thetic pathway for angiotensin action on the isolated rabbit aortic strips which was described previously[5]. ASTRÖM[10] found that local anaesthetics prevent the response of aortic strips to epinephrine and other stimulants, differently from their local anaesthetic activities. Our present results support ASTRÖM's findings for angiotensin but not for tyramine and cocaine interaction on the isolated rabbit aortic strips[11].

Zusammenfassung. An der isolierten Kaninchenaorta hebt Kokain erst in hoher Konzentration die kontraktile Wirkung von Angiotensin auf. Hingegen wird die Wirkung von Tyramin am selben Präparat in sehr viel niedrigerer Konzentration aufgehoben. Prokain antagonisiert bei gleicher Konzentration die Wirkung der beiden Pharmaka. Die Ergebnisse lassen annehmen, dass eine indirekt sympathomimetische Wirkung von Angiotensin an der isolierten Kaninchenaorta sehr fraglich ist. Ausserdem gibt die gefundene antagonistische Wirkung der verwendeten Lokalanästhetika gegenüber den myotropen Pharmaka den Eindruck, dass dieser Antagonismus unspezifisch ist und nichts mit ihrer lokalanästhetischen Wirkung zu tun hat.

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The in vivo Activity of Combinations of 5-Azacytidine and Cytidine on Leukemia L-1210

5-Azacytidine is an antitumor antibiotic which was isolated from a fermentation of *Streptoverticillium ladakanus*[1,2] and also synthesized, independently, by PISKALA and SORM[3]. Preliminary studies have shown that 5-azacytidine is active against the leukemia of AK mice[4,5], T-4 lymphoma[1] and leukemia L-1210[1]. 5-Azacytidine has been shown to inhibit the growth of *Escherichia coli* in a synthetic medium[1] and the inhibition can be reversed by the incorporation of cytidine or uridine into the medium. This paper extends the studies on the anti-leukemic activity of 5-azacytidine and shows that the anti-leukemic activity can be reversed in vivo by either cytidine or uridine.

Materials and methods. 5-Azacytidine, isolated from fermentations of *S. ladakanus*, was obtained from Mr. M. BERGY, The Upjohn Company, and synthetic 5-azacytidine was obtained through the Aldrich Chemical Co. and California Biochemical Corp. Cytidine and uridine were obtained from Nutritional Biochemical Corp.

Female mice ($B6D2F_1/J$), weighing 16–18 g each, were purchased from the Jackson Memorial Laboratories, Bar Harbor, Maine. The leukemia was induced by the i.p. injection of 1.6×10^6 cells obtained from the peritoneal fluid of a donor leukemic mouse. The dosage, route of administration and timing of the injections are given in the Tables. The mice were weighed at the start of each experiment and at intervals thereafter. The mice were checked daily for deaths. The median survival time was calculated for each group.

Results. The most effective dose of 5-azacytidine when given daily for 7 days starting 18 h after implanting the tumor cells was 10 mg/kg · day. This dose is very close to the toxic dose, since 12.5 mg/kg · day produced no increase in survival time and caused a marked loss in body weight during the injection period (Table I).

Studies using the combinations of cytidine and 5-aza-cytidine are shown in Table II. Administration of cytidine 1 h prior to the administration of 5-azacytidine or at the same time but at a different site results in a reduction in the toxicity, as evidenced by weight changes and an increase in the survival time. Administration of cytidine 1 or 2 h after the administration of 5-azacytidine or if the 2 drugs were mixed prior to injection produced no change in either the toxicity of the 5-azacytidine as evidenced by weight changes or survival. Similar observations were made when uridine was substituted for cytidine. From a therapeutic standpoint the combination of 5-azacytidine and either cytidine or uridine has no practical value over the administration of lower doses of 5-azacytidine in daily systemic treatment. The systemic use of either cytidine or uridine may be of value for the protection of the hemopoietic or other susceptible systems when a tumor mass is regionally perfused with 5-azacytidine.

A preliminary experiment indicated that intermittent therapy using relatively high doses of 5-azacytidine was effective in prolonging the survival time. A confirming study in early leukemia (Table III) shows that 20 or 30 mg/kg · day given every 3 days will prolong the survival time 3-fold without too severe loss in body weight. If treatment is delayed until the fifth day (48 h before the first control mouse dies), the treatment is not effective in prolonging the survival time. The concurrent administration of cytidine reduces the loss in body weight during treatment period and the median survival time.

Discussion. The results reported in this paper confirm and extend our work[1] and that of ŠORM[4,5]. We have shown that when the proper dosage regimens are used that a 3-fold increase in survival time can be obtained. It might be expected that if treatment was continued that further extensions of survival time might be expected.

