Antitumour Activity of Rhinacanthone against Dalton's Ascitic Lymphoma

Rathina Sabapathy THIRUMURUGAN,[a] S. KAVIMANI,[b] and Radhey Shyam SRIVASTAVA*,[a]

Department of Pharmaceutics, Institute of Technology, Banaras Hindu University,[a] Varanasi-221005, India and Department of Pharmacology, Pharmacy Division, Mother Teresa Institute of Health Sciences, (Govt. Pharmacy),[b] Gorimedu, Indira Nagar, Pondicherry-6, India. Received April 3, 2000; accepted July 13, 2000

The antitumour activity of Rhinacanthone (3,4-dihydro-3,3-dimethyl-2*H*-naphtho-[1,2-B] pyran-5,6-dione) has been evaluated against Dalton's ascitic lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time of tumour bearing mice and peritoneal cell count in normal mice was observed with respect to the control group. When these Rhinacanthone treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. After 14 d of inoculation, Rhinacanthone was able to reverse the changes in the haemotological parameters, protein and packed cellular volume consequent to tumour inoculation.

Key words Rhinacanthus nasutus; Dalton's ascitic lymphoma; haemotological study; life span

Rhinacanthone[1,2] (3,4-dihydro-3,3-dimethyl-2*H*-naphtho [1,2-B] pyran-5,6-dione) isolated from the aerial parts of Rhinacanthus nasutus[3,4] (Acanthaceae) has been reported for its antifungal activity against pyricularie oryzae with an ED_{50} value of 0.4 μg/ml. The methanolic extract of *Rhinacanthus nasutus* have shown significant cytotoxicity against P-388, A-549, HT-29 and HL-60 cell lines.[5—7] The present study was focussed on the evaluation of the antitumour activity of Rhinacanthone against Dalton's ascitic lymphoma in mice.

MATERIALS AND METHODS

Materials The aerial parts of Rhinacanthus nasutus (Acanthaceae) were collected from Alagarkoil Hills, Rajapalayam, Virudhunagar district of Tamilnadu (India). They were dried, pulverised and packed into a soxhlet apparatus (600 gm) and subjected to hot continuous percolation using petroleum ether (60—80°, 2.5 l) for 24 h. The extract was concentrated and treated with 10% sodium hydroxide (50 ml) and stored overnight. Then the aqueous alkali part was separated from petroleum ether part by using separating funnel, acidified with 2 N HCl and extracted with sufficient amount of solvent ether (3×100 ml). The solvent ether soluble part was concentrated and treated with hot hexane to separate hot hexane soluble part and hot hexane insoluble part. The hot hexane soluble part was concentrated, cooled and mixed with more amount of cold hexane and filtered. The cold hexane insoluble part was treated wih a further quantity of hexane which upon concentration and cooling yielded an orange coloured needle shaped crystals which was crystallised from hexane (56 mg, mp 150—151 °C). This compound was subjected to spectral studies and the results confirmed that the isolated compound was Rhinacanthone which has been already reported.[2] It was suspended in 2% gum tragacanth and used in the present study.

Animals Male Swiss albino mice (20—26 g) obtained from King Institute of preventive medicine, Guindy, Chennai were used throughout the experiment.

Cell Line Dalton's ascite lymphoma (DAL) cells were obtained by the courtesy of Cancer Research Centre (CRC), Adayar, Chennai, India (originally brought from Prof. G. Klein, Stockholm, Sweden) and given by intraperitoneal transplantation of 10^6 cells/mouse.[8,9]

Effect of Rhinacanthone on Survival Time[10] Animals were inoculated with 10^6 cells/mouse on day 0 and treatment with Rhinacanthone started 24 h after inoculation, at a dose of 10 mg/kg/d, *p.o.* (group A). The control group (group-B) was treated with the same volume of 2% gum tragacanth suspension. All treatments were carried for 9 d. Mean survival times (MST) of each group, containing 10 mice, were noted. The antitumour efficacy of Rhinacanthone (10 mg/kg/d, *p.o.* for 9 d) was compared with that of 5-fluorouracil (5-FU, 20 mg/kg/d, i.p. for 9 d). Survival times of treated group were compared with control group using the following equation.[11]

$$\text{increase of life} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

Tumour Cell Growth[10] Studies on *in vivo* tumour cell growth inhibition with Rhinacanthone were carried out under similar experiment conditions as stated above, using a dose of 10 mg/kg/d for 6 d. Animals were sacrificed on day 7 after transplantation and tumour cells were collected by repeated intraperitoneal wash with 0.9% sodium chloride. Viable tumour cell counts (Trypan blue test) were made with a haemocytometer.

