persistence of minimal residual disease in the bone marrow, detected either by immunohistochemistry or polymerase chain reaction suggests that some patients are at risk of relapse (6, 10). The purpose of this study is to determine the durability of remissions and relapse rate in patients with HCL treated with a single cycle of 2CdA.

Patients and Methods

Since 1994 when 2-CdA became available in this country we have administered 2-CdA in 19 patients with HCL.

The diagnosis of HCL was based on the presence of morphologically characteristic cells in the peripheral blood and/or the bone marrow, demonstration of tartarate resistant acid phosphatase activity in the neoplastic cells (20), typical histologic pattern in bone marrow biopsies with infiltration of malignant cells characteristically surrounded by a rim of pale cytoplasm resulting in clearly separated nuclei (2). In all 6 splenectomized patients the diagnosis of HCL has been reconfirmed by the histologic finding in the spleen showing heavy infiltration of the red pulp by abnormal interdigitating mononuclear cells and the presence of blood-filled spaces lined by hairy cells, so called pseudosinuses (15).

The levels of sIL-2R were determined by a sandwich enzyme immunoassay (Immunoenzymometric assay Test Kit cat. 0559, IMMUNOTECH) normal values obtained by determination of sIL-2R in 20 healthy blood donors were 30,4 ± 13,6 pM/l.

The RdW (in %) was determined by Coulter JT3.

Table 1: Patient Characteristics

Characteristics	
No of Patients	15
Age (yr)	
Median	61
Range	43-84
Sex	
Male	12
Female	3
Previous treatment	
None	7
Splenectomy	4
IFN-alpha	2
IFN-alpha, SPL	1
IFN-alpha, SPL, IFN-alpha	1
Duration of HCL before therapy (months)	2CdA
Median	13
Range	1-137
Bone marrow infiltration	
Diffuse	10
Interstitial to diffuse	2
Interstitial	3
Hgb (g/l)	
Median	107
Range	(88-149)
ACN ($x10^9$/l)	
Median	0,7
Range	(0,4-7,7)
Platelets ($x10^9$/l)	
Median	92
Range	(35-218)

SPL, splenectomy; ACN, absolute neutrophil count

The main patient characteristics are listed in table 1. Out of 19 patients 15 with follow up period more than 6 months were avaluated. There were 12 men and 3 women, with an age range of 43 to 84 years (median, 61 years). Seven patients were previously untreated. Eight patients were previously treated, four with splenectomy, two with interferon-alpha (IFN-alpha) only, one with splenectomy then IFN-alpha and one with IFN-alpha then splenectomy followed by IFN-alpha and then 2-CdA. The duration of HCL befor the start of 2-CdA therapy varied between 1 and 137 months. The bone marrow biopsy performed before the administration of 2-CdA revieled diffuse infiltration in 10 patients, interstitial with some areas of diffuse infiltration in 2 and interstitial infiltration in 3 patients. The hemoglobin levels were 88 to 149 g/l (median, 107 g/l) absolute neutrophil counts 0,4 to 7,7 x 10^9/l (median, 0,7 x 10^9/l), platelet counts 35 to 218 x 10^9/l (median, 92 x 10^9/l).

Eligibility

Eligibility criteria included the following:

(1) Confirmed diagnosis of HCL based on the criteria mentioned above (2) follow up at least six months, (3) evidence of active disease, including any of the following: neutropenia (absolute neutrophil count < 1,5 x 10^9/l), anemia (hemoglobin level < 120 g/l), thrombocytopenia (platelet count < 100 x 10^9/l), retroperitoneal lymphadenopathy.

Administration of 2-CdA

All patients received a single cycle of 2-CdA (Leustatin, Cladribine, Orthobiotech, Raritan, NY) at a dose of 0,1 mg/kg/d by continuous intravenous infusion for 7 days.

Supportive care

Neutropenic patients who developed fever greater than 38 °C were given broad-spectrum antibiotics. However many such patients with sterile blood cultures had no evidence of infection. Packed red blood cells were not routinely transfused, but rather were administered only for symptomatic anemia. Platelets were administered prophylactically if the platelet count was less than 10 to 15 x 10^9/l. Hematopoetic growth factors were administered in only 4 patients with severe neutropenia.

Initial evaluation

At the time of study entry, all patients had a complete history and physical examination; complete blood cell count (CBC) with differential and platelet count; computed tomographic (CT) or ultrasound (US) scans of the chest, abdomen, and pelvis; marrow aspirate and unilateral bone core biopsy.

Patients were monitored without other therapy and were then reevaluated at 3 and 6 months with a unilateral bone marrow aspirate and biopsy and CT or US scans of the abdomen and pelvis.

Response criteria

Patients were evaluated for response 6 months after the initiation of 2-CdA. CR (complete hematologic remission) required all of the following:

(1) complete absence of hairy cells in the peripheral blood

(2) normalization of peripheral blood counts (hemoglobin level > 120 g/l, white blood cell count > 3 x 10(/l, absolute neutrophil count > 1,5 x 10^9/l, platelet count > 100 x 10^9/l, disappearance of retroperitoneal lymphadenopathy and hepatosplenomegaly by CT or US scans.

A PR (partial remission) required all of the following:

(1) Failure of normalization in one of low peripheral blood counts

(2) Reduction of greater than 50 % in abnormal lymphadenopathy or hepatosplenomegaly.

Relaps was defined: as reappearance of hairy cells in the peripheral blood and decrease of blood cell count below the values required for CR; increase of lymphadenopathy and/or hepatosplenomegaly.

Results

Out of 15 assessable patients, 13 (87 %) achieved CR with a single cycle of 2-CdA; 2 (13 %) achieved PR. Therefore, the overall response rate with a single cycle was 100 %. Of the 2 patients achieving PR, the platelet count was 48 x 10^9/l in the first and 90 x 10^9/l in the second one (table 2). Retroperitoneal and mediastinal lymphadenopathy disappeared in all three patients in whom it was noticed before the initiation of 2-CdA therapy. In one patient with infiltrates of cornea these infiltrates disappeared as well as mediastinal and retroperitoneal lymphadenopathy which were present before the initiation of therapy with 2-CdA. No patient died and no patient relapsed during the follow up which varied between 6 and 37 months (median, 19 months).

Table 2: Blood cell counts in 15 patients with HCL before and after therapy with 2-CdA

No	Name	Sex	ANC (x10^9/l)		Hgb(g/l)		Platelets x10^9/l		Remission
			before	after	before	after	before	after	
1	Z.J	M	0,4	2,2	35	123	35	112	CR
2	L.Z	M	1,2	5,0	128	148	293	412	CR
3	T.D	F	1,4	2,6	102	139	218	344	CR
4	M.O	M	7,7	4,0	120	154	106	173	CR
5	B.J	M	1,3	7,0	130	159	110	268	CR
6	M.L	F	0,6	5,8	118	147	140	422	CR
7	Ž.J	M	0,4	2,9	88	161	92	185	CR
8	F.J	M	1,2	2,0	106	141	111	154	CR
9	Š.V	F	0,4	5,5	101	129	87	192	CR
10	D.S	M	0,6	3,6	132	152	89	182	CR
11	V.J.	M	1,6	9,6	60	128	190	178	CR
12	Z.S.	M	1,7	7,9	107	142	79	131	CR
13	G.A	M	0,7	3,3	88	125	25	48	PR
14	M.W	M	0,7	4,4	104	168	58	90	PR
15	H.M	M	0,7	3,0	149	161	217	359	CR
Median			0,7	4,0	107	147	92	183	

Vysvětlivky: ANC - absolute neutrophil count
CR - complete remission
PR - partial remission

The sIL-2R levels were increased in all patients before the initiation of the therapy with 2CdA in the range of 188 to 9000 pM/ml (median, 1350 pM/ml), they decreased after the therapy to 37 to 382 pM/ml (median, 84,3 pM/ml) -see fig. 1. The lowest value of sIL-2R before initiation of therapy was in a patient treated previously with IFN-alpha, splenectomy and the second course of IFN-alpha therapy. Complete normalization of sIL-2R level after therapy was reached only in 2 patients (34,7 and 30,7 pM/ml).

