Original article
UDC: 616-006:612.085.2:612.085.1:576.32/.36

The effect of nucleoside analogues on biochemical parameters in rats' sera

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BACKGROUND: *Nucleoside analogues are new chemotherapeutic agents which showed activity against various cancer cells in vitro and in vivo. They modulate signal transduction pathways causing growth inhibition, differentiation, apoptosis and modulation of gene expression through distinct mechanisms of action. Tiazofurin is a synthetic analogue of purine nucleosides with modification on C6 atom, while 8-Cl-cAMP belongs to C8 analogue group. In our study, we examined the effect of two nucleoside analogues, tiazofurin and 8-Cl-cAMP, on biochemical parameters in rats' sera.*

METHODS: *The experiments were carried out on adult Wistar rats. Tiazofurin and 8-Cl-cAMP were administered IP at a single dose (50 mg/kg/day) and four hours later the rats were sacrificed. In the sera of untreated and treated rats the following parameters were determined: urea, creatinine, uric acid, glucose, cholesterol, triglycerides, albumin, aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase, alfa-amilase, calcium, phosphate and iron.*

RESULTS: *The results showed that there were no significant statistical differences among biochemical parameters in the sera of treated animals in comparison to untreated rats.*

CONCLUSION: *These findings implicate that tiazofurin and 8-Cl-cAMP did not express toxic effects as measured by parameters above.*

KEY WORDS: *Antineoplastic Agents; Purine Nucleoside+analogs and derivates; Cyclic AMP; Blood Chemical Analysis; Rats, Wistar*

Archive of Oncology 2001,9(3):155-159©2001,Institute of Oncology Sremska Kamenica, Yugoslavia

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INTRODUCTION

The disturbance of homeostasis of endogenous nucleosides represents a particular field of scientific interest, because a lot of synthetic analogues become more important potential therapeutics in human oncology (1-7).

The pharmacological approach in the synthesis of novel drugs suggests that the use of purine nucleoside analogues in which heterocyclic structure or sugar moiety is altered in such a way that causes toxic effect when incorporated in different part of the cell. Various compounds used for chemotherapy differ in their chemical structure and mechanism of action (8,9). Widely used nucleoside analogues have substituents on purine or pirimidine ring, which do not exist in natural form. They change the pattern of base pairing or interaction of nucleotide with specific enzymes.

2-β-D-ribofuranosyl thiasole-4-carboxamide (tiazofurin)

Many studies have shown that certain imidazol nuclosides like ribavirin, which are potent inhibitors of guanine nucleotide synthesis, have a significant antiviral activity. Assuming that selective regulation of these synthetic pathways in nucleic acids biosynthesis may have an important chemoterapeutic application a series of thiasole-C-nucleosides with ribavirin-like structure, tiazofurin being one of them, were synthesized (10).

Tiazofurin is structural analogue of purine nucleosides with modification on C6 atom. Tiazofurin enters the cell through a simplified nucleoside transport mechanism and joins the cell metabolism. Tiazofurin is a pro-drug that undergoes metabolic conver-

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The manuscript was received: 05. 09. 2001.
Provisionally accepted: 18. 10. 2001.
Accepted for publication: 29. 10. 2001.

sion into adenindinucleotide analogue, tiazol-4-karboxamid adenindinucleotide (TAD), active metabolite (11). After that, enzyme adenosine kinase catalyses the conversion of tiazofurin into tiazofurinmonophosphate (TMP) which undergoes conversion into TAD in the presence of NAD pirophosphorilase. TAD is an active metabolite as well as potent inhibitor of enzyme inosine monophosphate dehydrogenase (IMPDH) (12). Inhibition of IMPDH decreases both DNA and RNA synthesis and proliferative ability of malignant cells (Natsumeda et al. 1989). TAD has stronger affinity for NAD/NADH binding site (10^{-7} M) in IMPDH molecule than endogenous coenzyme NAD (13).

There is a direct proportion between the degree of cellular import of tiazofurin, its conversion into TAD in target cells and its pharmacological effect (13).

