

Mechanism of Potentiation of the Carcinostatic Activity of 8-Aza-guanine by 4(or 5)-Aminoimidazole-5 (or 4)-carboxamide Analogs¹⁾

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(Received August 9, 1968)

The antitumor activity of 8-AG was potentiated by its administration with AICA or Aza-AICA to Ehrlich ascites carcinoma in mice. AICA or Aza-AICA alone had no carcinostatic actions, but Monomethyl-TICA alone was found to be slightly carcinostatic at the concentration used in these experiments.

AICA and Monomethyl-TICA inhibited liver 8-AG deaminase activity of normal mice, but Aza-AICA did not. Neither AICA nor Aza-AICA affected the activity of the liver xanthine oxidase of normal mice.

Therefore, the potentiating actions of AICA and Monomethyl-TICA on 8-AG may be to their inhibitory effects on 8-AG deaminase activity. That of Aza-AICA could be due to a different action from those of the former two compounds.

Introduction

Mandel and Law³⁾ demonstrated that the co-administration of the purine precursor, 4(or 5)-aminoimidazole-5(or 4)-carboxamide (AICA), and the carcinostatic guanine analog, 8-azaguanine (8-AG), potentiated the carcinostatic action of the latter. In their subsequent *in vitro* studies, AICA was shown to inhibit the deamination of 8-AG to the relatively nontoxic 8-azaxanthine.

Other investigators^{4,5)} reported that 2-amino-4-hydroxy-6-formylpteridine inhibited irreversibly the 8-AG deaminase activity of both mouse liver and adenocarcinoma-755, thereby increasing the carcinostatic potency of 8-AG on adenocarcinoma-755.

With adenocarcinoma-755, lymphoma-11 and mammary adenocarcinoma EO-771, combination therapy with 8-AG and a noncarcinostatic riboflavine analog, Flavotin (6-chloro-9-(1-D-sorbityl)isoalloxazine) resulted in enhanced inhibition of tumor growth.⁵⁻⁷⁾

Skipper⁸⁾ reported that the tumorcidal action of a combination of 6-mercaptopurine (6-MP) and 8-AG in mice bearing adenocarcinoma-755 was no greater than of 6-MP alone.

Accordingly, it is of interest to determine whether the carcinostatic properties of 8-AG are potentiated by AICA analogs.⁹⁻¹¹⁾

- 1) This investigation was supported by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education.
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This paper reports experiments on the effects of AICA, 4(or 5)-aminotriazole-5(or 4)-carboxamide (Aza-AICA) and 4(or 5)-monomethyltriazenoimidazole-5(or 4)-carboxamide (Monomethyl-TICA) on the carcinostatic activity of 8-AG. The results indicated that Aza-AICA potentiated the carcinostatic properties of 8-AG without inactivating 8-AG deaminase while Monomethyl-TICA potentiated the action of 8-AG by inactivation of the deaminase, as in the case of AICA.

Materials and Methods

8-AG was used as a carcinostatic agent, and AICA and some of its derivatives as combination agents: Their chemical structures are shown in Fig. 1.

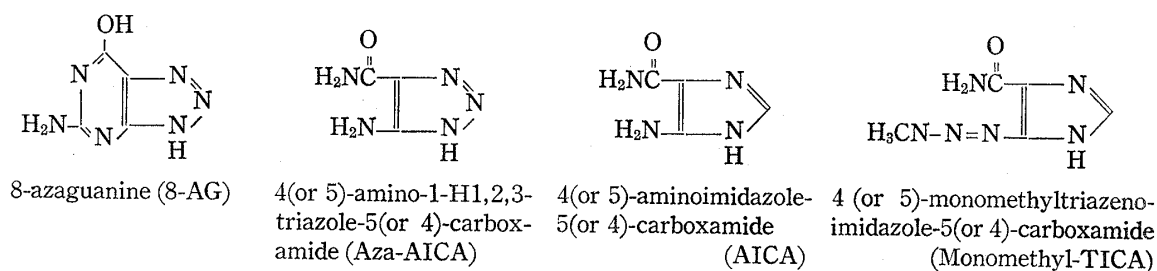


Fig. 1. Chemical Structures of the Compounds used

Male ddO strain mice weighing about 20 g were used. The Ehrlich ascites carcinoma cells used were harvested from tumor-bearing mice 7 days after their inoculation. The control and test group each contained 7 mice. Ascites carcinoma was inoculated intraperitoneally at a dose of 2×10^6 tumor cells per animal. Treatment was started 24 hr after the inoculation and continued once daily for 7 days. AICA or one of its analogs was administered intraperitoneally in weakly alkaline solution one hr before the intraperitoneal injection of 8-AG. The mean survival time of each group was compared with that of the control group. Solid carcinoma was inoculated subcutaneously into the inguinal region on both sides of each animal at a rate of 4×10^6 cells per animal. The mice were treated in the same way as with ascites carcinoma but received injections from the third day after inoculation of the tumor. The effect of the compounds are expressed as relative tumor weights on the 12th day after inoculation taking that of the control as 100.

