to zero. A number of smaller oscillations from the baseline immediately preceded and/or followed the large downward deflection. These oscillations may represent discharges of other electrically active units which are closely coupled to the large downward deflection. In some of the preparations examined, the electrical activity consisted of two tonically discharging action potentials. Each displayed the general shape described above. Their discharges were closely coupled and occurred within 5–60 ms of one another (fig. b).

The LNP is located in close proximity to the anterior region of the Y-organ, and the largest amplitudes were recorded at this location, thus verifying that the compound action potential was generated by the LNP. Moreover, various characteristics of this compound action potential can be correlated with known features of the LNP. For instance, the LNP is formed by the junction of several nerves, and serves as a neurohemal region containing terminals of NSCs whose cell bodies are located in the brain[9]. As seen in the present electrophysiological study, the action potential displayed a long duration indicative of NSC axons and terminals[15], and possessed a multi-component wave form reflecting the presence of a number of electrically active units. Transection of pathways linking the Y-organ to the brain also eliminated electrical activity indicating that the initiation of spontaneous activity occurred in regions outside the LNP. This result would be expected if the perikarya were located in the brain. The tonic discharge of the LNP differed from the discharge pattern of neurosecretory action potentials recorded from the sinus gland of the same animal (fig. c). Whereas electrical activity recorded from the sinus gland occurred in bursts with interburst frequencies as high as 90 Hz[12], the electrical activity associated with the Y-organ discharged in a slow tonic fashion ranging from 1 every 5 min to, at most, 40 per min. The tonic discharge suggests a sustained release of neurohormones. Such a release would be advantageous for a NSC whose role is to maintain a long-term inhibitory (or excitatory) control over the activity rate of an endocrine gland.

The above results demonstrate that electrical activity characteristic of NSCs can be recorded by an extracellular electrode placed near the Y-organ of terrestrial isopods. This activity eminates from the neurosecretory terminals in the LNP making it possible to compare the electrical activity of the LNP with that of the sinus gland which is also believed to release substances controlling Y-organ activity[16]. A description of the electrical activity of the LNP throughout the molt cycle will therefore provide additional insight into the neuroendocrine processes controlling molting in crustaceans.

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Effect of oridonin, a *Rabdosia* diterpenoid, on radiosensitization with misonidazole

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Summary. The radiosensitization brought about by oridonin, one of the *Rabdosia* diterpenoids, alone or in combination with misonidazole, was investigated in Chinese hamster V79 cells. The enhancement ratio of 1.92 was obtained when 0.01 mM oridonin and 1 mM misonidazole were administered to hypoxic cells under radiation. The enhancement ratios of oridonin and misonidazole for hypoxic cells were 1.16 and 1.59 respectively. Hence, a supra-additive effect was obtained by the combined treatment with these two drugs. Under aerobic conditions, no effect of 0.01 mM oridonin on the radiosensitization caused by misonidazole was observed.

Key words. Oridonin; *Rabdosia* diterpenoid; thiol-reactive agent; misonidazole; radiosensitizer.

Misonidazole (fig. 1, **1**) is known to be an effective radiosensitizer for hypoxic cells; clinical trials of the drug are being conducted in a number of countries throughout the world. In spite of a slight benefit in some tumor sites, the dose of misonidazole is generally too low to attain efficient sensitization of hypoxic tumor cells, because of its neurotoxicity[1,2]. In order to avoid the neurotoxic properties of misonidazole, there has been a great effort to develop new radiosensitizing drugs which are less lipophilic or more rapidly excreted[3,4]. Another appraoch has started; that is, to search for agents that will enhance the efficiency of existing radiosensitizers, hopefully without increasing their toxic side effects. This involves decreasing the endogenous radioprotective capacity by depleting glutathione (GSH) and other intracellular free thiols (e.g. cysteine), because such thiol compounds compete with radiosensitizers for repair or fixation of radiation-induced damage. Recently, several in vitro and in vivo studies have been reported in which diethyl maleate (DEM, a thiol alkylating agent) or buthionine sulphoximine (BSO, an inhibitor of GSH synthesis) has been used to deplete intracellular GSH; the radiosensitizing effectiveness of misonidazole has been improved by combining it with those drugs[5,6].