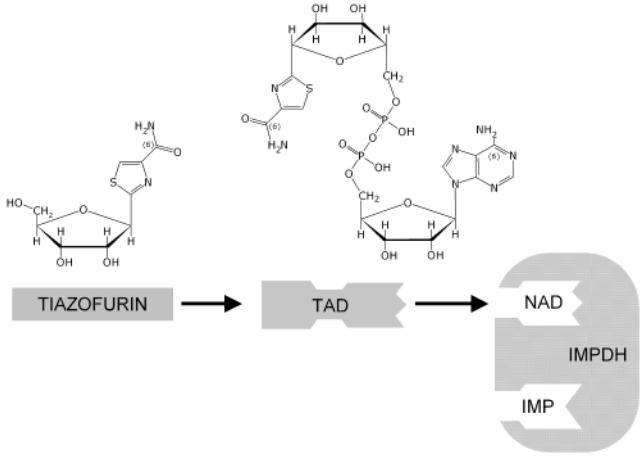

Figure 1. Tiazofurin mechanism of action

Studies of tissue distribution of tiazofurin and its metabolism in a great number of animal models (rodents, rabbits, and dogs) show a significant penetration of tiazofurin in many tissues (14).

In vitro studies of tiazofurin transport through humane erythrocyte membrane show that this molecule is transported by endogenous nucleosides transport system (15).

8-Chloro-cyclic adenosine 3´5´ monophosphate (8-Cl-cAMP)

8-Chloro-cyclic adenosine 3´5´monophosphate belongs to C8 analogue group; with modification on C8 atom of purine ring (Figure 2).

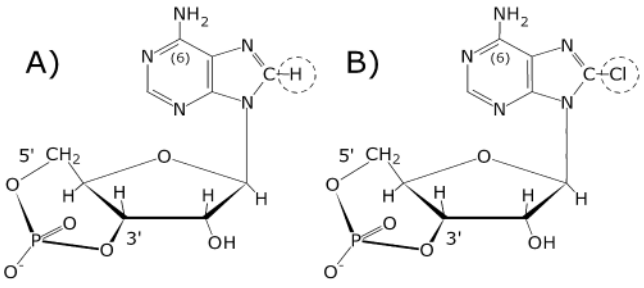

Figure 2. The structure of cyclic 3´5´- AMP (A); and 8-Cl-cAMP (B)

None of so far examined C2, C6 or C8 analogue of cAMP, showed such a great regulatory effects on the growth inhibition of human cancerous cell lines spectrum, as 8-Cl-cAMP did (16).

The mechanisms of normal differentiation in cancer cells are disturbed, but could be restored by action of 3´5´monophosphate analogues through specific receptors (17).

Cyclic AMP, as the intracellular regulating factor, plays a key role in differentiation and growth regulation in different cell types; however, the exact mechanism of action is still unknown (18). In mammal cells cAMP is acting via cAMP dependent protein kinase (PKA) receptors PKA I and PKA II (19,20).

The mechanism of action of 8-Cl-cAMP is still unknown. It is supposed that inhibitory growth effect of 8-Cl-cAMP might be related to decreased ratio of PKA I/PKA II isoforms in cancer cells (21). However, some results suggest that inhibitory growth effect of 8-Cl-cAMP could be mediated by its metabolite 8-Cl-adenosine as well (22).

The aim of this study was to examine the effect of two nucleoside analogues, tiazofurin and 8-Cl-cAMP, on biochemical parameters in rats' sera.

MATERIALS AND METHODS

Animals. Thirty Wistar rats, male and female, 250-300 g weight, bred at the Farm for Experimental Animals, ICN Yugoslavia Institute, were used in this study.

Agents. Tiazofurin and 8-Cl-cAMP were obtained from the ICN Yugoslavia Institute.

Application of tiazofurin and 8-Cl-cAMP. Tiazofurin and 8-Cl-cAMP were administered to Wistar rats intraperitoneally (IP) in a single dose (50 mg/kg/day). Four hours after administration of the agents, blood samples were taken by cardiac puncture. After that rats were sacrificed.

Determination of biochemical parameters. In the sera of untreated and treated rats, the following parameters were determined: urea, creatinine, uric acid, glucose, cholesterol, triglycerides, albumin, aspartate aminotransferase, alanine aminotranferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase, alpha-amylase, calcium, phosphate and iron (Table 1). We used EXPRESS 550 CIBA CORNING analyzer to determine the concentrations of biochemical parameters in rats' sera.

Statistics. The results are expressed as means (standard deviation (SD). Student's *t* test was used for the statistical analysis.

