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CYTOTOXIC ACTIVITY AND FRAGMENTATION OF AZIRIDINES IN MICROSOMES

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SUMMARY

The fragmentation reaction of 2-para-substituted phenyl-N-methylaziridines in rat liver microsomes was studied. The relative reactivity against the substituent group, estimated from the amount of styrenes produced in liver microsomal solution, was para-Cl > para-Me > H > para-NO_2 > para-OMe.

The cytotoxic activity of these aziridines was also studied using HeLa cells and L-1210 mouse leukemia cells in free-floating culture. The order of cytotoxic activity with HeLa and L-1210 cells was Cl ≡ Me > H > NO_2 ≡ OMe and Me > Cl ≡ OMe > H > NO_2, respectively. The results indicated that the orders of cytotoxic activity and fragmentation reactivity for the para-substituted aziridines have some parallel relationship. The nitrosomethane generated by fragmentation reaction of aziridine probably plays an important part in the biological activity of aziridines.

In studies of aziridine metabolism, two kinds of reactions have long been considered important. One is their hydrolysis to form aminoalcohols, which corresponds to the detoxification process of aziridines *in vivo*. The other process is an alkylation reaction on cellular macromolecules such as DNA, RNA and protein, which supposedly produces the carcinogenic effect of aziridines. However, recent investigations into the mechanisms of the carcinogenic effect of polyaromatic hydrocarbons have revealed that alkylation reaction by the arenoxide, which was thought to be a bioactivated intermediate the biopolymer, is clearly unrelated to the carcinogenic of the original aromatic

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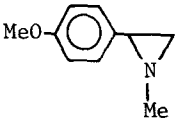
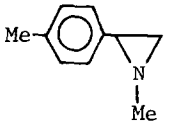
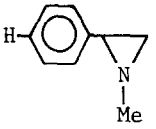
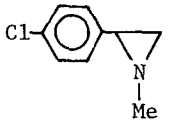
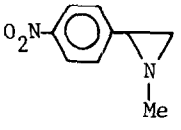
compound (1). Thus we doubt that the alkylation reaction on DNA is an essential mechanism for the carcinogenic activity of aziridines.

Previously we reported a third new metabolic reaction for aziridines occurring in rat liver microsomes (2). Aziridines gave olefin and nitrosoalkanes as main products of a fragmentation reaction catalyzed by the monooxygenase enzyme of the microsomes. We were interested in knowing what effect of the highly reactive nitrosoalkanes generated inside the body appears biologically. This time we studied the metabolic reaction of 2-para-substituted phenyl-N-methyl aziridines and found an interesting relationship between the intensity of the cytotoxicity of aziridines and the amount of nitrosomethane produced in hepatic microsomes by the fragmentation reaction of aziridines.

Fragmentation reaction of aziridines in hepatic microsomes

Metabolic reactions of 2-para-substituted phenyl N-methyl-aziridines in liver microsomes were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl, 10 mM $MgCl_2$, 1 mM nicotinamide, 1 mM pyrophosphate, 30 mg of protein of microsomes, 0.01 mM of aziridines, and an NADPH-generating system in a final volume of 5 ml. The reaction conditions were similar to those described previously (2). After an appropriate incubation time, the reaction mixture was quenched by adding 2N NaOH solution followed by extraction with CH_2Cl_2 for VPC and HLC analysis. The amount of para-substituted styrene generated by the fragmentation reaction of aziridine in microsomes was determined by comparison with an authentic sample. The experiments were repeated three or four times for each aziridine

Table 1 Cytotoxic activity and fragmentation of aziridines in Microsomes (37°C)

Compd.	Run[1]	Time (min)	X-⟨⟩-CH=CH$_2$ Yield % [2]	Rel. yield from II+III (60 min.)	ED_{50} (γ/ml) B-HeLa	L-1210
	II	60	3)		>10	4.8
	III	60	3)			
	II	60	3.9			
	III	5	1.3	1.6	2.3	1.4
	III	60	4.0			
	IV	15	2.3			
	I	60	1.3			
	II	60	1.2			
	III	5	1.2	1	6.3	>10
	III	60	3.7			
	IV	15	1.9			
	II	60	3.7			
	III	5	2.3	2.8	2.3	4.6
	III	60	10.0			
	IV	15	2.7			
	I	60	2.0			
	II	60	1.7	0.5	>10	13
	III	60	0.9			
	IV	15	0.8			

[1] Fragmentation reaction activity of microsomes depended on individual rat character. Consequently, comparison of the styrene yield should be done within the same experimental run.