The 8-AG deaminase activity was measured by determination of the amount of ammonia released enzymatically from 8-AG.¹²⁾

A solution of 0.5% 8-AG in 0.55M Na_2CO_3 was used as the substrate and 15% homogenate of normal mouse liver in 0.05M phosphate buffer (pH 7.3) was used as the crude enzyme preparation. A mixture of 1 ml of the substrate solution, 0.5 ml of the crude enzyme preparation and 0.5 ml of the buffer solution, containing the test compounds, was incubated for 30 min at 37.5°. Then the ammonia formed was measured colorimetrically by the indophenol method.¹³⁾

Xanthine oxidase activity was measured manometrically with xanthine as substrate and 10% normal mouse liver homogenate in 0.015M pyrophosphate buffer (pH 8.6) as the crude enzyme preparation.¹⁴⁾

Results

Potentiating Effect of AICA or Its Analogs on the Antitumor Activity of 8-Azaguanine against Ehrlich Ascites Carcinoma

The potentiating effect of AICA and its analogs on the antitumor activity of 8-AG against Ehrlich ascites carcinoma are shown in Fig. 2 as prolongation on mean survival time in the treated groups. AICA (200 mg/kg) or Aza-AICA (50 mg/kg) alone had no effect on the survival time of the tumor-bearing mice. Enhancement of the carcinostatic activity of 8-AG occurred when AICA or its analogs were given intraperitoneally 1 hr prior to the intraperitoneal injection of 8-AG. Combination therapy of AICA (200 mg/kg) with 8-AG (50 mg/kg) was virtually as effective as therapy with 8-AG (100 mg/kg) alone. Moreover, Aza-AICA

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was more potent than AICA in combination with 8-AG. The mean survival time of the group injected intraperitoneally with Aza-AICA (40 mg/kg) 1 hr prior to 8-AG (50 mg/kg) was 20.1 ± 1.1 days and that of group injected with 8-AG (50 mg/kg) alone was 13.6 ± 0.4 days. Intraperitoneal administration of Monomethyl-TICA (15 mg/kg) alone, unlike treatment with AICA or Aza-AICA alone, prolonged the mean survival time of tumor-bearing mice by 5.4 days. Moreover, Monomethyl-TICA (15 mg/kg) in combination with 8-AG (50 mg/kg) prolonged the life-span of the tumor-bearing mice even more 10.7 days.

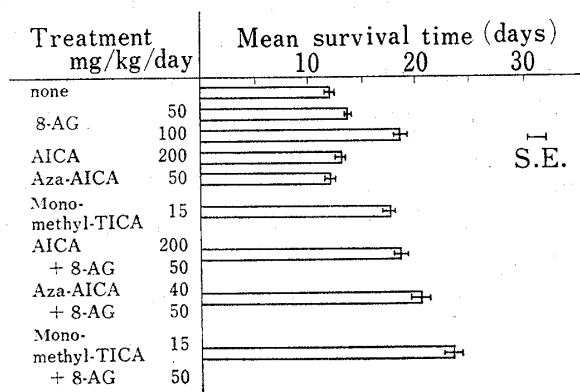


Fig. 2. Potentiating Effects of AICA Analogs on the Antitumor Activity of 8-AG against Ehrlich Ascites Carcinoma

Each bar represents the mean survival time of a group of 7 mice. AICA, Aza-AICA and Monomethyl-TICA were administered intraperitoneally 1 hr before 8-AG.
 AICA: 4(or 5)-aminoimidazole-5(or 4)-carboxamide
 Aza-AICA: 4(or 5)-aminotriazole-5(or 4)-carboxamide
 Monomethyl-TICA: 4(or 5)-monomethyltriazenoimidazole-5(or 4)-carboxamide
 8-AG: 8-azaguanine