The RdW values were before the therapy between 13,1 % to 25,0 % (median, 20,6 %), after therapy between 12,4 and 16,3 % (median, 13,7 %). The value 16,3 % was in the patient who reached only the PR (see Fig. 2).

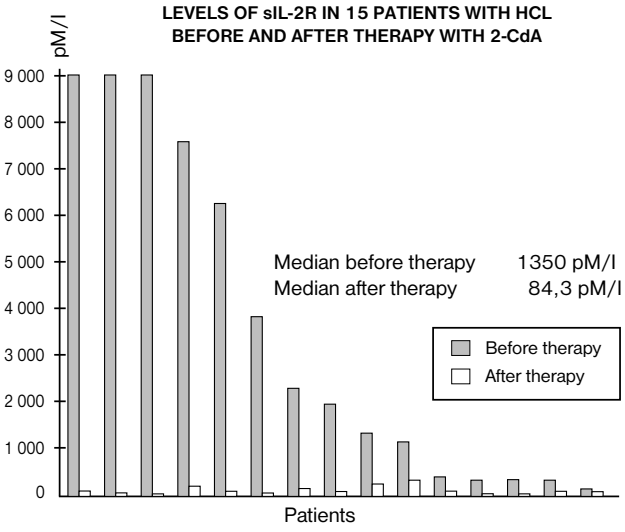

Fig. 1

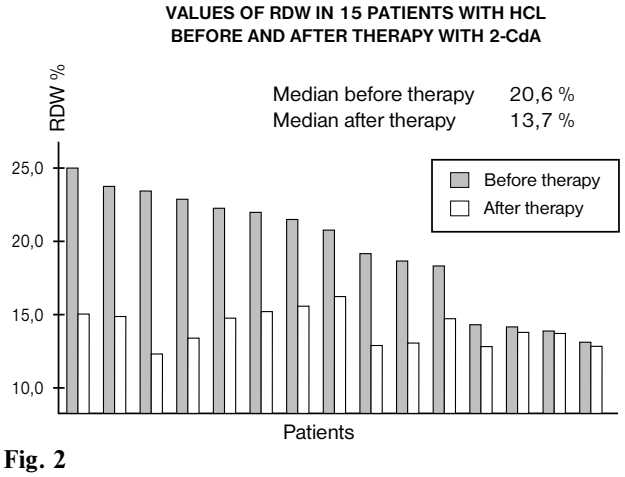

Fig. 2

Discussion

Our results confirm the effectiveness of 2-CdA in the treatment of HCL as reported also by other study groups (see Tab. 3). In 327 patients collected from 7 study groups com-

plete remission was achieved in 84 % of patients and partial remission in 14 %. Therefore, the overall response rate with a single cycle of 2-CdA therapy was 98 %. Mercieca et al (14) stressed differences in response in patients with and without abdominal lymphadenopaty. In 7 patients with abdominal lymphadenopathy he achieved complete remission only in 4 (57 %). Similar results, four CR and two PR were reported by Hakimian et al. (7). We concluded in our previous study that retroperitoneal lymphadenopathy is an unfavourable sign usually heralding the terminal stage of the disease (22). In this study complete remission was achieved in all three patients with retroperitoneal lymphadenopathy including one patient with leukemic infiltrates of the cornea on both eyes as reported previously (21). Assessment for response in HCL should not be performed to early, as responses found incomplete 1-3 months after therapy may turn to be complete responses when assessed later. More than 1 year may be needed before clearance of malignant cells from the bone marrow is complete (12). Response to 2-CdA seems to be unaffected by previous therapies (18). Increased sIL-2R levels which are considered as non-invasive marker of HCL burden dropped considerably after 2-CdA therapy to be mostly only slightly above the upper limit of normal values, but only in two patients they were within normal limits. This observation is in agreement with the opinion that complete eradication of tumoral cells may be very rare.

Tab. 3: Results of treatment in patients treated with 2 - CDA

Author	Number	treated: non-treat.	CR:	PR:	CR+PR	Failure
Merciece et al.[14]	23	18:5	20 (87%)	3 (13%)	23 (100%)	-
Tallman et al.[19]	50	13:39	40 (80%)	9 (18%)	49 (98%)	1 (2%)
Lauria et al.[13]	26	21:5	20 (77%)	6 (23%)	26 (100%)	-
Saven et al.[18]	143	74:69	123 (86%)	17 (12%)	140 (98%)	3 (2%)
Hoffman et al.[19]	48	27:21	42 (88%)	6 (12%)	48 (100%)	-
Filleul et al.[8]	22	12:10	17 (77%)	4 (18%)	21 (95%)	1 (5%)
Chrobák et al.	15	8:7	13 (87%)	2 (13%)	15 (100%)	-
Total	327	173:154 53%:47%	275 (84%)	42 (14%)	322 (98%)	5 (2%)

CR: complete remission, PR: partial remission

The RdW values reflecting the red cells anisocytosis were increased before the initiation of the therapy. This increased values were ascribed to the dyserythropoiesis and disappeared after successful therapy (5). Partial remission in one of our patients was associated only with a partial decrease of RdW from 20,6 to 16,3 %. In all the remaining patients almost normal values were achieved.

Whether the remission induced by 2-CdA may be considered as a cure is still unknown and is closely related to the discussion on eradication of malignant cells in complete responders. The present evidence suggests that residual disease persist in most if not in all complete remitters. Immunostaining of bone marrow biopsies with DBA 44 antibody in complete responders disclosed foci of malignant cells (6, 9).However, the persistence of residual disease is not necessarily predictive for relapse and only long-term follow up will settle this question. Data related to CR patients follow up are now available and suggest that relapses accumulate over time: median follow-up of 24, 13.5, 14, 23 and 19 months was associated with 22, 14, 7.6, 3.5, 14.2 and 0 relapse rate respectively (see table 4). The recognition that HCL may relapse or progress after responding to 2-CdA does not rule out the possibility that some remitters will enjoy indefinite remission which practically coincides with the cure. 2-CdA represents a major advance in the management of patients with HCL with an easy administration and long-term excellent quality of life that follows therapy with 2-CdA.

Tab. 4: Relapse of patients with HCL treated with 2-CDA

Author	No of pat.	Relapses		Median Folow-up	Folow-up (Months)	Relapse (Months)
		no	%			
Merciece et al.	23	5	22	25	6-30	6-17
Tallman et al.	50	7	14	24	12-44	CR: 12,24 (2x) 25, 35 PR: 6, 45
Lauria et al.	26	2	7,6	13,5	-	6, 12
Saven et al.	143	5	3,5	14	-	4-48
Hoffman et al.	48	5	10,4	-	-	-
Filleul et al.	22	3	14,2	23	-	28-37
Chrobák et al.	15	0	0	19	6-37	-
Total	327	27	8,2			

