



Some adverse effects of cytarabine in leukemic rats

M.G.^a, Elsayed ^a, Enas A.H. Farag^b and Hanan S. Khaled ^c

^a Department of pharmacology, Faculty of Veterinary Medicine, Benha University. ^b Animal health research institute Benha branch, ^c pharmaceutical company

ABSTRACT

In the present work, the biochemical and hematological parameters as well as histological changes following intravenous injection of 2 mg/ kg for 7 days and 3 mg cytarabine for 5 days in both normal and leukemic rats were studied. Sample were taken in the first, second and third week after end of administration of cytarabine. Both normal and leukemic rats showed significant increase in serum total bilirubin, AST, ALT, ALP, total protein and albumin after intravenous administration of cytarabine either 2 mg/kg body weight for 7 days or 3 mg/kg body weight for 5 days. The effect of intravenous injection of cytarabine either 2 mg/kg body weight for 7 days or 3 mg/kg body weight for 5 days on serum creatinine level in normal and leukemic rats showed a significant changes of kidney function through estimation of serum creatinine, urea and creatine kinase level. Intravenous injection of cytarabine either 2 mg/kg body weight for 7 days or 3 mg /kg body weight for 5 days induce significant increase on lipid profile (cholesterol and triglyceride) and MDA concentration in normal and leukemic rats.

Keywords: cytarabine, lipid profile, leukemic rats, MDA.

(<http://www.bvmj.bu.edu.eg>)

(BVMJ-31(2): 165-170, 2016)

1. INTRODUCTION

Leukemia is a broad term covering a spectrum of diseases. In turn, it is a part of the even broader group of diseases called hematological neoplasms (Mathers et al., 2001). Leukemia is the most common cancer during childhood, acute lymphoblastic leukemia accounts for 85% of all childhood (Gustafsson et al., 1998). Al-Badr and El-Subbagh (2009) reported that cytarabine is a pyrimidine nucleoside analog, antimetabolite, anti-neoplastic, which inhibits the synthesis of deoxyribonucleic acid. Its actions are specific for the S-shape of the cell cycle. It also has antiviral and immunosuppressant properties. Cytarabine is mainly used in the treatment of acute leukemia, especially acute non-lymphoblastic leukemia. Perry and Michael (2008) stated that cytosine arabinoside interferes with the synthesis of DNA. It is an antimetabolic agent with the chemical name of 1-β-arabinofuranosylcytosine. Its mode of action is due to its rapid conversion into cytosine arabinoside triphosphate, which damages DNA when the cell cycle holds in the S phase (synthesis of DNA). Rapidly dividing cells, which require DNA replication for mitosis, are therefore most affected. Cytosine arabinoside also inhibits both

DNA and RNA polymerases and nucleotide reductase enzymes needed for DNA synthesis.

The aim of the present work was to study the adverse effects of cytarabine by measuring some biochemical changes associated after administration of the cytarabine in different doses in normal and leukemic rats.

2. MATERIALS AND METHODS

2.1. Drugs

Cytarabine (cytosine arabinoside) $C_9H_{13}N_3O_5$ obtained from Hospira company limited Uk. Benzene (C_6H_6) was obtained from El-Gomhoria Company, El-Ameria, Cairo.

2.2. Rats:

Eighteen rats were divided into 3 groups: Group (1): Six rats were administered benzene 1mg/kg b.wt for 3 weeks for induction of leukemia. Group (2): Six rats were administered benzene for induction of leukemia as in group 2 then followed by intravenous injection f cytarabine 2 mg/kg b.wt. for 7 days. Group (3): Six rats were administered benzene for induction of leukemia as

in group 2 then followed by intravenous injection of cytarabine 3 mg/kg b.wt

2.3. Blood Samples:

Blood samples were taken at first, second and third week post- treatment in all groups after the end of administration of cytarabine. blood samples were taken from each rat in the group for some biochemical studies. Blood sample was collected without anticoagulant for separation of clear serum for biochemical analysis to determine serum total bilirubin (Jendrasski, 1938), serum transaminases activities (AST and ALT) (Reitmans and Frankel, 1957), ALP, (Chairman, 1983), total protein; albumin (Doumas, 1975), blood creatinine (Folin, 1934), blood urea (March et al., 1965); creatinine kinase (Morin, 1977), blood cholesterol (Allain, 1974), blood triglyceride (Schettler and Nussel, 1975) and MDA.