[2] 50-90% of starting materials were recovered.

[3] Undetectable.

derivative; the results are shown in Table 1. Relative yield of styrenes was calculated on the basis of the average value

of Runs II and III (60 min) using 2-phenyl-N-methylaziridine as the reference. 2-p-Anisyl-N-methylaziridine was very unstable in aqueous solution being converted into aminoalcohol. Thus, under our fragmentation reaction conditions with microsomes, we could not obtain p-methoxystyrene as a fragmentation product.

Cytotoxicity of aziridines

The in vitro cytotoxic effect of aziridine derivatives was tested against HeLa cells and L1210 mouse leukemia cells in free-floating culture. HeLa cells were grown in Eagle's minimum essential medium (MEM) containing 10% bovine serum. For subcultivation, the suspension of HeLa cells was prepared by trypsinization of the stock culture. Next, the cells were resuspended in a fresh medium, diluted to a concentration of 10^5 cells/ml, then transferred into small test tubes. Three cultures thus prepared were used as a set for each experiment. L1210 mouse leukemia cells were cultivated in MEM supplemented with 10% fetal calf serum.

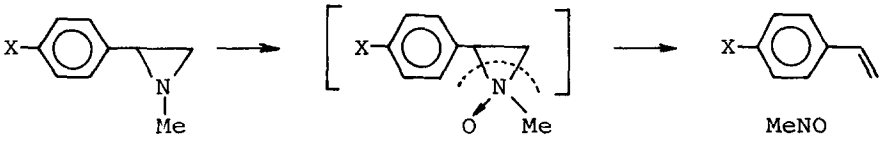
The sample was added to the cell cultures on the second day of cultivation. After 2 days of incubation, the cell population was measured using a TOA micro-cell counter (TOA Electronics, Kobe, Japan). The growth rate (%) was calculated from the following formula:

$$\text{Growth } \% = (T - Co/C - Co) \times 100$$

where C = final cell number in controls, T = final cell number in treated tube, and Co = cell number in the tube at the time of sample addition. The effective dose for 50% growth inhibition (ED_{50}) was determined by plotting the logarithmic curve of the drug concentration against the growth rate. The values are listed in Table 1.

Results and discussion

The aziridines introduced into the microsomal suspension gave N-oxide as an unstable intermediate which then decomposed into styrenes and nitrosomethane as shown below (2).



Nitrosomethane is very unstable and probably highly reactive with an amino group bound to a biopolymer such as DNA, RNA and protein (3,4). However, another kind of fragment, para-substituted styrenes, was stable enough to allow estimation of the amount of nitrosomethane generated in the metabolic reactions.

The yield of styrenes indicated that the metabolic reaction was independent of the electronic effect of the substituent group observed in the ordinal chemical reaction. The yield of styrenes vs. the substituent constant σ proposed by Hammett et al. (5) is far from a linear relationship. The order of styrene formation of the substituted aziridines in the microsomes was Cl > Me > H > NO_2 > OMe.

Surprisingly, we found the same tendency for the ED_{50} value of cytotoxicity of the corresponding aziridines. Thus, the order of the cytotoxicity of aziridines on HeLa and L-1210 was Cl ≡ Me > H > NO_2 ≡ OMe and Me > Cl ≡ OMe > H > NO_2, respectively. Only 2-paraanisyl N-methyl aziridine, which was very unstable in the microsomal solution, had a value slightly higher than that expected from the above styrene formation. Our study revealed that aziridine, which gives a higher yield of nitrosomethane upon metabolism in microsomal solution, also has a higher cytotoxicity for HeLa cells and L1210 mouse

leukemia cells. Although the mechanism of the cytotoxic activity of aziridine remains ambiguous (6), we suggest that nitrosomethane generated by a fragmentation reaction of aziridine probably plays an important part in the biological activities of aziridines (7,8).

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7. The carcinogenicity of 2-β-naphthyl-N-isopropylaziridine was discussed by R. Howe, Nature 207, 594 (1965). Strong carcinogenic activity of 2-β-naphthyl-N-methylaziridine was also found in our laboratory. Y. Harada, Shionogi Res. Lab. private communication.
8. All substituted styrenes appeared in this report did not indicate any cytotoxic activity on HeLa cells and L1210 mouse leukemia cells.