References

1. Ambrosetti A, Semenzato G, Prior M et al. Serum levels of soluble interleukin-2-receptor in hairy cell leukemia: a reliable marker of neoplastic bulk. Br J Heamatol 1989;73:181-6.
2. Burke JS. The value of the bone marrow biopsy in the diagnosis of hairy cell leukemia. Am J Clin Pathol 1978;70:876-84.
3. Chrobák L, Podzimek K, Kerekeš Z et al. Long-term results in hairy cell leukemia treated by splenectomy. Neoplasma 1993;40:133-6.
4. Chrobák L, Podzimek K, Plíšková L. Serum soluble IL-2 receptor as reliable and non-invasive marker of disease ac-

tivity in patients with hairy cell leukemia. Acta Medica (Hradec Králové) 1996;39:3-6.
5. Chrobák L, Žák P, Podzimek K et al. Dyserythropoiesis and increased RdW in hairy cell leukemia. The chronic leukeamias, International Conference, Prague (Abstract) 1996:p. 72.
6. Ellison DJ, Sharpe RW, Robbins BA et al. Immunomorphologic analysis of bone marrow biopsies after treatment with 2-chlorodeoxyadenosine for hairy cell leukemia. Blood 1994;84:4310-5.
7. Estey EH, Kurzrock R, Kantarjian H et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine for hairy cell leukemia. Blood 1992;79:882-7.
8. Filleul B, Delannoy A, Ferrant A et al. A single course of 2-chlorodeoxyadenosine does not eradicate leukemic cells in hairy cell leukemia patients in complete remission. Leukemia 1994;7:1153-6.
9. Hakimian D, Tallman MS, Hogan DK et al. Prospective evaluation of internal adenopathy in a cohort of 43 patients with hairy cell leukemia. J Clin Oncol 1994;12:268-72.
10. Hakimian D, Tallman MS, Kiley C et al. Detection of minimal residual disease by immunostaining of bone marrow biopsies after 2-chlorodeoxyadenosine for hairy cell leukemia. Blood 1993;82:1798-1802.
11. Hoffman M, Janson D, Aggarwal A et al. Long term results after treatment of hairy cell leukemia (HCL) with 2-chlorodeoxyadenosine (2-CdA). Blood 1995;86(Suppl. 1):351a.
12. Juliusson G, Heldal D, Hippe E et al. Subcutaneous injections of 2-chlorodeoxyadenosine for symptomatic hairy cell leukemia. J Clin Oncol 1995;13:989-95.
13. Lauria F, Benfenati D, Raspadori D et al. High complete remission rate in hairy cell leukemia treated with 2-chlorodeoxyadenosine. Leuk Lymph 1993;11:399-404.
14. Mercieca J, Matutes E, Emmett E et al. 2-Chlorodeoxyadenosine in the treatment of hairy cell leukaemia: differences in response in patients with and without abdominal lymphadenopathy. Br J Haematol 1996;93:409-11.
15. Nanba K, Soban EJ, Bowling MC et al. Splenic pseudosinuses and hepatic angiomatous lesions. Distinctive feature of hairy cell leukemia. Am J Clin Pathol 1977;67: 415-26.
16. Piro LD, Carrera CJ, Carson DA et al. Lasting remissions in hairy-cell leukemia induced by a single infusion of 2-chlorodeoxyadenosine. N Engl J Med 1990;322:1117-21.
17. Richards JM, Mick R, Latta JM. Serum soluble interleukin - 2R receptor is associated with clinical and pathologic disease status in hairy cell leukemia. Blood 1990;76:1941-5.
18. Saven A, Piro LD. 2-chlorodeoxyadenosine in treatment of hairy cell leukemia and chronic lymphocytic leukemia. Leuk Lymph 1994;11(Suppl. 2):109-14.
19. Tallman MS, Hakimian D, Rademaker AW et al. Relapse of hairy cell leukemia after 2-chlorodeoxyadenosine: long-term follow-up of the Northwestern university experience. Blood 1996;88:1954-9.
20. Yam LT, Li CY, LAM KW. Tartarate resistant acid phosphotase isoenzyme in the reticulum cell of leukemia reticuloendotheliosis. N Engl J Med 1971;284:357-60.
21. Žák P, Chrobák L, Podzimek K et al. Neobvyklý průběh leukémie s vlasatými buňkami s výraznou abdominální lymphoadenopatií, leukemickými infiltráty oční rohovky a kožními změnami. Vnitr Lek 1996;42:463-6.
22. Žák P, Chrobák L, Šimáková A et al. Zvětšení uzlin u leukémie s vlasatými buňkami a problematika výrazné abdominální lympfadenopatie. Vnitr Lek 1993;39:896-901.

Submitted May 1997.
Accepted June 1997.

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ORIGINAL ARTICLE

VISUAL FUNCTIONS AFTER PHOTOREFRACTIVE KERATECTOMY

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Summary: 1. 45 myopes (-3.0 to -6.0 D) were examined before and 1 and 6 months after photorefractive keratectomy (PRK). Visual acuity (VA) was tested using Snellen and logMAR charts. Contrast sensitivity (CS) was measured using a computerized system. 2. Preoperative best corrected VA (BCVA) in myopes was significantly lower in comparison with a control group using logMAR charts only. A reduction of BCVA by both methods at 1. month and its return after 6 months nearly to original values was noted. 3. Significantly lower values of CS were found in patients before PRK compared to the control group. After 1 and 6 months stayed the values on preoperative level.

Key words: *Photorefractive keratectomy; Moderate myopia; Contrast sensitivity; Visual acuity; LogMAR charts*

Introduction

Since the original 1983 description by Trokel et al. (10) of the effect of 193 nm laser energy on the corneal stroma, numerous investigators have further defined the clinical usefulness of this modality. An active area of investigation has been the use of the excimer laser in the surgical treatment of refractive errors by modification of the corneal surface contour. This procedure is referred to as photorefractive keratectomy (PRK). By ablating more tissue centrally than peripherally in the treatment zone, a flattening of the contour is achieved, resulting in a reduced corneal dioptric power (Piebenga et al.(5)).

Pallikaris et al. (4), McCarty et al.(3) and other authors agree that PRK is a safe, effective and predictable method for treatment of low and moderate myopia. Most of patients achieve emmetropia, the refraction is stable till 6 months after PRK and complications are very rare and mostly not vision threatening. Optical complications including various degrees of myopic regression call for reoperations, or decrease of best corrected visual acuity in consequence of haze, appearance of central islands after irregular healing of corneal epithelium and other complications grow with the level of myopia (McCarty et al.(3)). A new, more suitable methods for treatment of high and extreme myopia (up -10.0 D) are appearing (Tong et al.(9)).

In our clinic the patients before PRK were given complete ophthalmologic examination including slit lamp, ophthal-moscopy, tonometry, corneal topography and Schirmers test. Uncorrected and best corrected visual acuities were tested using Snellen charts. Refraction was measured using autorefractometer.

Postoperative examinations were scheduled for 4. day or to full reepitelization of the cornea and then for 1,3,6,9 and 12 months. In addition to visual acuity, refraction, tonometry, slit lamp examination and keratotopography were performed on each visit.

Considering that VA is mostly examined only using Snellen charts and CS is not measured at all, we decided to complete this data using logMAR charts with Landolt rings and computerized method for CS.

Materials and methods

1. PRK was performed on 45 right eyes of 45 patients with myopia -3.0 to -6.0 diopters (D). Median age of the 20 women and 25 men was 27,5 years (range 19 - 46). We examined them before and 1 month after PRK, 40 patients were examined also after 6 months.

As a control group we examined 20 eyes of 20 people with normal intraocular findings and uncorrected VA (UCVA) 6/6 or better using Snellen charts. There were 12 women and 8 men with median age of 26 years (range 20 - 40 years).

2. We examined UCVA and BCVA using Snellen and logMAR optotype charts. CS was tested using computerized Contrast sensitivity 8010 system (Neuroscientific corp., Farmingdale, USA) in spatial frequencies from 0.74 to 29.55 c/deg.

The distance for examination of VA using logMAR charts was 4 meters. The patient determined the position of the gap in Landolt rings that could be in one of four basic directions. Each row contained 10 Landolt rings. Their size in the subsequent rows had a logarithmic progression. We noted the number of the right answers and for the calcula-

tion of the threshold VA the method of Ferris et al.(1) was used.