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Table I. Effect of 5-azacytidine on leukemia L-1210

5-Azacytidine mg/kg · day	Route	Body weight T_0	T_8	T/C ratio[a]
0		15.3 g	16.1 g	1.00
0.5	SC	16.1	18.3	1.59
1.0	SC	15.5	16.3	1.76
1.75	SC	17.3	17.3	1.94
5.0	IP	16.0	15.3	3.06
10.0	SC	16.2	14.0	3.35
10.0	IP	16.2	12.6	3.50
12.5	IP	16.4	12.5	1.00
20.0	SC	15.7	11.0	0.94
20.0	IP	16.3	13.1	1.00

[a] Ratio of median survival time of the treated to that of the control mice. – The 5-azacytidine was given daily for 7 days starting 24 h (T_1) after the implanting of the leukemic cells. The median survival time in our studies varied from 8.0–8.5 days.

Table II. Effect of combinations of 5-azacytidine and cytidine on leukemia L-1210

5-Azacytidine mg/kg · day	Cytidine dosage schedule[a] 400 mg/kg · day	Body weight T_0	T_8	T/C[b]
0	0	15.9 g	17.6 g	1.00
20	0	16.3	13.1	1.00
20	Injected 1 h before 5-azacytidine	15.5	13.5	2.88
20	Injected at same time as 5-azacytidine	16.0	15.6	2.44
20	Injected 1 h after 5-azacytidine	16.3	12.0	1.13
20	Injected 2 h after 5-azacytidine	16.1	11.6	1.00
20	Mixed prior to injection and given i.p.	16.1	17.4	1.44
20	Mixed prior to injection and given s.c.	16.1	18.1	1.31

[a] 5-Azacytidine and cytidine were given daily for 7 days starting on T_1. [b] See footnote to Table I.

Table III. Effect of varying dosage schedules on the response of leukemia L-1210 to 5-azacytidine

5-Azacytidine mg/kg · day i.p.	Cytidine mg/kg · day s.c.	Dosage schedule	Body weight (g)		T/C ratio
0	–	$T_1 \rightarrow T_7$	15.9	17.6	1.00
10	–	T_1, T_4, T_7	16.0	16.4	2.14
20	–	T_1, T_4, T_7	16.0	14.8	3.00
30	–	T_1, T_4, T_7	16.7	14.8	3.06
30	400	T_1, T_4, T_7	16.1	16.8	2.00
10	–	T_3, T_6, T_9	16.6	18.8	1.63
20	–	T_3, T_6, T_9	16.7	15.6	2.13
30	–	T_3, T_6, T_9	16.8	14.5	2.06
30	400	T_3, T_6, T_9	16.8	18.4	1.38
10	–	T_5, T_8, T_{11}	17.9	–	1.13
20	–	T_5, T_8, T_{11}	17.2	–	1.13
30	–	T_5, T_8, T_{11}	18.7	–	1.13
30	400	T_5, T_8, T_{11}	16.7	–	1.19

Further increases in survival may be limited, however, by either accumulative toxicity of the drug or by the development of resistant forms of the leukemic cell in susceptible mice.

CIHAK and ŠORM[6] have studied the metabolism of 5-azacytidine and showed that it was phosphorylated and incorporated into nucleic acids. The same authors postulated an alternate pathway involving 5-azauridine and 5-azauracil which was believed to block uridine phosphorylase. RAŠKA[7] showed that the administration of large doses of 5-azacytidine to mice is accompanied by an excretion of 5-azacytidine followed by the excretion of other metabolites. The excretion of 5-azacytidine is accompanied by an increased excretion of orotic acid and orotidine in the urine. One effect of 5-azacytidine would thus be an interference with the decarboxylation of orotodylic acid. RAŠKA and co-workers do not feel that this mechanism of action could account for the activity of 5-azacytidine since 6-azacytidine acts in a similar fashion and is less active biologically than 5-azacytidine.

RAŠKA and co-workers showed[8] that the phosphorylation of 5-azacytidine was inhibited by cytidine in an in vitro system using isolated nuclei from calf thymus cells. In the same system protein synthesis was inhibited by 5-azacytidine in 60–90 min. They attributed the inhibition of protein to an inhibition of messenger RNA since actinomycin D acts in a similar manner with similar time relationships. CIHAK, TYKOVA and ŠORM[9] showed that when Ehrlich ascites cells are exposed to 5-azacytidine-4(^{14}C) and cytidine(^{3}H), the incorporation of both radioactive compounds into RNA is markedly reduced. This explains the reduction in biological activity noted in our studies using combinations of 5-azacytidine and cytidine in various time cycles. The use of cytidine or uridine for the protection of certain metabolic functions requires that these materials get to metabolic sites either immediately before or at the same time as the 5-azacytidine in order for them to be effective[10].

Zusammenfassung. Cytidin- oder Uridinzufuhr, bei an Leukämie L-1210 erkrankten Mäusen entweder 1 h vor oder gleichzeitig mit 5-Azacytidin verabreicht, bewirkt eine Herabsetzung der Toxizität von 5-Azacytidin, die sich in der Gewichtsveränderung und Verlängerung der Lebenszeit zeigt. Aufgrund der gefundenen zeitlichen Verhältnisse wird die Hypothese aufgestellt, dass Cytidin oder Uridin nur dann wirksam werden, wenn der Ort der metabolischen Vorgänge gleichzeitig mit 5-Azacytidin erreicht wird.

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