2.4. Statistical analysis

Statistical analysis were conducted with the Statistical Package for Social Science (SPSS Inc. Released, 2009) to determine if variables differed between groups, according to Snedecor and Cochran (1989). The Shapiro-Willk test was used to test the normal distribution of the data before statistical analysis was performed. Compare between means were conducted by one-way ANOVA and subsequent Duncan's multiple range test (Duncan, 1955). Probability values of less than 5% ($P < 0.05$) were considered significant.

3. RESULTS

3.1. Effect of cytarabine on liver function

Leukemic and treated rats showed significant increase in serum total bilirubin, AST, ALT, ALP, total protein and albumin after intravenous administration of cytarabine either 2 mg/kg body weight for 7 days or 3 mg/kg body weight for 5 days for three weeks after intravenous injection table (1).

3.2. Effect of cytarabine on kidney function

The effect of intravenous injection of cytarabine either 2mg/kg body weight for 7 days or 3 mg/kg body weight for 5days on serum creatinine level in leukemic and treated rats showed a significant increase in kidney function parameters (serum creatinine, urea and creatine kinase level) table (2).

3.3. Effect of cytarabine on lipid profile

Intravenous injection of cytarabine either 2 mg/kg body weight for 7 days or 3 mg /kg body weight for 5 days on serum lipid profile (cholesterol, triglyceride and MDA concentration in normal and leukemic rats, induced significant increase on lipid profile table (3).

4. DISCUSSION

Cytarabine is one of the most power full cytotoxic drugs available for the treatment of acute leukemia. Cytarabine based regimens are the gold standard for induction therapy of acute myeloid leukemia. The present study is to investigate the adverse effect of cytarabine following intravenous injection of cytarabine either 2 mg /kg body weight for 7 days or 3 mg/kg body weight for 5 days in leukemic and normal rats by measuring the changes in both.

Both normal and leukemic rats showed significant increase in serum total bilirubin, AST, ALT, ALP, total protein and albumin after intravenous administration of cytarabine either 2 mg/kg body weight for 7 days or 3 mg/kg body weight for 5 days. the obtained results came in agreement with that obtained by Herzig et al. (1983), who studied cytosine arabinoside therapy for refractory leukemia (transient elevations in transaminase, alkaline phosphatase, or bilirubin) was frequently observed. Another similar result was obtained by Pizzuto et al. (1983) who, reported that cytosine arabinoside induced liver damage. Two patients with acute leukemia developed abnormal liver function tests after the administration of cytosine. The obtained results were inconsistent with Gustafsson et al. (1998) who, found that hepatic dysfunction and jaundice following high-dose cytosine arabinoside. A similar result was obtained by Herzig et al. (1983) who reported that acute leukemia complicated by hyper bilirubinemia due to high dose cytosine arabinoside therapy. Tanaka et al. (2007) et al., (1994) evaluated the adverse effects after administration of arabinoside cytosine in high doses to children with acute myelogenous leukemia. In individual cases jaundice with elevated activity of aminotransferases, paralytic ileus and pulmonary edema were observed. Another similar result was obtained by Tanaka et al. (2007) who investigated that low-dose cytarabine-induced hepatic and renal dysfunction in a patient with myelodysplastic syndrome.

Some adverse effects of cytarabine in leukemic rats

Table (1): Effect of intravenous injection of cytarabine either 2mg/kg body weight for 7 days or 3 mg/kg body weight for 5days on liver in normal and leukemic rats.