The distance for examination of CS was 2.2 meters so that the range of spatial frequencies from 0.74 to 29.55 c/deg was achieved when the size of monitor was 5 x 3.5°. An adjustment method with ascendent and descendent approach of the threshold contrast determination (Langrova (2)) was used.

Results

Visual acuity

- In the control group UCVA using Snellen chart was 6/6 or better, in myopes up to 6/12.
- threshold BCVA using logMAR charts in myopes was significantly lower compared to the control group only using logMAR charts.
- 1 month after PRK BCVA decreased significantly by both methods (Snellen charts: $P < 0.05$, logMAR charts: $P < 0.001$).
- after 6 months BCVA returned nearly to its preoperative level using both methods (Fig. 1, Tab.1, Tab. 2).

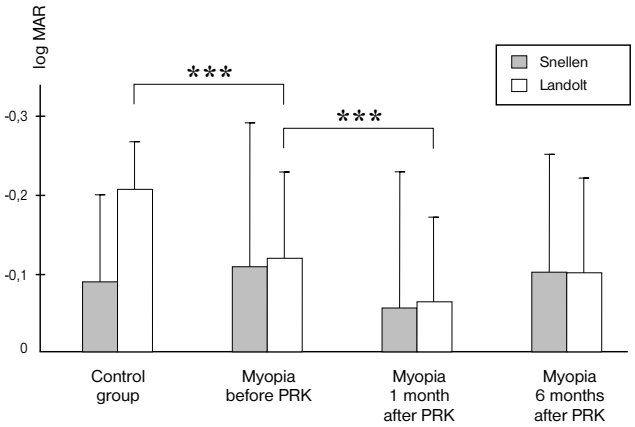

Fig. 1: Visual acuity values (in logMAR units) using Snellen (Sn) and logMAR charts with Landolt rings (L). The control group consisted of 20 subjects, patients group of 45 persons. n.s. nonsignificant differences, *** $p < 0.001$.

Visual acuity	After 1 month (%)	After 6 months (%)
6/6 or better	51	54
6/12 or better	100	93
Refraction		
± 1 D	88	74
± 2 D	100	98

Table 1: The percentage of eyes 1 and 6 months after PRK which achieve BCVA 6/6 or better and 6/12 or better using Snellen charts and refraction (1 D and 2 D from emmetropia).

Lines of BCVA	Gained (Nr. of eyes) 1 month	6 months	Lost (Nr. of eyes) 1 month	6 months
1	6	7	12	9
2	1	2	6	0
3	1	1	1	0

Table 2: The number of eyes 1 and 6 months after PRK which gain or loose 1,2 and 3 lines of BCVA using Snellen charts.

Contrast sensitivity

- CS in myopes was significantly lower compared to the control group, especially in moderate and higher spatial frequencies (Fig. 2).
- Nonsignificant changes in CS after PRK were noted except the value in frequency of 29.55 c/deg which increased significantly in both terms after PRK (Fig. 3).

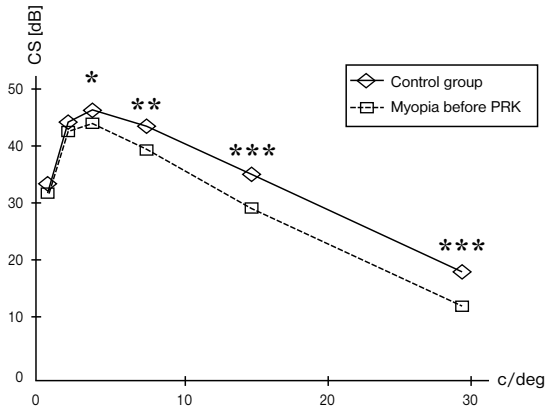

Fig. 2: Contrast sensitivity (CS) values in decibels (dB) for spatial frequencies of 0.74, 0.97, 3.69, 7.39, 14.77 and 29.55 c/deg. The differences in CS between the control group [black ring] and myopic patients [white rectangle] before PRK are statistically significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ at spatial frequencies from 3.69 to 29.55 c/deg.

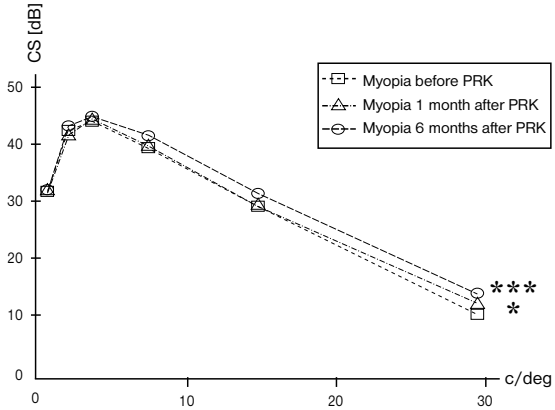

Fig. 3: The changes of CS in decibels (dB) after PRK were nonsignificant except significant increase of the CS in spatial frequency of 29.55 c/deg ($p < 0.05$ after 1 month, $p < 0.001$ after 6 months).

Discussion

The changes of VA after PRK using logMAR charts are similar to those of McCarty (3). She described a significant loss of VA 1 month after PRK and its return to preoperative level after 3 months. Our results are better than those of Tong et al (9) who used Snellen charts for testing of VA in myopes up -5 D and Salz et al (6) who tested myopes up -7.5 D (Tab. 3).

Visual acuity	After 1 month (%)			After 6 months (%)		
	This paper	McCarty (3)	Tong (9)	This paper	McCarty (3)	Tong (9)
6/6 or better	51	40	23	54	52	28
6/12 or better	100	84	55	93	89	61
Refraction						
± 1 D	88	82		74	86	
± 2 D	100	97		98	97	

Table 3: Comparison of the results of three studies concerning the changes of visual acuity and refraction.

In contrast to Pallikaris et al.(4), who noted the loss of BCVA of 1 - 1,5 lines using logMAR charts, we described the loss of VA of 2 - 3 lines after PRK.

Contrary to Pallikaris et al.(4) who describe the decrease of CS using CSV-1000 (Vector Vision) in spatial frequencies 3, 12 and 18 c/deg without glare 1 month after PRK and the return to preoperative level in 3 months, we noted nonsignificant changes in all but one frequencies tested . Also Piebenga et al.(5) noted lower values in all frequencies 6 months after PRK, significantly only in frequency of 18 c/deg. Identically with us saw Sher et al.(7) nonsignificant changes of CS using Vistech charts 3 months after PRK and Shimizu et al.(8) in CS without glare 6 months after PRK.

Conclusions

Our results indicate that PRK is a suitable method for the treatment of moderate myopia from the functional point of view.

Acknowledgement

This work was supported by the Grant Agency of Charles University (Grant No 59/97).

References

1. Ferris FL, Krasoff A, Bresnick GH, Bailey l. New visual acuity charts for clinical research. Am J Ophthalmol 1982; 94:91-6.
2. Langrová H, Hejcmanová D, Peregrin J. Reproducibility of contrast sensitivity testing (In Czech).Cesk Ophthalmol 1996; 3:152-7.
3. McCarty CA, Aldred GF, Taylor HR and the Melbourne excimer laser group. Comparison of results of excimer laser correction of all degrees of myopia at 12 months postoperatively. Am J Ophthalmol 1996;121:372-83.
4. Pallikaris I et al. Tracker-Assisted photorefractive keratectomy for myopia of -1 to -6 diopters 1996;12:240-7.
5. Piebenga LW, Matta CS, Deitz MR, Tauber J. Excimer photorefractive keratectomy for moderate myopia. Six to 18 month follow up.Ophthalmol 1995;102:1054-9.
6. Salz JJ et al. A Two-year experience with excimer laser photorefractive keratectomy for myopia. Ophthalmol 1993;100:873-882.
7. Sher NA et al. The use of the 193-nm excimer laser for myopic photorefractive keratectomy in sighted eyes. A multicenter study. Arch Ophthalmol 1991;109:1525-30.
8. Shimizu K, Amano S, Tanaka S. Photorefractive keratectomy for myopia: One-year follow-up in 97 eyes. Suppl J Refract Corn Surg 1994;10:178-87.
9. Tong PPC et al. Excimer laser photorefractive keratectomy for myopia: Six-month follow-up. J Cat Refract Surg 1995;21:150-5.
10. Trokel SL, Srinivasan R, Braren B. Excimer laser surgery of the cornea. Am J Ophthalmol 1983;96:710-5.

