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Acute toxicity of novel *N*-sulfonylpyrimidine derivatives *in vivo*

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ABSTRACT

The aim of the present study was to investigate *in vivo* toxic effects and to find acute toxic doses (LD_{50}) of novel *N*-sulfonyl derivatives of pyrimidine nucleobases uracil and cytosine. Six *N*-1-sulfonyluracil derivatives (1, 2, 6, 7, 8, and 9) and two *N*-1-sulfonylcytosine (4, 12) and *N*-1,NH-4-disulfonylcytosine (13) were evaluated in this study. All experiments were performed on 10-14- week-old male and female c57bl/6 zgr mice weighing 22-25 g at the time of treatment. The obtained data showed that derivatives 1, 2, 7, 12 and 13 cause less toxicity than derivatives 4, 6, 8 and 9. Compounds 2, 7, 12 and 13 did not cause death in mice in doses of 3000 mg/kg. Uracil derivative 8 has shown the highest toxicity, its acute toxic dose being 150 mg/kg, similar to the acute toxic dose of 5-fluorouracil.

Key words: uracil, cytosine, *N*-sulfonylpyrimidine derivatives, mice

Introduction

N-sulfonyl derivatives of pyrimidine nucleobases are very interesting compounds since they consist of two biologically active components: a sulfonylcyclourea fragment and a nucleobase. Presently, it is known that sulfonylurea and its derivatives have anticholesterolemic (SLIŠKOVIĆ et al., 1994), antihyperglycemic (AGUILAR-BRYAN et al., 1990; HATZIDIMITROU et al., 1993),), herbicidal (BROWN, 1962; HAY, 1990) and anti-tumour (MOHAMADI et al., 1992; HOWBERT et al., 1990) activities, and they have been found to have many biological and pharmacological interests. There are also a great number

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of biologically active nucleoside and nucleobase derivatives with antineoplastic activity (RUIZ VAN HAPEREN and PETERS, 1994; WILT and PETERS, 1994). 5-fluorouracil is the first analogue of pyrimidine nucleobase uracil, currently used as a chemotherapeutic agent for the treatment of many different tumour cells (YAMASHITA et al., 1982). At the same time, however, 5-fluorouracil causes dose-limiting toxicities, just like many antineoplastic agents, derivatives of nucleobase (YAMASHITA et al., 1982). Therefore, there are many reasons for searching for new agents that will cause less toxicity and which will have much greater therapeutic effects. Consequently, novel *N*-sulfonylpyrimidine derivatives, could be potentially biologically active compounds very sparsely elaborated in the literature.

The aim of the present study was, firstly, to investigate in vivo toxic effects and to find acute toxic doses (LD_{50}) of novel synthesized *N*-sulfonyl derivatives of pyrimidine nucleobase that have shown cytotoxic effects on some human carcinoma cells (GLAVAŠ-OBROVAC et al., 2001), and secondly, to investigate which of these pyrimidine derivatives, and in which doses, could be evaluated in experimental studies as potential anti-tumour drugs.

Materials and methods

Animals. All experiments were performed on 10-14-week-old male and female c57bl/6 zgr mice weighing 22-25 g at the time of treatment. Eight to ten mice were used in each group per experiment. In each experiment animals were treated with different doses of examined drugs. Each experiment was repeated twice. Mice were obtained from the ruđer bošković institute breeding colony. During the experiment three to four animals were kept in a cage. Food and water were supplied ad libitum.

After the administration of drug, animals were observed over a 24-hour period. Animals that died were examined pathoanatomically immediately when death was established. Other animals were also observed and sacrificed when the death was esTablished, or at the end of the research.

All procedures performed in this study were consistent with the european document "directive for the protection of vertebrate animals used for experimental and other scientific purposes" (86/609/eec).