Table 1. The biochemical parameters, their units and methods for the quantitative determination in rats' sera

Biochemical parameters	Methods
1. Urea (mmol/L)	Urease/glutamate-dehydrogenase
2. Creatinine (μmol/L)	Alkaline-picrate (Jaffe)
3. Uric Acid (μmol/L)	Uricase- peroxidase
4. Glucose(mmol/L)	Glucose oxidase
5. Cholesterol (mmol/L)	Cholesterol oxidase
6. Triglycerides(mmol/L)	Lipase/glycerol
7. Albumin (g/L)	Bromcresol green
8. Aspartate aminotransferase (U/L)	AST, pyridoxal 5- phosphate
9. Alanine aminotransferase (U/L)	ALT, pyridoxal 5- phosphate
10. Creatine kinase (U/L)	CK – NAC (NAC=activator: N-acetylcysteine)
11. Lactate dehydrogenase (U/L)	Pyruvate, > 0.7 mmol/L
12. γ-Glutamyltransferase (U/L)	γ-glutamyl-3carboxy-4-nitroanilide
13. Alkaline phosphatase(U/L)	p-nitrophenyl phosphate
14. α-Amylase (U/L)	Maltoheptaoside
15. Calcium (mmolL)	Orto-cresolphtaleine
16. Phosphate(mmol/L)	Phosphomolybdate
17. Iron (μmol/L)	Colorimetric test without deproteinisation

RESULTS

Because biochemical parameters have not been evaluated so far in sera of Wistar rats, we determined their concentrations in control samples. The control group consisted of ten rats' sera samples (five males and five females). In the sera of untreated and treated rats the following parameters were determined: urea, creatinine, uric acid, glucose, cholesterol, triglycerides, albumin, aspartate aminotransferase, alanine aminotranferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase, alfa-amilase, calcium, phosphate and iron (Table 2).

Table 2. The concentrations of biochemical parameters in control group expressed as a range of two standard deviations

Biochemical parameters	Control group	
	male	female
1. Urea (mmol/L)	4.4-8.4	6.6-7.2
2. Creatinine (μmol/L)	57-92	78-82
3. Uruc Acid (μmol/L)	32.7-71.3	19.7 – 138.3
4. Glucose (mmol/L)	7.1-7.8	4.6-8.8
5. Cholesterol (mmol/L)	2.14-3.1	2.45-3.11
6. Triglycerides (mmol/L)	1.67-2.19	0.68-2.6
7. Albumin (g/L)	36-62	31-59
8. Aspartate aminotransferase (U/L)	129-142	93-227
9. Alanine aminotransferase (U/L)	50-66	35.8-83.4
10. Creatine kinase (U/L)	522-938	599-997
11. Lactate dehydrogenase (U/L)	1366-2036	597-3023
12. γ-Glutamyltransferase (U/L)	23.4-38.2	26.6-31.8
13. Alkaline phosphatase (U/L)	461-657	279-593
14. α-Amylase (U/L)	3611-5984	2721-5607
15. Calcium (mmol/L)	1.95-3.05	2.15-2.85
16. Phosphate (mmol/L)	1.71-2.17	1.56-2.08
17. Iron (μmol/L)	32.2-41.92	33.12-59.76

The group treated with tiazofurin consisted of ten rats (five males and five females). Four hours after administration of a single dose of tiazofurin we determined the concentrations of biochemical parameters in rats' sera (Table 3).

The group treated with 8-Cl-cAMP consisted of ten rats (five males and five females). Four hours after administration of a single dose of 8-Cl-cAMP we determined concentration of biochemical parameters in rats' sera (Table 4).

Table 3. The mean values of biochemical parameters in rats' sera after administration of single dose of tiazofurin

Biochemical parameters	Treated rats Tiazofurin	
	male	female
1. Urea (mmol/L)	6.1	5.82
2. Creatinine (μmol/L)	66.16	69.18
3. Uruc Acid (μmol/L)	41	61
4. Glucose(mmol/L)	7.5	6.5
5. Cholesterol (mmol/L)	2.18	2.76
6. Triglycerides(mmol/L)	1.65	1.14
7. Albumin (g/L)	39	42
8. Aspartate aminotransferase(U/L)	145	138
9. Alanine aminotransferase(U/L)	75.3	52.8
10. Creatine kinase (U/L)	730	750
11. Lactate dehydrogenase (U/L)	1664	1780
12. γ-Glutamyltransferase (U/L)	24.4	27.6
13. Alkaline phosphatase(U/L)	551.4	411.8
14. α-Amylase (U/L)	5792.2	4802.6
15. Calcium (mmol/L)	2.5	2.46
16. Phosphate(mmol/L)	1.92	1.7
17. Iron (μmol/L)	39.18	49.46

Table 4. The mean values of biochemical parameters in rats' sera after administration of single dose of 8-Cl-cAMP