Animal groups	first week					week 2					third week									
	total bilirubin	ast	alt	alp	total protein	total bilirubin	ast	alt	alp	total protein	total bilirubin	ast	alt	alp	total protein	total bilirubin	ast	alt	alp	total protein
G(2)	0.86 ± 0.09 ^b	55.20 ± 2.95 ^c	34.33 ± 0.667 ^c	113.0 ± 1.15 ^c	6.09 ± 0.234 ^b	3.90 ± 0.179 ^b	1.12 ± 0.19 ^b	52.80 ± 3.30 ^c	34.33 ± 1.45 ^b	129.3 ± 3.71 ^b	5.95 ± 0.380 ^b	4.24 ± 0.052 ^b	0.95 ± 0.02 ^c	56.74 ± 1.96 ^c	34.60 ± 1.91 ^c	132.0 ± 5.03 ^b	5.59 ± 0.704 ^b	4.04 ± 0.254 ^b		
G(5)	1.56 ± 0.06 ^a	85.74 ± 0.786 ^b	40.34 ± 0.453 ^a	163.7 ± 2.03 ^a	9.20 ± 0.100 ^a	5.20 ± 0.35 ^o	2.28 ± 1.47 ^a	97.99 ± 1.12 ^a	54.70 ± 2.71 ^a	169.7 ± 2.04 ^a	9.25 ± 0.104 ^a	5.29 ± 0.32 ^a	3.26 ± 0.135 ^b	123.67 ± 2.97 ^{ab}	63.07 ± 1.32 ^a	184.0 ± 3.08 ^a	9.15 ± 0.366 ^a	5.30 ± 0.56 ^a		
G(6)	1.62 ± 0.09 ^a	90.80 ± 0.821 ^a	40.90 ± 1.94 ^a	165.0 ± 2.04 ^a	9.33 ± 0.058 ^a	5.12 ± 0.33 ^a	2.35 ± 0.142 ^a	98.01 ± 1.14 ^a	55.26 ± 2.59 ^a	170.3 ± 1.90 ^a	9.33 ± 0.114 ^a	5.40 ± 0.35 ^a	3.54 ± 0.104 ^a	129.83 ± 2.77 ^a	63.73 ± 1.63 ^a	184.6 ± 3.08 ^a	9.25 ± 0.402 ^a	5.35 ± 0.46 ^a		

Table (2): Effect of intravenous injection of cytarabine either 2mg/kg body weight for 7 days or 3 mg/kg body weight for 5days on kidney in normal and leukemic rats .

Animal groups	first week			second week			third week		
	creatinine	urea	ck	creatinine	urea	ck	creatinine	urea	ck
G(1)	3.90 ± 0.179 ^b	45.00 ± 5.77 ^b	1435.7 ± 1.76 ^b	4.24 ± 0.052 ^b	45.67 ± 5.93 ^b	1434.7 ± 4.33 ^b	4.04 ± 0.254 ^b	46.67 ± 5.21 ^b	1436 ± 3.28 ^b
G(2)	5.20 ± 0.53 ^a	58.18 ± 4.24 ^a	1888 ± 26.65 ^a	5.29 ± 0.32 ^a	58.69 ± 2.08 ^a	1917.7 ± 59.4 ^a	5.30 ± 0.56 ^a	65.88 ± 0.071 ^a	1961.3 ± 45.8 ^a
G(3)	5.12 ± 0.33 ^a	59.18 ± 4.51 ^a	1898 ± 26.65 ^a	5.40 ± 0.35 ^a	58.69 ± 2.94 ^a	1924 ± 61.87 ^a	5.35 ± 0.46 ^a	66.62 ± 0.230 ^a	1971.3 ± 45.7 ^a

Table (3): Effect of intravenous injection of cytarabine either 2mg/kg body weight for 7 days or 3 mg/kg body weight for 5days on lipid profile in normal and leukemic rats.

Animal groups	first week			second week			third week		
	cholesterol	triglyceride	mda	cholesterol	triglyceride	mda	cholesterol	triglyceride	mda
G(1)	64.60 ± 2.88 b	142.6 ± 0.33 c	11.74 ± 0.197 d	77.00 ± 3.30 b	143.9 ± 3.5 c	11.43 ± 0.261 c	76.00 ± 3.80 b	143.7 ± 0.89 d	11.72 ± 0.460 c
G(2)	100.7 ± 2.64 a	204.0 ± 2.08 a	15.05 ± 0.275 ab	110.3 ± 3.93 a	204.3 ± 2.4 a	17.91 ± 0.038 a	107.3 ± 0.88 a	174.7 ± 2.91 b	17.66 ± 0.332 ab
G(3)	100.8 ± 0.74 a	205.1 ± 3.01 a	15.17 ± 0.264 a	111.5 ± 4.38 a	205.3 ± 2.5 a	17.95 ± 0.032 a	108.1 ± 1.02 a	175.4 ± 3.0 b	17.84 ± 0.262 ab