Drugs. Acute toxicity (LD_{50}) was examined for nine *N*-sulfonylpyrimidine derivatives: six *N*-1-sulfonyl derivatives of pyrimidine nucleobase uracil (1, 2, 6, 7, 8, and 9) and two *N*-1-sulfonyl (4, 12) and *N*-1,NH-4-disulfonyl (13) derivatives of pyrimidine nucleobase cytosine. (Fig. 1). All drugs were synthesized at the Ruđer Bošković Institute (Zagreb, Croatia). The synthesis procedures have previously been described by KAŠNAR et al. (1997). All drugs were dissolved in isotonic physiological saline immediately prior to intraperitoneal injection (i/p). The drug was given according to the body weight of mice, i.e. 0.05 ml/25g. For calculation of $LD_{50,}$ GraphPad PRISM, ver. 4.0 (MILLER, 2003) was applied.

Results and discussion

The results of the examined *N*-sulfonylpyrimidine derivatives of nucleobases uracil and cytosine for acute toxicity (LD_{50}) are shown in Tables 1 and 2.

Table 1. Acute toxic doses (LD_{50}) for *N*-sulfonyl derivatives of pyrimidine nucleobase uracil

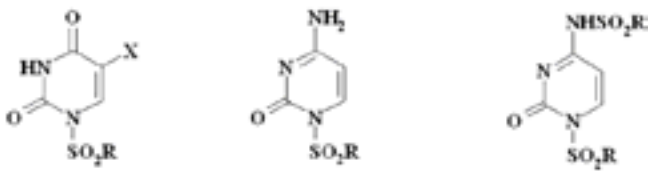
N-sulfonyl derivatives of pyrimidine nucleobase uracil	LD_{50} (mg/kg)
1	>2100
2	>3000
6	≈750
7	>3000
8	≈150
9	≈400

Table 2. Acute toxic doses (LD_{50}) for *N*-sulfonyl derivatives of pyrimidine Nucleobase cytosine

N-sulfonyl derivatives of pyrimidine nucleobase cytosine	LD_{50} (mg/kg)
4	≈1600
12	>3000
13	>3000

As shown in Table 1, *N*-1-sulfonyluracil derivatives 6, 8, and 9 cause higher toxicity than derivatives 1, 2 and 7. The *N*-1-sulfonyluracil derivatives 2 and 7 did not cause death in 50% of mice, even when high doses (LD_{50}=3000 mg/kg) were administered. The same results were obtained after administration of *N*-1-sulfonylcytosine derivative 12 and *N*-1,NH-4-disulfonylcytosine derivative 13, as shown in Table 2. The same Table shows that when the toxicity of *N*-sulfonylpyrimidine derivatives of cytosine is compared, the toxicity of *N*-1-sulfonylcytosine derivative 4 is two times higher in comparison with *N*-1-sulfonylcytosine derivative 12 and *N*-1,NH-4-disulfonylcytosine derivative 13.

The synthesis of novel *N*-sulfonyl derivatives of nucleobases, which at the same time represent sulfonylcycloureas, was followed from the hypothesis that novel synthesized compounds could have significant biological activity because they consist of a combination of biologically active components as well as fragment of sulfonylurea and pyrimidine nucleobase (KAŠNAR et al., 1997). Sulfonylurea and its derivatives are biologically active compounds which have been used in medicine for a long time (AGUILAR-BRYAN et al., 1990; HATZIDIMITROU et al., 1993, SLIŠKOVIĆ et al., 1994), but it is only in the last 20 years that they begun to be used as potential antitumor agents (MOHAMADI et al., 1992; HOWBERT et al., 1990).