In 1970, Fujita's group[7] isolated a kaurene-type diterpene, oridonin (fig. 1, **2**), from *Rabdosia trichocarpa* KUDO (Labia-

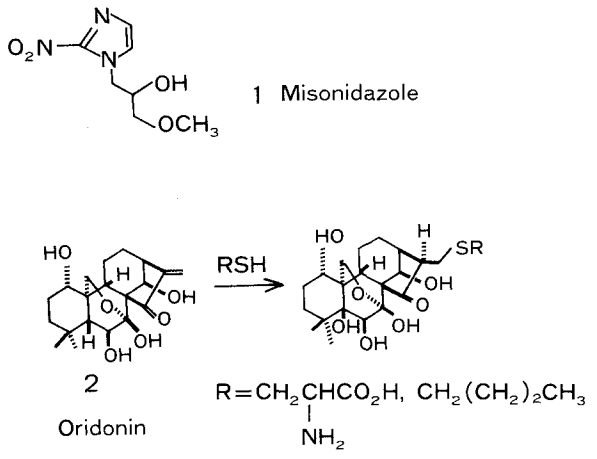

Figure 1. Chemical structures of misonidazole (**1**) and oridonin (**2**) and the product of its reaction with thiols[9].

tae) which has been used as a home remedy in Japan. Antitumor activity of compound **2** against Ehrlich ascites tumor and P 388 lymphocytic leukemia in mice was disclosed by the same group[8–11]. Oridonin proved to react with some thiol compounds such as L-cysteine and butane thiol under neutral conditions, yielding the corresponding thiol adducts (fig. 1)[9]. On the basis of previous studies[8–11] on oridonin, we anticipated that compound **2** would exhibit the same property of increasing the radiosensitivity of cells and improving the effect of hypoxic cell radiosensitizers as DEM or BSO. The radiosensitizing effect of oridonin and misonidazole on Chinese hamster V79 cells in vitro was therefore investigated, according to the method described previously[12]. Oridonin, obtained from the plant *Rabdosia trichocarpa* KUDO by a conventional isolation procedure, was employed. Misonidazole was supplied by Nippon Roche K. K. Test samples were added to 0.5 ml of the cell suspension ($3–4 \times 10^5$ cells/ml) in Eagle's minimum essential medium (MEM) in glass tubes. After hypoxic treatment of the medium by flushing with N_2+5% CO_2, it was irradiated with ^{60}Co γ-rays at a dose rate of 2.34 Gy/min at room temperature. In aerobic experiments, air+5% CO_2 was used instead of the N_2-CO_2 mixture. After irradiation, the medium was removed, and the cells were washed with Hanks' balanced salt solution, resuspended in MEM, and then diluted and plated in plastic dishes containing 6 ml of MEM+10% fetal bovine serum. The cells were incubated for 6 days at 37 °C. The surviving fraction was determined by the colony-formation method. Figure 2 shows the survival curves for oridonin-treated and untreated V79 cells at various concentrations irradiated in the absence or presence of oxygen. It is obvious that an increase in the concentration of oridonin caused a progressive increase in sensitization of both aerobically treated and hypoxic cells. Enhancement ratios (ER) for each concentration of oridonin under aerobic and hypoxic conditions are shown in table 1 and the ER for misonidazole under hypoxic conditions is also presented for comparison. The ER was calculated from the ratio of the radiation dose with and without the sensitizer required to reduce the surviving fraction to 1%. The ERs of 0.01, 0.025 and 0.05 mM oridonin for hypoxic cells were 1.16, 1.66 and 2.26, respectively. In the case of aerobically treated cells, the sensitizing effect of oridonin was slightly lower than on the hypoxic cells; the ERs of 0.01 and 0.0.5 mM oridonin for aerobic cells were 1.14 and 1.90, respectively. Under hypoxic conditions, the ER obtained with 1 and 5 mM misonidazole was similar to that obtained with 0.025 and 0.05 mM oridonin. This means the ER obtained with 5 mM misonidazole (2.20) could be achieved with only 1/100 of the concentration (0.05 mM) of oridonin. However, unfortunately the improvement in the oxygen enhancement ratio (OER) for cells by oridonin was slight; the OER for control cells in the 1% surviving fraction was 2.98, whereas the OER for cells treated with 0.01 and 0.05 mM oridonin was 2.92 and 2.50, respectively. Therefore we investigated the combination of hypoxic cell radiosensitizer with oridonin at a small cytotoxic dose level.