Another similar results were obtained by Sun et al. (2009) who found that severe liver damage and pathological changes of the liver were able to alleviate: First, the number of white blood cells in the peripheral blood was significantly lower and with less transplanted K562 leukemia cells; Second, liver function damage was alleviated as liver function tests showed that alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TbIL) were significantly reduced, while the albumin (Alb) was notably increased; Third, liver antioxidant ability was improved as the activities of the antioxidant enzymes glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) were significantly increased, and the contents of GSH and malonaldehyde (MDA) were decreased significantly in the liver; Fourth, the inflammation of the liver was relieved as the level of IL-1beta and IL-6, the inflammatory cytokines, were decreased significantly in the liver. Fifth, liver index was increased as the pathological observation showed that leukemia cells with diffused infiltration into the liver lobules were significantly reduced and with a remarkable increase of apoptotic positive cell rate by TUNEL test.

The effect of intravenous injection of cytarabine either 2 mg/kg body weight for 7 days or 3 mg/kg body weight for 5 days on serum creatinine level in normal and leukemic rats showed a significant increase in kidney function tests (serum creatinine, urea and creatine kinase level). This results were similar to that obtained by Tanaka et al. (2007) who, investigated that low-dose cytarabine-

induced hepatic and renal dysfunction in a patient with myelodysplastic syndrome. Eagle et al. (2012) found that Unilateral Hydronephrosis and Renal Damage after Acute Leukemia. A 14-year-old boy presented with asymptomatic right hydronephrosis detected on routine yearly ultrasound examination. Previously, he had at least two normal renal ultrasonograms, 4 years after remission of acute myeloblastic leukemia, treated by AML-BFM-93 protocol. A function of the right kidney and no damage on the left was confirmed by a DMSA scan. Right retro peritono-scopic nephrectomy revealed 3 renal arteries with the lower pole artery lying on the pelviureteric junction. Herzig et al. (1983) investigated tumor lysis syndrome as a new therapeutic strategy and classification Tumor lysis syndrome described the metabolic derangements that occur with tumor breakdown following the initiation of cytotoxic therapy. Tumor lysis syndrome results from the rapid destruction of malignant cells and the abrupt release of intracellular ions, nucleic acids, proteins and their metabolites into the extracellular space. These metabolites can overwhelm the body's normal homeostatic mechanisms and cause hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia and uremia.

Intravenous injection of cytarabine either 2 mg/kg body weight for 7 days or 3 mg /kg body weight for 5days on serum lipid profile (cholesterol, triglyceride and MDA concentration in normal and leukemic rats, induced significant increase on lipid profile. The obtained results were consistent with Deborah et al. (2004) et al.,(2004) who, studied that Cholesterol synthesis and import

contribute to protective cholesterol increments in acute myeloid leukemia cells. Cholesterol levels are abnormally increased in many acute myeloid leukemia samples exposed *in vitro* to chemotherapy. Blocking these acute cholesterol responses selectively sensitizes acute myeloid leukemia cells to therapeutics. Thus, defining the molecular mechanisms by which acute myeloid leukemia cells complete these protective cholesterol increments which might elucidate novel therapeutic targets. Authors reported that the levels of mRNAs encoding the cholesterol synthesis-regulating enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase, and the cholesterol-importing low-density lipoprotein receptor were both increased by daunorubicin or cytarabine treatments in almost three fourths of cultured acute myeloid leukemia samples. However, less than one third of acute myeloid leukemia samples. Kornblau et al. (2007) reported blockade of adaptive defensive changes in cholesterol uptake and synthesis in acute myeloid leukemia by the addition of pravastatin to idarubicin and high-dose cytarabine phase I study. Following exposure to cytotoxic agents, acute myeloid leukemia blasts elevated cellular cholesterol in a defensive adaptation that increases chemo resistance.

5. CONCLUSION

In the present work, it was concluded that the repeated intravenous administration of 3 mg/kg body weight of cytarabine for five consecutive days in or 2mg /kg body weight in normal and leukemic rats is model for treatment of leukemia, however cytarabine had the following side effects. Hepatotoxicity of cytarabine is a result of metabolism of the drug in the liver that includes increased serum total bilirubin, serum transaminases (serum AST, serum albumin, and serum total protein. Renal toxicity of cytarabine is due to excretion in urine causing (increased serum creatinine, increased serum urea and serum creatine kinase). Lipid profile affected by intravenous injection of cytarabine showing increase in serum cholesterol level and serum triglyceride level. It was clear that cytarabine increase serum MDA, glucose and insulin level.

