Cancer Chemopreventive Activity of Odorine and Odorinol from Aglaia odorata

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In the course of our continuing search for novel cancer chemopreventive agents from natural sources, we have carried out a primary screening in vitro assay of the compounds isolated from Aglaia odorata. Consequently, aminopyrrolidine-diamides, odorine and odorinol, were obtained as active constituents. These compounds exhibited potent anti-carcinogenic effects in a two-stage carcinogenesis test of mouse skin induced by 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter. Further, both compounds showed remarkable inhibitory effects in two-stage mouse skin carcinogenesis models induced by nitric oxide (NO) donors such as (±)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide (NOR-1) or peroxynitrile as an initiator and TPA as a promoter. From these results, it was concluded that odorine and odorinol inhibited both the initiation and promotion stages of two-stage skin carcinogenesis.

Key words anti-carcinogenic activity; Aglaia odorata; aminopyrrolidine-diamide; odorine; odorinol

The leaves and flowers of Aglaia odorata Lour. (Meliacaeae) are used in China as a herbal remedy for treatment of bruises, vertigo, cough etc. and the flowers are also used for scenting tea on account of its fragrance. From this plant, the aminopyrrolidine-diamides odorine and odorinol (=2-hydroxyodorine), and other constituents, had been isolated. In addition, the anti-leukemic activity of odorinol against P-388 lymphocytic leukemia of mice has already been reported. As part of our search for possible cancer chemopreventive agents from natural sources, we have already reported a primary in vitro assay indicating the inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) activation in Raji cells induced by 12-O-tetradecanoylphorbol-13-acetate (TPA). Because chemical carcinogenesis is a two-stage or multi-stage process, including initiation, promotion, and progression stages, anti-tumor promoters and initiators have been regarded as most promising agents for the chemoprevention of cancer.

As a result, we have characterized the potent anti-tumor promoting activity of odorine and odorinol isolated from A. odorata. In the present study, we carried out in vivo two-stage carcinogenesis tests of these compounds on mouse skin using 7,12-dimethylbenz[a]anthracene (DMBA)/TPA since many compounds inhibiting EBV-EA activation have been shown to act as inhibitors of tumor promotion in in vivo systems. Further, on the basis of experimental proof that nitric oxide (NO) strongly initiated carcinogenesis on mouse skin, the inhibitory effects of odorine and odorinol on tumor-initiation by NO donors such as (±)-(E)-methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide (NOR-1) and peroxynitrile were also investigated.

MATERIALS AND METHODS

Chemicals DMBA and TPA were purchased from Nacalai Tesque (Kyoto, Japan). NOR-1 and peroxynitrile were obtained from Dojindo Laboratories (Kumamoto, Japan).

Animals Specific pathogen-free female ICR- (6-week-old) and SENCAR-mice (6-week-old) were obtained from Japan SLC Inc. (Shizuoka, Japan). These animals were housed, five per polycarbonate cage, in a temperature-controlled room at 24±2°C and given food and water ad libitum.

Isolation of Odorine and Odorinol from Aglaia odorata

Leaves of A. odorata (700 g) were harvested in January, 1993 in the Herbarium Bogoriense (Bogor, Indonesia). The ethyl acetate-soluble fraction was prepared (47.6 g from 78.0 g of the MeOH extract) and aminopyrrolidine-diamides, odorine (160 mg) and odorinol (230 mg), were isolated in 99.9% purity from the crude fraction by the previously reported method.

Two-Stage Mouse Skin Carcinogenesis Test Induced by DMBA/TPA

The animals (female ICR mice, 6-week-old) were divided into three experimental groups of 15 mice each. The back of each mouse was shaved with surgical clippers, and the mice were carcinogenically initiated with DMBA (100 μg, 390 nmol) in acetone (0.1 ml) as an initiation treatment. For group I (positive control group), one week after initiation with DMBA, mice were promoted by application with TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) twice a week. Groups II and III received a topical application of odorine (85 nmol) in acetone (0.1 ml) and odorinol (85 nmol) in acetone (0.1 ml) 1 h before each promotion treatment, respectively. The incidence of papillomas was observed weekly for 20 weeks; the percentage of mice bearing papillomas (Fig. 2A) and the average number of papillomas per mouse were...
recorded (Fig. 2B). The differences in mouse papillomas between control and the experiments were analyzed by means of the Student’s t-test at 20 weeks after promotion.

Two-Stage Mouse Skin Carcinogenesis Test Induced by NOR-1/TPA The animals (female SENCAR mice, 6-week-old) were divided into three experimental groups of 15 mice each. The back of each mouse was shaved with surgical clippers and the mice were carcinogenically initiated with NOR-1 (90 μg, 390 nmol) in acetone (0.1 ml) as an initiation treatment. For group I (positive control group), one week after initiation with NOR-1, mice were promoted by application with TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) twice a week. For groups II, odorine (0.0025%, 2.5 mg/100 ml drinking water) and III, odorinol (0.0025%, 2.5 mg/100 ml drinking water) were orally given from one week before to one week after the initiation treatment with NOR-1, and then promoted by application with TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) twice a week for 20 weeks, respectively. The incidence of papillomas was observed weekly for 20 weeks; the percentage of mice bearing papillomas (Fig. 3A) and the average number of papillomas per mouse were recorded (Fig. 3B). Differences in mouse papillomas between control and the experiments were analyzed by means of the Student’s t-test at 20 weeks after promotion.