The cytotoxicity of oridonin was expressed in terms of the plating efficiency of unirradiated V79 cells. Under both aerobic and hypoxic conditions, no severe cell killing effect was observed when V79 cells were exposed to up to 0.025 mM

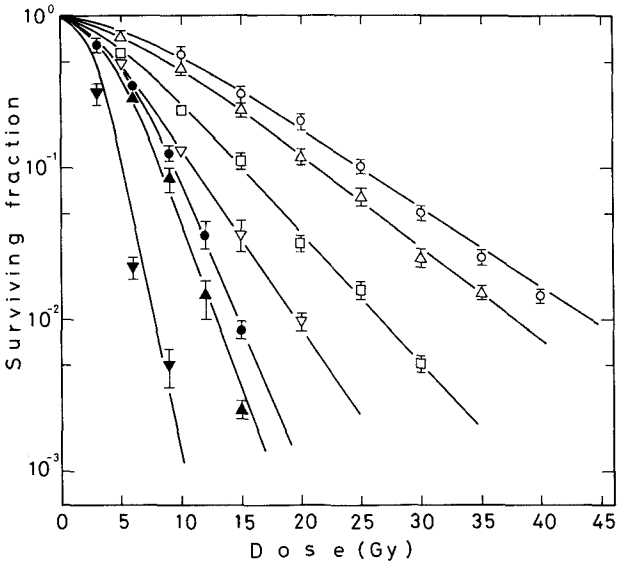

Figure 2. Survival curves of irradiated Chinese hamster V79 cells under hypoxic (open symbols) and aerobic (closed symbols) conditions. (○), hypoxic control; (△, ▲), 0.01 mM oridonin; (□), 0.025 mM oridonin; (▽, ▼), 0.05 mM oridonin; (●), aerobic control. Bars, SE (3 experiments).

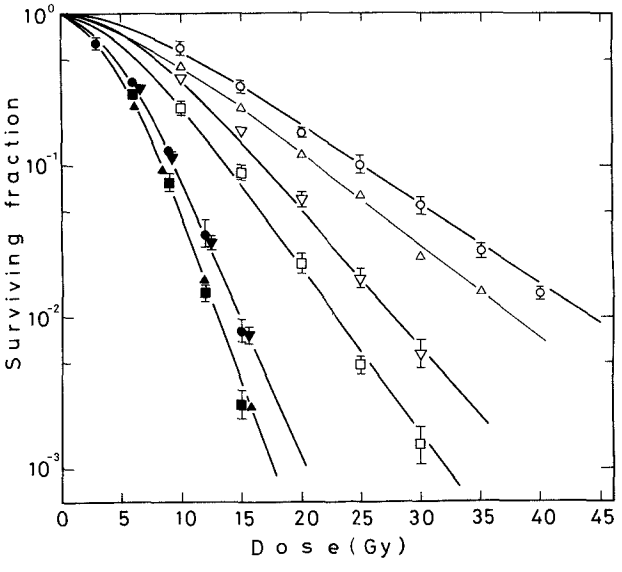

Figure 3. Survival curves of irradiated Chinese hamster V79 cells under hypoxic (open symbols) and aerobic (closed symbols) conditions. (○), hypoxic control; (▽, ▼), 1 mM misonidazole; (□, ■), 1 mM misonidazole +0.01 mM oridonin; (●), aerobic control. Misonidazole and oridonin were added simultaneously before aerobic or hypoxic treatment followed by irradiation. (△, ▲), 0.01 mM oridonin (redrawn from fig. 2). Bars, SE (3 experiments).