6. REFERENCES

- Al-Badr, A.A., El-Subbagh, H.I., 2009. Chapter 5 - Itraconazole: Comprehensive Profile, In: Profiles of Drug Substances, Excipients and Related Methodology. Academic Press, pp. 193-264.
- Allain, C.C., 1974. Clin. Chim. 20, 470.
- Chairman, N.W., 1983. A reference method for measurement of alkaline phosphatase activity in human serum study group on alkaline phosphatase. Clin. Chem. 29, 75.
- Deborah, E.B., Sasha, J.M., Henry, Y.L., Cheryl, L.W., Frederick, R.A., Richard, Z., 2004. Cholesterol synthesis and import protective cholesterol increments in acute myeloid leukemia cells. Blood 104.
- Doumas, B.T., 1975. Colorimetric determination of total proteins. Clin. chem. 21, 1159-1166.
- Duncan, D.B., 1955. Multiple range and multiple F tests Biometrics 11, 1-42.
- Eagle, S., Valentina, D., Arvydas, L., Ugnius, M., Vaida, S., Goda, V., Gilvydas, V., 2012. Unilateral Hydronephrosis and Renal Damage after Acute Leukemia Hindawi Case Reports in Medicine.
- Folin, O.Z., 1934. Colorimetric determination of creatinine. Phys. Chem. 268, 228.
- Gustafsson, G., Langmark, F., Pihkala, U., Deverdier, B., Lilleaas, 1998. Childhood cancer in the Nordic countries, report on epidemiologic and therapeutic results, from registus and working groups. NOPHO Annual Meeting 11, 122.
- Herzig, R.H., Wolff, S.N., Lazarus, H.M., Phillips, G.L., Karanes, C., Herzig, G.P., 1983. High-dose cytosine arabinoside therapy for refractory leukemia. Blood 62, 361-369.
- Jendrascki, 1938. Biochem 7297, 81.
- Kornblau, S.M., Banker, D.E., Stirewalt, D., Shen, D., Lemker, E., Verstovsek, S., Estrov, Z., Faderl, S., Cortes, J., Beran, M., Jackson, C.E., Chen, W., Estey, E., Appelbaum, F.R., 2007. Blockade of adaptive defensive changes in cholesterol uptake and synthesis in AML by the addition of pravastatin to idarubicin + high-dose Ara-C: a phase I study. Blood 109, 2999-3006.
- March, W.H., Fingerhut, B., Miller, H., 1965. Clinical chemistry 11 624-627.
- Mathers, C.D., Cynthia Boschi-Pinto, Alan, D.L., Christopher, J.L.M., 2001 Cancer incidence, Mortality and survival by site for 14 regions of the world Global Programme on Evidence for Health Policy Discussion 13.
- Morin, L.G., 1977. Clin. Chem. 23, 646.
- Perry, D., Michael, J., 2008. The Chemotherapy source book. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia.
- Pizzuto, J., Avilés, A., Ramos, E., Cervera, J., J., A., 1983. Hepatic Cytosine arabinoside

- induced liver damage: histopathologic demonstration. *Med Pediatr Oncol* 11, 287-290.
- Reitmans, S., Frankel, S., 1957. Reitman-Frankel colourimetric method of Got/ As T and GPT/ALT Tromsaminales. *Am. J. Clin. Path.* 28 56-63.
- Schettler, G., Nussel, E., 1975. Colorimetric method for determination of cholesterol activity. *Arb. Med. Sozial Med. Prav. Med.* 22, 759-761.
- Snedecor, G.W., Cochran, W.C., 1989. *Statistical methods*, 8th ed. Iowa University Press, Ames, Iowa, USA.
- Sun, B., Wakame, K., Sato, E., Nishioka, H., Aruoma, O.I., Fujii, H., 2009. The effect of hexose correlated compound in modulating cytosine arabinoside-induced hair loss, and 6-mercaptopurine-and methotrexate-induced liver injury in rodents. *cancer epidemiol* 33, 293-299.
- Tanaka, M., Kanamori, H., Yamaji, S., Mishima, A., Fujita, H., Fujisawa, Wasim, E., Helmi, A., Farouq, M., 2007. Leukemia among children in the university pediatric hospital . *Alex. Univ. (EGY)*.