Biochemical parameters	Treated rats 8-Cl-cAMP	
	male	female
1. Urea (mmol/L)	7.4	6.78
2. Creatinine (μmol/L)	74.12	76.2
3. Uruc Acid (μmol/L)	71	84
4. Glucose(mmol/L)	6.1	6.3
5. Cholesterol (mmol/L)	2.34	2.8
6. Triglycerides(mmol/L)	1.42	1.15
7. Albumin (g/L)	41	42
8. Aspartate aminotransferase (U/L)	204	174
9. Alanine aminotransferase (U/L)	74.5	61.5
10. Creatine kinase (U/L)	770	831
11. Lactate dehydrogenase(U/L)	2267	2354
12. γ-Glutamyltransferase (U/L)	43.4	35.2
13. Alkaline phosphatase(U/L)	574	502.4
14. α-Amylase (U/L)	6140	4385.8
15. Calcium (mmol/L)	2.57	2.44
16. Phosphate(mmol/L)	2.02	1.74
17. Iron (μmol/L)	40.82	46.6

DISCUSSION

We examined the effect of two nucleoside analogues, tiazofurin and 8-Cl-cAMP, on biochemical parameters in rats' sera. To our knowledge, the biochemical parameters (enzymes, metabolites and oligoelements) have not been determined in Wistar rats' sera so far. Our aim was to determine their concentrations prior to treatment. In the sera of ten untreated rats the following parameters were determined: urea, creatinine, uric acid, glucose, cholesterol, triglycerides, albumin, aspartate aminotransferase, alanine aminotranferase, creatine kinase, lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase, alfa-amilase, calcium, phosphate and iron (Table 2). After determination of control values of biochemical parameters, we determined the concentrations of the same parameters after administration of a single dose of tiazofurin and 8-Cl-cAMP. Both drugs were applied at doses that induce antitumor and/or antiproliferative effects on C6 cell line (24-26). The mean values of all investigated biochemical parameters after administration of tiazofurin were within range of

two standard deviations compared to control values, except for creatinine. The difference between concentration of creatinine in treated female and creatinine concentration in untreated female rats was statistically significant ($p < 0.05$; Table 3). The inhibition of IMPDH leads to the decrease of guanilate synthesis, depletion of guanilate in cells and reduction of DNA and RNA synthesis, which causes the reduction of malignant cells' proliferative ability (5). Antitumor effect of tiazofurin is shown *in vitro* and *in vivo* in a number of solid tumors. Except for anti-proliferative effect, tiazofurin also leads to differentiation of breast cancer cells - MCF-7, ovarian carcinoma cells OVAR-5, and differentiation and apoptosis of leukemia cell line - K-562 (23). Examination of antitumor effect of tiazofurin in human glioma line has shown that tiazofurin has significant antiproliferative and differentiation potential, but it has no cytotoxic effect on these cells (24). The mean values of all investigated biochemical parameters after administration of 8-Cl-cAMP were within the range of two standard deviations as compared to control values, except for glucose. The difference between concentration of glucose of treated males and untreated males was statistically significant ($p < 0.05$; Table 4). 8-Cl-cAMP showed an inhibitory effect to breast cancer and colon cancer cell line growth, without toxic signs (27). It is showed that 8-Cl-cAMP significantly inhibits proliferation of stomach cancer cells (19). Application of previously examined cAMP analogues, such as dibutiril-cAMP or medicines that increase cAMP levels in cells, led to controversial opinions about the cAMP effect on cancer cells growth. 8-Cl-cAMP effect to inhibition of growth was compared with dibutiril-cAMP effect on wide spectrum of human cancer cell lines (21).

The anti-cancer compound tiazofurin is currently being tested in patients (Phase II/III clinical trials) with chronic myelogenous leukemia (CML) in accelerated phase or blast crisis, ovarian cancer, and multiple myeloma. (28).

Experimental results obtained *in vivo* and *in vitro* with 8-Cl-cAMP make basis for clinical investigation of the compound in the therapy of human glioma (26).

CONCLUSION

Our results showed that there were no significant differences among investigated biochemical parameters in the sera of treated animals by tiazofurin and 8-Cl-cAMP in comparison to untreated rats. These findings implicate that tiazofurin and 8-Cl-cAMP do not express toxic effect. This is an important conclusion in our further investigation of tiazofurin and 8-Cl-cAMP in clinical treatment.

Acknowledgments

We would like to thank Assist. Prof. Aleksandar Kerenji for critical reading of the manuscript and Dimitar Jakimov for excellent technical asisstance.

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