Table 1. Sensitizer enhancement ratios (at a surviving fraction of 1%)

Concentration (mM)	Oridonin Aerobic	Hypoxic	Misonidazole Hypoxic
0.01	1.14	1.16	-
0.025	-	1.66	-
0.05	1.90	2.26	-
0.1	-	-	1.13
0.5	-	-	1.40
1.0	-	-	1.59
5.0	-	-	2.22

Table 2. Sensitizer enhancement ratios after the combined treatment (at a surviving fraction of 1%)

	Aerobic	Hypoxic
Oridonin (0.1 mM)	1.14	1.16
Misonidazole (1 mM)	1.0	1.59
Oridonin+misonidazole	1.14	1.92

oridonin, but when the concentration was increased to 0.05 mM, severe cytotoxicity appeared and the plating efficiency decreased to around 0.1. On the basis of these results 0.01 mM oridonin was used in the combination experiment.

Figure 3 shows the survival curves for combined drug-treated and untreated V79 cells irradiated in the absence or presence of oxygen. The combination of 0.01 mM oridonin and 1 mM misonidazole resulted in greater sensitization than with either drug alone under hypoxic conditions. On the other hand, under aerobic conditions no sensitization was obtained by the same combination of those drugs. Table 2 summarizes the ERs calculated from figure 3. An ER of 1.92 was obtained when 0.01 mM oridonin and 1 mM misonidazole were administered to hypoxic cells with radiation. Since the ERs of oridonin and misonidazole for hypoxic cells were 1.16 and 1.59 respectively, a supra-additive effect was obtained by combined treatment with these two drugs. Because there are no differences in the ER between the combination of oridonin with misonidazole and oridonin alone under aerobic conditions, it is clear that 0.01 mM oridonin had no effect on the radiosensitization of aerobic cells by misonidazole.

As mentioned above, it is important to develop a potentiator to enhance the effect of a hypoxic cell radiosensitizer. The present results suggest the possibility that oridonin can be used as a bifunctional agent which has an antitumor effect as a potentiator of misonidazole. Therefore, we propose the hypothesis that improvement in the radiosensitizing effectiveness of misonidazole would be caused by depletion of intracellular glutathione and nonprotein and protein thiol after the treatment with oridonin. This hypothesis can be supported by the facts that the center of physiological activity of oridonin is considered to be the α-methylene cyclopentanone function[9] and that the appearance of the physiological activity may be due to the deactivation of the SH enzymes (DNA and RNA polymerase) by oridonin[10]. More detailed investigations, e.g. measurement of nonprotein and protein thiols, the time schedule of administration of the combination and also in vivo experiments, are needed to clarify whether or not oridonin has a therapeutic potential.

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Collagen synthesis by human bone marrow fibroblasts[1]

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Summary. Collagen synthesis was measured in fibroblast cultures derived from normal and acute lymphoblastic leukemia (ALL) bone marrow. Collagen production was higher in normal than in ALL fibroblasts. These cells elaborate type I and type III procollagens in a ratio that depends on cell density and whether cells originate from normal or ALL bone marrow.
Key words. Collagen; bone marrow; acute lymphoblastic leukemia; fibroblasts.

Bone marrow stromal cells and their products (growth factors and extracellular matrix) form a hemopoietic microenvironment for stem cell proliferation and differentiation[2]. Among extracellular matrix (ECM) components, collagen, apart from its structural role, has numerous developmental and physiological functions. As judged by studies with hemopoietic long-term culture systems, collagen possibly affects growth factor production or has a direct influence on the hemopoietic stem cell[3].

Recent data have shown that bone marrow obtained from patients with acute lymphoblastic leukemia (ALL) either at diagnosis or during therapy, have a population of damaged stromal cells with impaired growth capacity[4]. Improvement after successful therapy suggests reversible damage[5]. Whether the damaged population of stromal cells in ALL also presents an impaired production of ECM components is not known. The current studies were undertaken to assess and compare the production and nature of collagen synthe-