1 X = H, R = a
2 X = Br, R = a
6 X = H, R = b
7 X = I, R = a
8 X = Br, R = b
9 X = I, R = b
4 R = a
12 R = b
13 R = b

Fig. 1. Chemical structure of *N*-sulfonylpyrimidine derivatives

In this research of toxicity of *N*-sulfonylpyrimidine derivatives pyrimidine nucleobases *in vivo*, acute toxic doses of derivatives of different nucleobases - uracil and cytosine on which were bound R-SO_2-fragments - were examined. The examined derivatives showed differences in R-substituent in R-SO_2 fragment, which was either aromatic (tosyl) or aliphatic (mesyl). On derivatives 1, 2, 4 and 7 on the *N*-1 site *p*-toluenesufonyl or tosyl was bound, while on derivatives 6, 8, 9, 12 and 13 on the same site (*N*-1) metanesulfonyl or mesyl was bound (Fig. 1). On compound 13 one more mesyl group on the *N*-4 site was also bound, and that compound has no free amino group. Furthermore, either bromine or iodine was introduced onto the C-5 site in some of the *N*-1-sulfonyl derivatives of uracil (derivatives 2, 7, 8, 9) (Fig. 1). These structural differences among studied *N*-sulfonylpyrimidine derivatives showed some differences in acute toxicity. Examining the relationship between the toxicity and the structure of *N*-1-sulfonyluracil derivatives, it can be noted that uracil derivatives with mesyl (derivatives 6, 8, 9) showed stronger toxicity in comparison with the uracil derivatives with tosyl (derivatives 1, 2, 7). Namely, the administration of derivatives 2 and 7 did not result in the death of 50% of examined animals in doses of 3000 mg/kg (Table1). When comparing derivatives with mesyl (derivatives 6, 8, and 9) with each other, it can be seen that derivatives 8 (containing bromine) and 9 (containing iodine), showed stronger toxicity than derivative 6, which has no halogen element. The strongest toxicity was shown by derivative 8, 5-bromo-1-mesyluracil (Fig. 1, Table 1). No differences in toxicity were found between 5-bromo-1-tosyluracil (derivative 2) and 5-jodo-1-tosyluracil (derivative 7). The study of acute toxicity of *N*-sulfonyl derivatives of cytosine has shown opposite results. Namely, by administration of *N*-1-tosylcytosine (derivative 4) the acute

toxic dose was two times higher than by administration of derivatives *N*-1-mesylcytosine (derivative 12) and *N*-1,NH-4-dimesylcytosine (derivative 13). Our results have shown compatibility with the results for the same derivatives obtained in vitro. Comparing our results with results of cytotoxicity in vitro by GLAVAŠ-OBROVAC et al. (2001), it can be noted that uracil derivatives with the mesyl group have also shown better cytotoxic activity than derivatives with the tosyl group.

Comparing the results of toxicity of *N*-1-sulfonyl derivatives of uracil to 5-fluorouracil that have been used in therapy of many different tumours, we can observe that the dose of derivative 8 administrated intraperitoneally in mice (LD_{50} = 150 mg/kg) was slightly higher than an acute toxic dose of 5-fluorouracil (LD_{50} = 117 mg/kg) (WANG and CHEN, 1995).

Results of pathological examination showed gastric mucosal lesions in animals which died within 24 hour after administration of *N*-1-sulfonyluracil derivatives with low LD_{50} (derivatives 8 and 9) and *N*-1-sulfonylcytosine derivative 4.

On the basis of the results of this study it can be concluded that *N*-sulfonyl derivatives of pyrimidine nucleobases uracil and cytosine cause different toxic effects. Although some derivatives have not shown strong toxicity, it would probably be very difficult to use them in cancer therapy because of a very high lethal dose. Therefore, novel *N*-sulfonyl derivative with a lower lethal dose could be tested in anti-cancer treatment, thereby representing a valuable contribution to the development of new anti-cancer chemotherapeutic agents.

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SAŽETAK

U radu je istražen toksični učinak odnosno akutna toksična doza (LD_{50}) novosintetiziranih N-sulfonil-derivata pirimidinskih nukleobaza uracila i citozina. Akutna toksična doza istražena je u šest *N*-1-sulfonil-derivata uracila (1,2,6,7,8,9) i dva *N*-1-sulfonil-derivata citozina (4,12) i *N*-1,NH-4-disulfonil-derivata citozina. Toksični učinak istražen je na miševima soja C57BL/6 Zgr u dobi 10-14 tjedana koji su težili 22-25 g. Polučeni rezultati ukazuju na manju toksičnost derivata 1, 2, 7, 12 i 13 u usporedbi na derivate 4, 6, 8 i 9. Nakon aplikacije derivata 2, 7, 12 i 13 u dozi od 3000 mg/kg nije došlo do uginuća u 50% promatranih miševa. Najveća toksičnost zapažena je kod derivata 8, derivata uracila čija je LD_{50} iznosila 150 mg/kg. Akutna toksičnost ovog derivata pokazuje sličnosti s toksičnošću citostatika 5-fluorouracila.

Ključne riječi: uracil, citozin, *N*-sulfonil-derivati pirimidinskih nukleobaza, miš