Effect of Rhinacanthone on Normal Peritoneal Cells[10] Three groups of normal mice ($n=5$) were used for the study. One group was treated with 10 mg/kg *p.o.* of Rhinacanthone and the second group received the same treatment for 2 consecutive days. The untreated third group was used as control. Peritoneal exudate cells were counted 24 h after treatment of each treated group and compared with those of the untreated group.

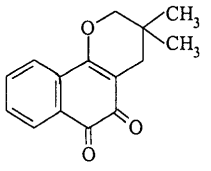

Fig. 1. Rhinacanthone

* To whom correspondence should be addressed.

Effect of Rhinacanthone on Haemotological Parameters[10] In order to detect the influence of Rhinacanthone on the haemotological status of DAL bearing mice, comparison was made amongst three groups ($n=5$) of mice on the 14th day after inoculation. The 3 groups comprised of (1) tumour bearing mice (2) tumour bearing mice treated with Rhinacanthone (10 mg/kg/d, *p.o.*) for 9 d and (3) normal mice. Blood was drawn from each mouse in the conventional way and the white blood cell count, red blood cell count (using a Neubauer counting chamber), haemoglobin (using Shali's acid haematin method), protein, differential count and packed cellular volume (using Wintrobe's method) were determined.[12—14] All the results were analysed by analysis of variance.[15]

RESULTS

The effect of Rhinacanthone on the survival of tumour bearing mice showed MST for the control group to be 23 d, while it was 36 and 40 d for the groups treated with Rhincanthone (30 mg/kg/d, *p.o.*) and 5-FU (20 mg/kg/d i.p.) respectively (Table 1).

The average number of peritoneal exudate cells per normal mouse was found to be ($6.2\pm0.70\times10^6$). Rhinacanthone (10 mg/kg) treatment increased the number of peritoneal cells as shown in Table 2. Single treatment enhanced the peritoneal cells to ($7.1\pm0.60\times10^6$) while two consecutive treatments enhanced the number to ($8.4\pm0.25\times10^6$).

Haemotological parameters (Table 3) of tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total white blood cells count, protein and packed cell volume were found to be increased with a reduction of the haemoglobin content of red blood cells. In a differential count of WBC, the percent of neutrophils increased while the lymphocyte count decreased. At the same time interval, Rhinacanthone (10 mg/kg/d, *p.o.*) treatment could change these altered parameters to near normal.

Table 1. Effect of Rhinacanthone Treatment on the Survival of Tumour Bearing Mice

Treatment	MST (d)	Life span (%)
Control	23±1.02	100.00
5-FU (20 mg/kg, i.p.)	40±0.67	173.91
Rhinacanthone (10 mg/kg, *p.o.*)	36±1.06*	156.52

* $p<0.001$ *vs.* control. Number of animals used=10 in each group. Days of drug treatment=9. Values were expressed as mean±S.E.

Table 2. Effect of Rhinacanthone (10 mg/kg, *p.o.*) Treatment on Enhancement of Peritoneal Cell Count in Normal Mice

Experiment	Number of peritoneal cells (1×10^6)/mouse
Control	6.2±0.70
Treated once	7.1±0.60
Treated twice on two consecutive days	8.4±0.25*

* $p<0.001$ *vs.* control. Number of animals used=5 in each group. Values were expressed as mean±S.E.

DISCUSSION

The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal[16] and reduction of WBC from blood.[17] The above results demonstrated the antitumour effect of Rhinacanthone against DAL in Swiss albino mice. A significant enhancement of MST was found. Dry treated tumour bearing mice shows the enhancement of number of lymphocytes that indicates the immunomodulatory effect. The harvested viable cells (Trypan blue method) after Rhinacanthone treatment showed morphological changes as revealed by the reduction in size of the cells.