Submitted May 1997.
Accepted June 1997.

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ORIGINAL ARTICLE

UNUSUAL OCULAR FINDINGS IN CHILDREN'S ACUTE LEUKEMIA CASES

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Summary: The most frequent ocular findings in acute leukemia cases of children and two of our own observations are presented in this study. Emphasized are all difficulties in fixing the diagnosis and the necessity of close cooperation between ophthalmologist, pediatrist and radiologist to prevent the irreversible ocular changes.

Key words: *Leukemic iris infiltration; Leukemic optic nerve head infiltration*

Supported by Grant No 59/97 from Charles University Grant Agency.

Leukemia is one of the systemic diseases of all age groups that appears as an infiltration of systemic organs (10).

Allen and Straatsma (1) proved in their clinical studies the occurrence of ocular involvement in 50 - 70% of patients with acute leukemia, especially in tissues that are rich in vessels (uvea, retina). On the other hand, the avascular tissues (cornea, lens) have been proved not to be affected often (6). Leukemic manifestations in vitreous body are very rare (18).

Soylu et al. (17) emphasized that ophthalmologic evaluation of leukemic patients contributes in making a prognosis for the disease's advancement and may guide the theraupetic regimen choice. The team detected mainly hemorrhages of retina in 36 of 84 children suffering from acute leukemia. Neither platelet nor hematrocit values correlated with the occurrence of hemorrhages.

Ohkoshi et al. (15) noted a positive ophthalmologic finding in 28 of 63 patients with acute leukemia. In this study 27 of 29 (96.4%) patients with ocular involvement died within 28 months after the diagnosis had been fixed. Bone marrow or CNS relapse has later developed in all of the disease victims (2).

All of these facts showed a high prognostic value of ocular involvement in acute leukemia.

Hatvani et al. (12) and Ells et al. (8) mention that the ocular involvement could be the first site of acute leukemia.

Ocular involvement is more common in the acute than in chronic leukemia cases. Virtually any or all of the ocular structures may be involved.

It is, however, important to distinguish primary leukemic infiltration, which is rare, from the more common secondary changes such as those due to associated anaemia, thrombocytopenia, hyperviscosity, opportunistic infections, and the side effects of cytostatic (9) or corticosteroid treatment (e.g. steroid-induced cataracts).

Ocular features

Orbit

Orbital involvement is more common in acute than in chronic leukemia cases. Orbital infiltration usually presents itself with painful proptosis, lid oedema and chemosis (Fig. 1). It occurs more frequently in the lymphoblastic than in the myeloid type of leukemia.

Anterior segment

Changes in form of nodular or diffuse infiltration of iris (Fig. 2), infiltration of the cornea (Fig. 3), spontaneous subconjunctival hemorrhages or spontaneous bleeding into the anterior chamber occur mostly in the acute myeloid leukemia patients and, very rarely, in the chronic leukemia patients.

Retina

Manifestations in retina are very frequent. In adults, we can see light tinge of retina and arteries along with venous tortuosity and dilatation of the vessels (Fig. 4), flame-shaped hemorrhages at the posterior pole of the eye, or so-called Roth's spots (i.e. yellow infiltrates surrounded by hemorrhages) may also be present (Fig. 5).

Optic neuropathy (ON)

Disc involvement is characterized by the fluffy infiltrates associated with variable disc oedema and hemorrhages (Fig. 6). It is important to differentiate leukemic optic neuropat-

hy from papilloedema caused by raised intracranial pressure - secondary to meningeal infiltration. Echography of the eye according to Pierro et al (16) enables a better interpretation of the ON involvement. In the same way, the examination by magnetic resonance clarifies the diagnosis (19).

Case reports

1. Isolated leukemic infiltration of the iris

The involvement of the anterior part of uvea is rare (1) and leukemic infiltration of the iris is only sporadic (12, 20, 11). The rise of pseudohypophyon represents the first sign of the disease's relapse, but its occurrence is rare (5,2).

We are presenting the case of a 3-1/2-year-old boy. The acute lymphoblastic leukemia diagnosis was fixed at the boy's age of nine months. The child was treated according to the schedule for acute lymphocytic leukemia (ALL-BFM-83), but the treatment had to be repeatedly interrupted because of bone marrow recession. The basic treatment was finished with considerable difficulties and the final aktinotherapy was done only partly due to continuous leukopenia. Neither was the treatment with L-asparaginosis brought to an end due to the allergic manifestations of the skin. The boy was in good clinical and hematological state. He was checked at the outpatient department and was treated with Merkaptopurin and Methotraxat.

The first examination by an ophthalmologist was performed on Nov.13,1991 upon the request of the boy's pediatrist who noticed anisokoria and marced iris heterochromia of the left eye.

Ocular finding:

VA OR: 5/5

VA OL: certa

OR: Normal outer and intraocular finding.

OL: In the anterior chamber of completely calm eye, there was a grayish-white exudate 2mm high with horizontal surface; diffuse thickening of the iris, iris' gray color, and considerable dilatations of the vessels were also noticed. Red reflex was apparent but the evaluation of the retina was not possible due to the opacity in the anterior chamber. The intraocular pressure was normal.

We diagnosed the case as acute iritis of the left eye. We started topical mydriatics and corticosteroids treatment and prescribed oral corticosteroids and antibiotics. The maintaining dose of cytostatics remained at the same level. After a short-lasting improvement of the condition, during which the anterior chamber had become clearer and the visual acuity recovered to 6/12, we discovered the oedema of the macula to the extent of 2 PD in the affected eye. The child's general health condition was satisfactory; repeated results of the sternal and lumbal punctures were negative. Nevertheless, we clarified the diagnosis as an isolated infiltration of the iris. Our decision was based upon the facts that the appearance in the eye (Fig. 7) was completely different from the appearance of common iritis and that the eye did not respond to all therapy applied. The echography examination showed thickening of the uveal tissue (Fig. 8). The iris was still infiltrated, grayish colored in spite of all treatment. The exudate in the anterior chamber was not receding; on the other hand, the posterior synechiae was not appearing. The eye seemed to be completely calm. The visual acuity decreased to finger counting because of opacities in the anterior chamber. Therefore we proposed a systemic treatment with cytostatics and local aktinotherapy. After a consultation with the radiologist, we administered radiation to the eye in 18 doses 0.25 Gy and 140 kW three times a week to the total dose of 4.5 Gy. Shortly thereafter, the eye condition turned distinctively better. The exudate in the anterior chamber disappeared, the infiltration of the iris stroma was lower and the leakage to the posterior pole also decreased.

At this time a spherical, painless, reddish infiltrate was noticed under the skin. Excision proved lymphoblostoma with a higher risk of malignance. Therefore, a systemic therapy according to the relapse schedule (ALL-BFM-87 REZ) in a modification with high doses of Methotraxat was started on March 16, 1992. However, the treatment could not be finished. The boy died of the cytostatic therapy complication on May 4, 1992.

Histopathologic study proved leukemic infiltrates of chorioidea, the same changes were detected in corpus ciliare and iris. There was no evidence of leukemia in other structures of the left eye as shown in the Fig. 9. Histopathologic study proved infiltration of the spleen and both testes.

Discussion

The diagnosis of ocular involvement is usually set after the diagnosis of acute leukemia or during the relapse of the disease.