Two-Stage Mouse Skin Carcinogenesis Test Induced by Peroxynitrite/TPA The animals (female SENCAR mice, 6-weeks-old) were divided into three experimental groups of 15 mice each. The back of each mouse was shaved with surgical clippers and the mice were topically initiated with peroxynitrite [(33.1 μg, 390 nmol in 1 mM NaOH) in acetone (0.1 ml)] as an initiation treatment. For group I (positive control group), one week after initiation with peroxynitrite, the mice were promoted by application with TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) twice a week. For groups II, odorine (0.0025%, 2.5 mg/100 ml drinking water) and III, odorinol (0.0025%, 2.5 mg/100 ml drinking water) were orally given from one week before to one week after the initiation treatment with peroxynitrite, and then promoted by application with TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) twice a week for 20 weeks, respectively. The incidence of papillomas was observed weekly for 20 weeks; the percentage of mice bearing papillomas (Fig. 4A) and the average number of papillomas per mouse were recorded (Fig. 4B). Differences in mouse papillomas between control and the experiments were analyzed by means of the Student’s t-test at 20 weeks after promotion.

RESULTS AND DISCUSSION

As shown in Fig. 2, aminopyrrolidine-diamide, odorinol remarkably delayed the formation of papillomas on mouse skin and exhibited a significant inhibitory effect on tumor-promotion induced by DMBA and TPA.

The positive control group, which received treatment with DMBA and TPA, showed a 100% incidence of papillomas (Fig. 2A: % of papilloma bearers) within 10 weeks. The animals which were treated with DMBA, TPA and odorinol showed less than 15% at 10 weeks, 15 weeks to show less than 70% and 20 weeks to show 93.3% papilloma formation. These tumor inhibitory effects were also seen as a reduction in the multiplicity of papillomas (Fig. 2B: the number of papillomas per mouse) over a 10 week period. In the positive control, 3.9, 8.1, 8.8 papillomas were formed per mouse after 10, 15, and 20 weeks of promotion, respectively. On the other hand, in the groups treated with DMBA, TPA, and odorinol, less than 0.3, 2.6 and 3.6 were formed per mouse under the same periods, respectively. Another aminopyrrolidine-diamide, odorine also delayed the formation of papillomas and reduced the average number of papillomas per mouse (4.3 at 20 weeks) but did not reduce the rate of papilloma-bearing mice at 20 weeks compared with the control group (see Fig. 2). Next, the inhibitory effects of these compounds on two-stage skin carcinogenesis using NO donors such as NOR-1 and peroxynitrite as an initiator, and TPA as a promoter, were investigated. Because it has been ascertained that the overproduction of NO induced damage at the gene, cell and tissue levels, and consequently NO strongly initiated mutagenesis and carcinogenesis. Since the original plant has gen-
erally been used by oral administration, the inhibitory effects of both compounds on two-stage skin carcinogenesis using NO donors were examined by the same oral administration methods. As a result, both compounds delayed the formation of papillomas and exhibited inhibitory effects by oral feeding, as shown in Fig. 3.

The positive control group, which received treatment with NOR-1 and TPA, showed a 100% incidence of papillomas (Fig. 3A: % of papilloma bearers) within 11 weeks. The animals which were treated with NOR-1, TPA and odorinol at 10 weeks showed less than 30% incidence, at 15 weeks showed 60%, and at 20 weeks showed 80.0% papilloma formation. Figure 3B shows papillomas per mouse and reduction in the number of papillomas per mouse. Thus for the positive control, 6.1 and 8.1 papillomas were formed per mouse after 15 and 20 weeks of promotion, respectively. In the group treated with odorinol, less than 2.6 and 3.3 (60% reduction compared with control) were formed per mouse after 15 and 20 weeks of promotion. The other compound odorine also exhibited inhibitory activity [86.6% papilloma bearers at 20 weeks; 3.9 papillomas per mouse at 20 weeks (see Fig. 3)]. In these experiments, no statistically significant difference was observed between each group in terms of mean intake of drinking water, and the increase in body weight of the treated mice was not affected by treatment with both compounds. Finally, Fig. 4 shows the inhibitory effects of odorine and odorinol on two-stage skin carcinogenesis using peroxynitrite as initiator by oral feeding. Again, both compounds delayed the formation of papillomas and exhibited inhibitory effects.

In the positive control group, which received treatment with peroxynitrite and TPA, a 100% incidence of papillomas
(Fig. 4A: % of papilloma bearers) was observed within 10 weeks. Animals which were treated with peroxynitrite, TPA and odorinol took 10 weeks to show less than 30% incidence, 15 weeks to show 60% and 20 weeks to show 86.7% incidence of papilloma formation. These tumor inhibitory effects were also seen as a reduction in the multiplicity of papillomas (Fig. 4B) and odorinol reduced the average number of papillomas per mouse (about 46% reduction at 20 weeks compared with the control). Odorine also showed inhibitory activity (93.3% papilloma bearers and 5.1 papillomas per mouse at 20 weeks, respectively) (see Fig. 4). Again, no statistically significant difference was observed between each group in terms of mean intake of drinking water. From the results of these two-stage carcinogenesis tests, it was concluded that odorine and odorinol inhibited both the initiation and promotion stages of two-stage skin carcinogenesis. The present cancer chemopreventive activity of odorine and odorinol together with the direct anti-leukemic activity of odorinol\(^3\) show that these compounds might be valuable anti-carcinogenic agents in chemical carcinogenesis. Investigations on the details of the inhibitory mechanisms of these compounds on chemical carcinogenesis are being studied.

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**REFERENCES**