To evaluate whether Rhinacanthone treatment indirectly inhibited the tumour cell growth, the effect of Rhinacanthone was examined on the peritoneal exudate of normal mice. Normaly each mouse contain about 5×10^6 intraperitoneal cells, 50% of which are macrophages. Rhinacanthone treatment was found to enhance peritoneal cell counts. When these Rhinacanthone treated animals underwent i.p. inoculation with DAL cells, tumour cell growth was found to be inhibited. These results demonstrated the indirect effect of Rhinacanthone on DAL cells, probably mediated through redox cycling and/or reaction of semiquinone with SH groups could participate in their activity against malignant cells.[18]

Analysis of the haemotological parameters showed minimum toxic effects in the mice treated with Rhinacanthone. After 14 d of transplantation, Rhinacanthone treated groups were able to reverse the changes in haemotological parameters consequent to tumour inoculation.

REFERENCES

1) Kodama O., Ichikama H., Akatsuka T., Santisopasri V., Kato A., Hayashi Y., *J. Nat. Prod.*, **56**, 292—294 (1993).
2) Kuwahara S., Awai N., Kodama O., *J. Nat. Prod.*, **58**, 1455—1458 (1995).
3) "The wealth of India," Raw Materials, Vol. IX, Publication and Information Directorate, CSIR, New Delhi, 1972, p. 6.
4) Chopra R. N., Nayar S. L., Chopra I. C., "Glossary of Indian Medicinal Plants," CSIR, New Delhi, 1957, p. 212.
5) Wu T. S., Tien H. J., Yeh M. Y., Lee K. H., *Phytochemistry*, **27**, 3787—3788 (1988).

Table 3. Effect of Rhinacanthone (10 mg/kg, *p.o.*) on Haemotological Parameters in Mice

	Hb (g%)	RBC (cells/ml×10^{10})	WBC (cells/ml×10^6)	Protein (g%)	PCV (mm)	Differential count %		
						Lymphocytes	Neutrophils	Monocytes
Normal mice	14.2±0.42	1.30±0.22	7.2±0.44	8.6±0.4	15.2±0.64	64±1.3	30±2.1	6±0.13
Tumour bearing mice (14 d)	12.1±0.34	1.12±0.08	14.1±0.87	13.7±0.3	32.0±0.51	42±1.5	54±1.6	4±0.07
Treated tumour bearing mice	12.8±0.39	1.32±0.16*	8.8±0.63*	9.1±0.5*	17.6±0.83	58±1.6	35±1.3	7±0.08

* $p<0.001$ *vs.* control. Number of animals=5 in each group. Days of drug treatment=9.

6) Wu T. S., Hsu H. C., Wu P. L., Leu Y. L., Chan Y. Y., Chern C. Y., Yeh M. Y., Tien H. J., *Chem. Pharm. Bull.*, **46**, 413—418 (1998).
7) Wu T. S., Hsu H. C., Wu P. L., Teng C. M., Wu. Y. C., *Phytochemistry*, **49**, 2001—2003 (1998).
8) Majumdar U. K., Gupta M., Maiti S., Mukherjee M., *Indian J. Expt. Biol.*, **35**, 473—477 (1997).
9) Gothaskar S. V., Ranadive K. J., *Indian J. Expt. Biol.*, **9**, 372—375 (1971).
10) Sur P., Ganguly D. K., *Planta Med.*, **60**, 106—110 (1994).
11) Umadevi P., Emerson Solomon F., Shavada A. C., *Indian J. Expt. Biol.*, **32**, 523—527 (1997).
12) D'Amour F. E., Blood F. R., Belden D. A., "Manual for Laboratory work in Mammalian Physiology," 3rd ed., The University of Chicago Press, 1965, pp. 148—150.
13) Dacie J. V., "Practical Haemotology," 2nd ed., J & A Churchill Ltd., London, 1958, pp. 38—42.
14) Lowry O. H., *J. Biol. Chem.*, **193**, 265—267 (1951).
15) Armitage P., "Statistical Methods in Medical Research," Blackwell Scientific Publication, 1971, pp. 217—219.
16) Clarkson B. D., Burchenal J. H., *Prog. Clin. Cancer*, **1**, 625—629 (1965).
17) Feringer L. G., Mider D. B., "Advances in Cancer Res. II," Academic Press, New York, 1954, pp. 406—410.
18) Powis G., *Free Rad. Biol. Med.*, **6**, 63 (1989).