Ells et al (8) bring to attention the possibility of the fact that the initial symptom of acute leukemia can be found in the eye. They reported a case of an 11-month-old child with unilateral hypopyon and nodules of the iris. The first signs of acute myeloid leukemia appeared 3 weeks after their ocular findings.

Our patient's leukemic infiltration of the iris with pseudohypopyon arose in spite of the fact that there was repeatedly no evidence of relapse of the disease in the bone marrow and central nervous system.

Pracentesis of the anterior chamber and cytologic examination of the aqueous humour is very useful in determining the differential diagnosis (2,11,12,18,20,) since it helps a fast diagnosis of extramedullar leukemic infiltration of the iris. It also enables the administration of adequate therapy - especially to patients in remission of the disease.

We were reluctant to use these methods of examination not only because of the preceding administration of corticosteroids and cytostatics but also because of the poorly-healing incision - the result of the catheter application that was necessary for the cytostatics therapy.

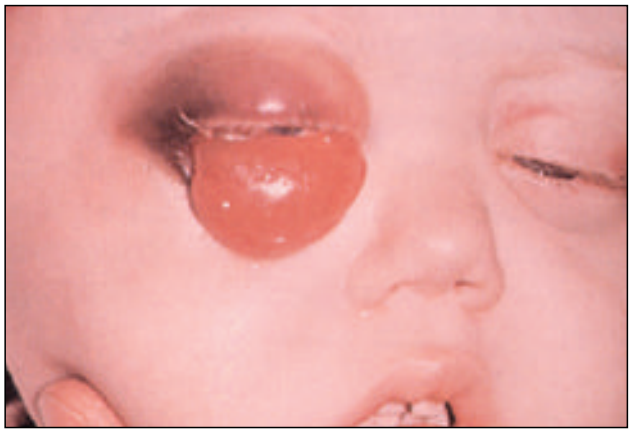

Fig. 1: Orbital infiltration with painful proptosis, lid oedema and chemosis

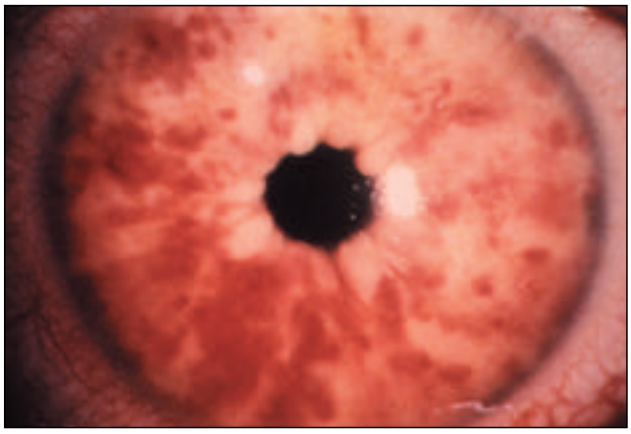

Fig. 2: Diffuse iris infiltrates and scattered stromal hemorrhages

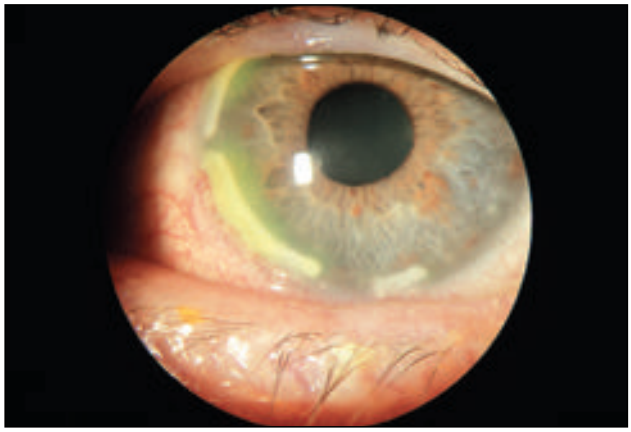

Fig. 3: Infiltrates at the limbus of the cornea

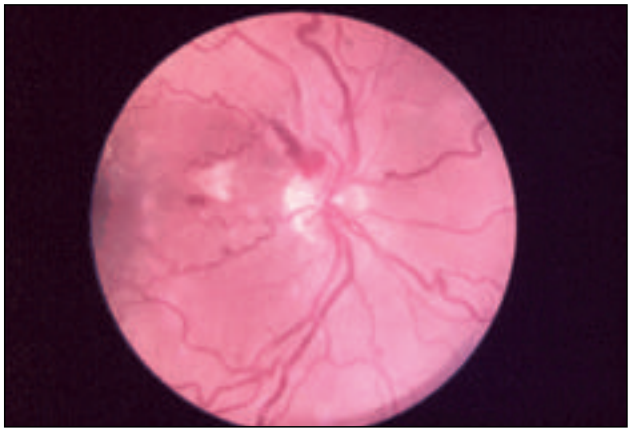

Fig. 4: Venous tortuosity and dilatation with flame - shaped retinal hemorrhages

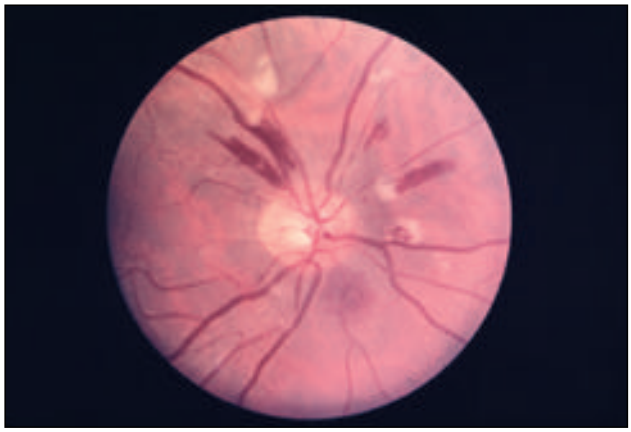

Fig. 5: Flame - shaped hemorrhages with pale centres (Roth's spots)

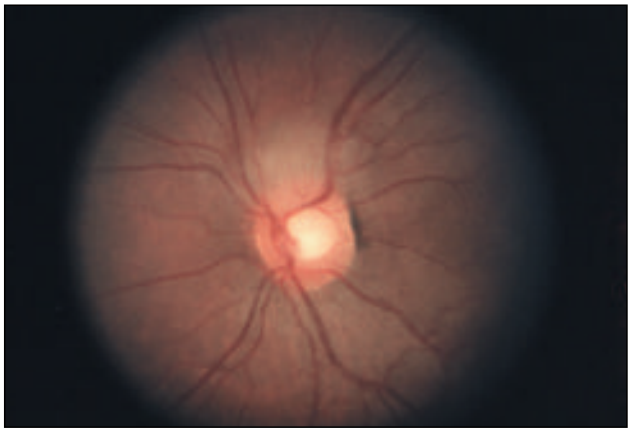

Fig. 6: Leukemic infiltration of the optic nerve

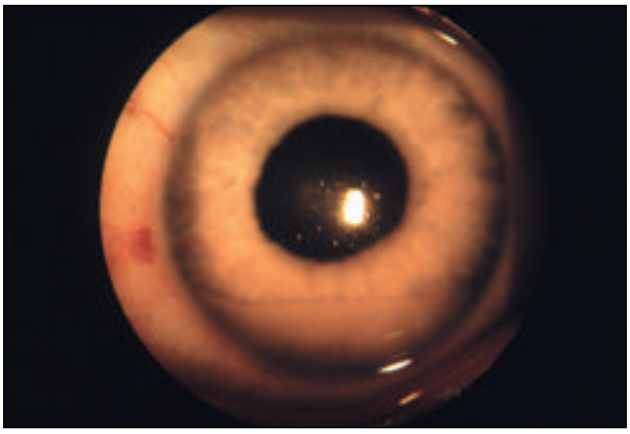

Fig. 7: Leukemic hypopyon in the anterior chamber of the left eye

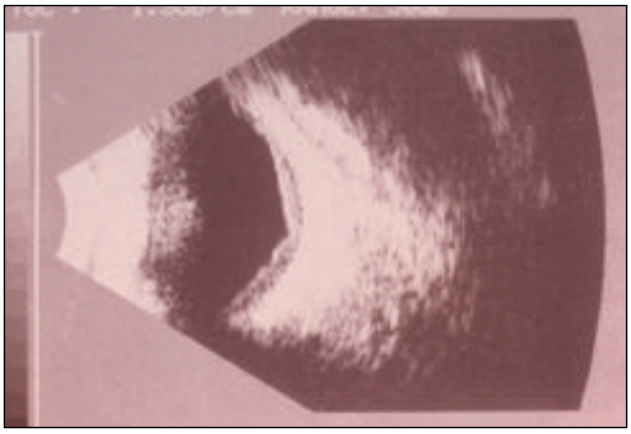

Fig. 8: Ultrasonogram of the left eye shows leukemic infiltration of the chorioidea