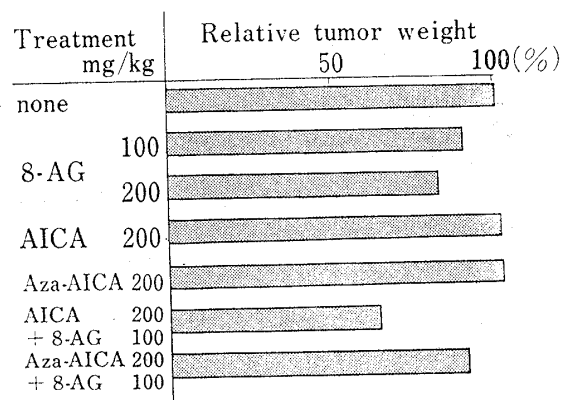


Fig. 3. Effect of AICA and Aza-AICA on the Antitumor Activity of 8-AG against Ehrlich Carcinoma (Solid Tumor)

Each bar represents the relative tumor weight of a group of 7 mice. The weight of tumor in the untreated control group was taken as 100%. AICA and Aza-AICA was administered intraperitoneally 1 hr before 8-AG.
 AICA: 4(or 5)-aminoimidazole-5(or 4)-carboxamide
 Aza-AICA: 4(or 5)-aminotriazole-5(or 4)-carboxamide

Effects of AICA and Aza-AICA on the Antitumor Activity of 8-AG against Solid Form of Ehrlich Carcinoma

The *in vivo* effects of several AICA analogs in combination with 8-AG on Ehrlich solid carcinoma are shown in Fig. 3 as ratios of the tumor weights on the 12th day after incubation. The potentiation of the carcinostatic activity of 8-AG by AICA or Aza-AICA seen with ascites carcinoma was not observed with the solid tumor.

Effects of AICA and Its Analogs on the Activity of Liver 8-Azaguanine Deaminase and Xanthine Oxidase in Normal Mice *in Vitro*

The effects of AICA and its analogs on the activity of liver 8-AG deaminase and xanthine oxidase in normal mice are shown in Fig. 4. It is evident that 8-AG deaminase catalyzes a metabolic change of 8-AG into non-carcinostatic 8-azaxanthine. AICA analogs which enhance the carcinostatic action of 8-AG, as shown in Fig. 2, might inhibit 8-AG deaminase activity, as 6-formylpteridine does.⁷⁾ Accordingly, the inhibitory effects of AICA analogs on 8-AG deaminase were tested. The result in Fig. 4 demonstrates that AICA and Monomethyl-TICA inhibit strongly 8-AG deaminase, while Aza-AICA had no inhibitory effect *in vitro* at a final concentration of 10^{-3} M or 10^{-4} M.

Dietrich and Shapiro⁶⁾ found that the carcinostatic action of 8-AG was potentiated by flavotin. They suggested that xanthine oxidase was inhibited by flavotin, resulting in a higher level of xanthine which would inhibit guanase. To test whether AICA and its analogs had a flavotin-like action, the effects of these compounds on the activity of liver xanthine oxidase in normal mice were tested manometrically *in vitro*. Neither

aqueous conditions.¹⁰⁾ Therefore, the effect of Monomethyl-TICA in potentiating activity *in vitro* seems similar to that of AICA. On the other hand, Aza-AICA potentiated the carcinostatic action of 8-AG without causing inactivation of 8-AG deaminase. Thus, it seems that the biochemical mechanisms of potentiation of the two AICA analogs on the action of 8-AG are not the same.

Dietrich and Shapiro⁶⁾ considered that potentiation of the action of 8-AG by flavotin could be ascribed to an inhibitory effect of the flavotin on xanthine oxidase, thereby indirectly inhibiting guanase by product inhibition. Unlike flavotin, AICA inhibits 8-AG deaminase, but not xanthine oxidase, while Aza-AICA inhibits neither 8-AG deaminase nor xanthine oxidase. Potentiation by AICA could be understood as due to inhibition of 8-AG deaminase. The mechanism of potentiation by Aza-AICA is not apparently due to that of AICA or flavotin, but it can not be explained clearly at present. The above conclusions are illustrated schematically in Fig. 5.

Acknowledgement Our thanks are due to Prof. Z. Horii of the Department of Medicinal Chemistry of this Faculty for providing 4(or 5)-monomethyltriazenoimidazole-5(or 4)-carboxamide.