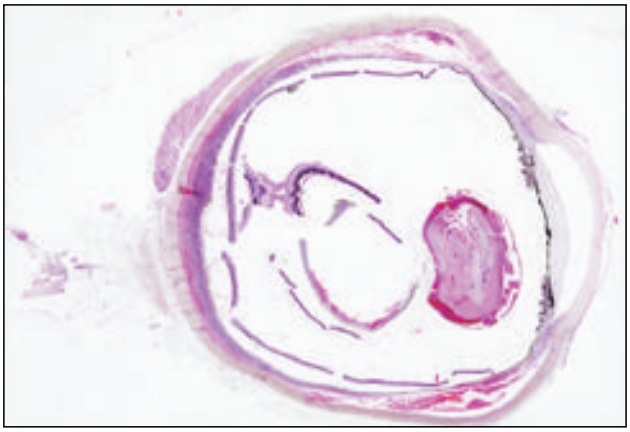

Fig. 9: Section through left globe revealing leukemic infiltration of iris, ciliary body and chorioidea

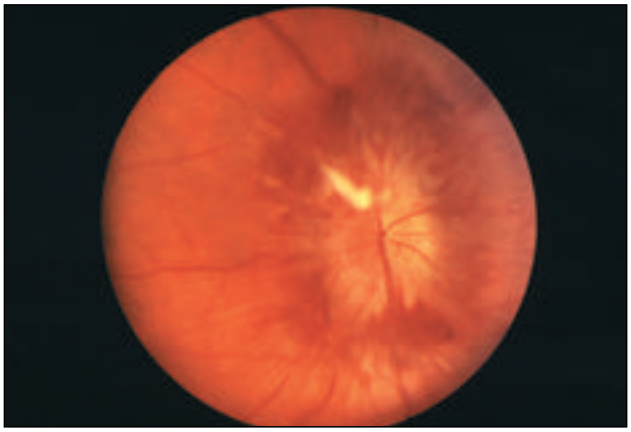

Fig. 10: Leukemic infiltration of the optic nerve with hemorrhages

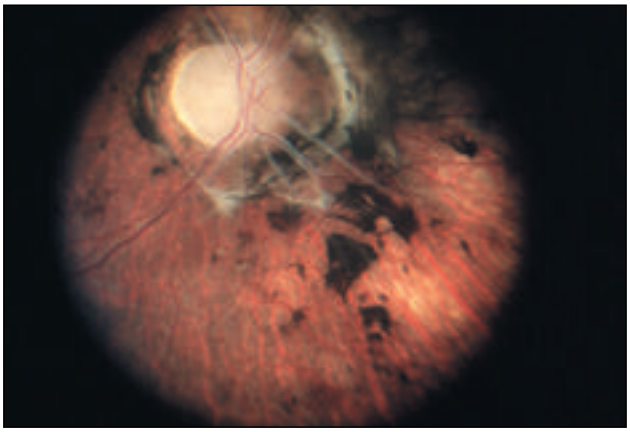

Fig. 11: Atrophy of the optic disc, white retinal vessels and peripapillary pigmentations

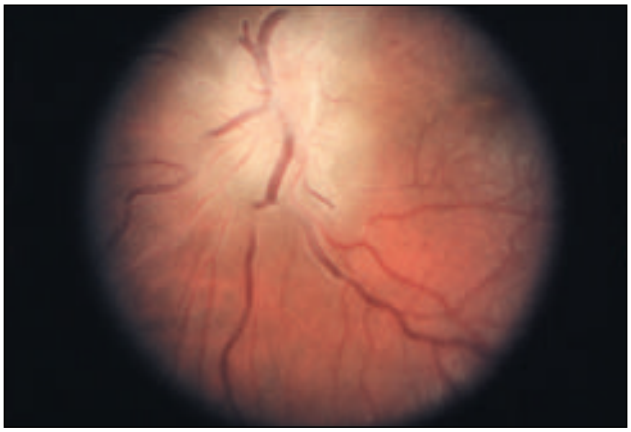

Fig. 12: Oedema of the optic nerve with venous tortuosity and dilatation

The course the disease took in our case was different from the course that Britt et al (3) described. They noticed the leukemic iris infiltration in a form of heterochromia which was proved by the autopsy 7 months later.

Also in studies of Zakka et al. (20) and Hatvani et al. (12) the diagnosis of isolated leukemic iritis was done when the illness had already been complicated by secondary glaucoma. The leukemic infiltration in chamber's angle caused the bleeding into the anterior chamber.

Isolated leukemic infiltration in form of iritis can be the first sign of the disease as described by Ells et al. (8). Decker et Burnstine (5) described it as the only sign of the disease relapse or it could be preceding the relapse (2). Harrer et al. (11) draws our attention to the typical picture of grayish-white exudate - pseudohypopyon and frequent hemorrhage into the the anterior chamber. The distinction of isolated leukemic infiltration in form of iritis is necessary for an early therapy application.

Hatvani et al. (12) asserts that the doses 1,25 - 4,5 Gy divided into single doses administered during the course of 4 weeks should not have any cataractogenous effects. In spite of this fact, tiny opacities in the anterior and posterior pole of the lens were noted in our case as the result of the same therapy.

The diagnosis of the relapse of the disease was done according to the clinical picture and the echography. The effect of the therapy and the follow-up histopathologic examination, which proved infiltrates of the iris, corpus ciliare and choroidea, showed that the diagnosis of the disease relapse was correct.

2. Leukemic infiltrates of the optic nerve

The studies on leukemic infiltrates of the optic nerve are sporadic (4, 13, 19). Echography (4) and magnetic resonance tests (19) are the most important examinations for fixing the differential diagnosis and, according to Yamamoto et al. (19) the differential diagnosis should be determined as soon as possible in order to begin an adequate therapy.

We present a case of a 4-1/2-year-old girl with the diagnosis of acute lymhoblastic leukemia that was fixed in November, 1980. The child was treated in compliance with Maurer's method. The basic therapy was discontinued three years later due to the patient's good clinical state.

In October, 1987, fever occurred and the patient's ability to walk quickly diminished to spastic paraparesis of the legs with preservation of the sensivity. The examination proved lymphoblastic lymphoma in the region of Th 5 - 6.

The laminectomy of Th 4 - 7 with aktinotherapy and cytostatic therapy according to schedule ALL-BMF-83 with high doses of Methotraxat was done. After the rehabilitation, the legs movement was totally restored and the child didn't experienced any difficulties till November, 1991.

At that time, the girl was examined at the eye clinic in Košice for the decrease of the visual acuity to 5/15 and for pains behind the right eye. The diagnosis was neuritis of the right eye. The patient was examined on November 25, 1991 at the eye clinic in Hradec Králové.

Ocular finding:

VA OR: 6/60. The eye was completely calm, all spaces were transparent, the disc of the optic nerve was oedematic with the prominence to +5,0 D, with deposits of exudates (Fig. 10), and with numerous hemorrhages on the disc. The exudates were also found around the disc and in the middle periphery.

VA OL: 6/6. The left eye was calm, all spaces were transparent, the disc was restricted and on the same level. There were tiny hemorrhages and deposits of exudates around the disc and in the middle periphery. Our prognosis indicated the relapse of the diseases. The examination of cerebrospinal fluid proved the prognosis' correctness. Thereafter, the treatments according to the relapse schedule (BFM-REZ-83 at first; BFM-REZ-87 later) were commenced. The cerebrospinal fluid findings turned to a normal state again.

However, the visual acuity of the right eye decreased to incerta. The disc was restricted, on the same level, atrophic, pale. In the distance of 1/2 PD nasally, there was a light ring with well-noticeable pigmentations which continued nasally as deposits of bone-like cells, retinal vessels were sheated to the middle part of retina (Fig. 11). On the left side, there was visual acuity of 6/6, outer and intraocular findings were normal.

Until December, 1993 (i.e. 13 years after the diagnosis), the child experienced no difficulties except for the common signs of recession of bone marrow. However, at the end of December 1993, the girl was repeatedly examined for temporary failure of visual acuity of the left eye at the eye clinic in Košice. Allegedly, the failure always settled. The diagnosis was fixed as neuritis of the left side. VA was 5/15, the girl was treated with Prednison and Azamun.

The patient came to the eye clinic in Hradec Králové in May, 1994.

VA OR light projection: incerta.

VA OL light projection: certa. Both eyes showed afferent pupillary defect.

OR: The eye was in a devergency of 10 degrees. Retina showed the same findings as shown in Fig. 11.

OL: The eye was calm, the disc was swollen to +2,0 D with dilated vessels, no hemorrhages were detected (Fig. 12). The echography of the left orbit proved the prominence of the disc and diffuse thickening of the left optic nerve.

The state was closed as both-sided atrophy of the discs due to the infiltrates in the region of the optic nerves during the course of the basic disease. Computed tomography of the brain did not prove the signs of intracranial expansive process.

The patient died within a short period of time at the department of hemato-oncology in Košice with both-sided amarousis due to the irreversible course of the disease.

Discussion

According to Ellis et al (7), the studies on infiltration of the optic nerve are rare. The infiltrations are almost always

the signs of the extramedullar manifestations. We encounter them only in acute leukemia states. They could present the isolated extramedullar relapse of the disease (4).

Nikaido et al. (14) informed about five patients who were treated with combined therapy including chemotherapy, intrathecal injections of cytostatics together with radiotherapy. The results of visual functions of such therapy were always disappointing.

Better results were described by Yamamoto et al. (19). They were the first team to observe their patient's recovery of visual functions in leukemic infiltration of the right optic nerve after administrating 40 Gy of radiation to the brain and right orbit. The visual acuity improved from movement vision in front of the eye to 20/20 within 3 weeks.

Also Camera et al. (4) observed the recovery of visual functions in one of his two patients who were treated immediately for the isolated relapse of the optic nerve. He recommended the use of the most sensitive method - A scan echography for an early diagnosis of the eye involvement in acute leukemia cases.

Kaikov (13) described visual acuity improvement in infiltration of the optic nerve after the immediate irradiation of the orbit as well as the whole CNS. Also, according to him, an early children diagnosis determination is very difficult most of the time. Children ophthalmologists should keep in mind the infiltration of the optic nerve so that the diagnosis can be fixed immediately and the proper treatment can be launched at once. In such event, the visual acuity can be saved.

Conclusions

The probability of extramedullar complications occurrences is higher when prolonging the lives of patients who suffer from acute leukemia. We can find such occurrences especially in organs with high barrier such are the CNS or the eye. In these organs, there is lower concentration of drugs and, therefore, the number of complications is quite high and their frequent occurrence is not surprising. It is necessary to know of such complications so that the patient can receive an immediate and adequate treatment. The close cooperation of ophthalmologist, radiologist and physician can lead to an early diagnosis, adequate therapy and, consequently, to the visual functions improvement in such suffering patients.

References

1. Allen RA, Straatsma BR. Ocular involvement in leukaemia and allied disorders. Arch of Ophthal 1961;66:490-508.
2. Ayliffe W, Foster CS, Marcoux P. et al. Relapsing acute myeloid leukemia manifesting as hypopyon uveitis. Am J Ophthalmol 1995;119:361-4.
3. Britt JM, Karr DJ, Kalina RE. Leukemic iris infiltration in recurrent acute lymphocytic leukemia. Arch Ophthal 1991; 109:1456.
4. Camera A, Piccririllo G, Cennamo G et al. Optic nerve involvement in acute lymphoblastic leukemia. Leuk Lymphoma 1993;11(1-2):153-5.
5. Decker EB, Burnstine RA. Leukemic relapse presenting as acute unilateral hypopyon in acute lymphotic leukemia. Ann Ophthalmol 1993;25(9):346-9.
6. Duke-Elder Sir St. System of Ophthalmology. Vol IX. Kimpton London 1966;634.
7. Ellis W, Little HL. Leukemic infiltration of the optic nerve head. Amer J Ophthalmol 1973;75:867-71.
8. Ells A, Clarke WN, Noel LP. Pseudohypopyon in acute myelogeneous leukemia. J Pediastr Ophthalmol Strabismus 1995;32(2):123-4.
9. Fischman ML, Bean SCh, Cogan DG. Optic atrophy following prophylactic chemotherapy and cranial radiation for acute lymphocytic leukemia. Amer J Ophthalmol 1976;82:571-6.
10. Friedman B. Hematologie v praxi. Státní nakladatelství Galen:1994.
11. Harrer S, Krieger O, Rigal K, Rubey F, Weber R, Lutz D. Seltene Form der Augenbeteiligung bei akuter lymphatischer Leukämie. Klin Mbl Augenheilk 1984;185:451-3.
12. Hatvani I, Balázs E. Isolierte Leukämische Iritis im Kinderalter. Klin Mbl Augenheilk 1988;192:703-5.
13. Kaikov Y. Optic nerve head infiltration in acute leukemia in children: an indication for emergency optic nerve radiation therapy. Med Pediatr Oncol 1996;26(2): 101-4.
14. Nikaido H, Mishima H, Ono H, Choshi K, Dohy H. Leukemic involvement of the optic nerve. Amer J Ophthalmol 1988;105: 294-8.
15. Ohkoshi K, Tsiaras WG. Prognostic importance of ophthalmic manifestations in childhood leukaemia. Br J Ophthal 1992;76(11):651-5.
16. Pierro L, Brancato R, Zaganelli E, Guarisco L, Lanzetta P. Ocular involvement in acute lymphoblastic leukemia: an echographic study. Int Ophthalmol 1992;16(3):159-62.
17. Soylu M, Tanyeli A, Ozdemir N, Eroglu A,Ersoz TR. Ocular involvement in childhood leukemias. Turk J Pediatr 1994; 36(1):35-41.
18. Schwartz M, Schumann GB. Acute leukemic infiltration of the vitreous diagnosed by pars plana aspiration. Am Ophthalmol 1980;90:326-30.
19. Yamamoto N, Kiyosawa M, Kawasaki T, Miki T, Fujino T, Tokoro T. Successfully treated optic nerve infiltration with adult T-cell lymphoma. J Neuroophthalmol 1994;14(2):81-3.
20. Zakka KA, Yee RD, Shorr N, Smith GS, Petit TH, Foos RY. Leukemic iris infiltration. Amer J Ophthalmol 1980;89:204-9.

Submitted May 1997.
Accepted June 1997.

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