thyroid hormone (PTH) secretion and PTH-independent diminution of tubular Ca reabsorption by WR-2721, a unique hypocalcemic agent. *J Clin Invest* 1985, **76**, 1854–1856.
10. Hirschel-Scholtz S, Caverzasio J, Rizzoli R, Bonjour JP. Normalization of hypercalcemia associated with a decrease in renal calcium reabsorption in Leydig cell tumor-bearing rats treated with WR-2721. *J Clin Invest* 1986, **78**, 319–322.
11. Fiske C, Subbarow T. The colorimetric determination of phosphorus. *J Biol Chem* 1925, **66**, 375–400.
12. Taussky HH. A microcolorimetric determination of creatinine in urine by the Jaffé reaction. *J Biol Chem* 1954, **208**, 853–861.
13. Rizzoli R, Fleisch H. The Walker 256/B carcinosarcoma in thyroparathyroidectomized rats: a model to evaluate inhibitors of bone resorption. *Calcif Tissue Int* 1987, **41**, 202–207.
14. Ralston SH, Gardner MD, Jenkins AS, McKillop JH, Boyle IT. Malignancy-associated hypercalcaemia: relationship between mechanisms of hypercalcaemia and response to antihypercalcaemic therapy. *Bone Mineral* 1987, **2**, 227–242.
15. Harinck HIJ, Bijvoet OLM, Plantingh AST, *et al.* Role of bone and kidney in tumor-induced hypercalcemia and its treatment with bisphosphonate and sodium chloride. *Am J Med* 1987, **82**, 1133–1142.
16. Rizzoli R, Caverzasio J, Chapuy MC, Martin TJ, Bonjour JP. Role of bone and kidney in parathyroid hormone-related peptide-induced hypercalcemia in rats. *J Bone Mineral Res* 1989, **4**, 759–765.
17. Attie MF, Fallon D, Spar B, Wolf JS, Slatopolsky E, Goldfarb S. Bone and parathyroid inhibitory effects of S-2(3-aminopropylamino)etylphosphorothioic acid. *J Clin Invest* 1985, **74**, 1191–1197.
18. Weiss J, Walker T, Fallon M, Goldfarb S. *In vivo* and *in vitro* effects of WR-2721 in experimental hypercalcemia in the rat. *J Pharmacol Exp Ther* 1986, **238**, 969–973.
19. Hirschel-Scholz S, Paunier L, Bonjour JP. Interference of WR-2721 with magnesium metabolism: Mechanism of action. *Mineral Electrolyte Metab* 1988, **14**, 114–120.
20. Klahr S, Hruska K. Effects of parathyroid hormone on the renal reabsorption of phosphorus and divalent cations. In: Peck WA, ed. *Bone Mineral Res* Amsterdam, Elsevier, 1983, 65–124.
21. Massry SG, Coburn JW, Chapman LW, Kleeman CR. Effect of NaCl infusion on urinary Ca^{++} and Mg^{++} during reduction in their filtered loads. *Am J Physiol* 1967, **213**, 1218–1224.
22. Bonjour JP, Rizzoli R. Pathophysiological aspects and therapeutic approaches of tumoral osteolysis and hypercalcemia. In: Brunner KW, Fleisch H, Senn H, eds. *Recent Results in Cancer Research*. Berlin, Springer, 1989, 29–39.

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Induction of Natural Killer Activity by Xanthenone Analogues of Flavone Acetic Acid: Relation with Antitumour Activity

Lai-Ming Ching, Wayne R. Joseph, Li Zhuang, Graham J. Atwell, Gordon W. Rewcastle, William A. Denny and Bruce C. Baguley

Flavone-8-acetic acid (FAA) induces haemorrhagic necrosis and tumour regression in experimental tumours and induces natural killer (NK) activity. Xanthenone-4-acetic acid (XAA) forms the basis of a series of analogues of FAA which vary in antitumour potency. FAA, XAA and 15 XAA derivatives were tested for their ability to induce either NK activity in mouse spleens or haemorrhagic necrosis in mouse colon 38 tumours. Some derivatives were active in both assays (one at a dose 8-fold lower than that of FAA). When both assays were quantitated, a significant correlation ($r = 0.85$; $P < 0.001$) was found. NK assays could be useful in screening compounds such as FAA and XAA analogues which appear to mediate their antitumour activity by biological response modification. Since tumour necrosis may not be mediated directly by NK cells, FAA and active XAA derivatives may exert pleiotropic effects that include NK induction and tumour necrosis by acting on host cells to release cytokines.
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INTRODUCTION

FLAVONE-8-ACETIC ACID (FAA) is a synthetic flavonoid with high activity against several tumours in mice [1, 2] but low clinical antitumour activity [3]. The mechanism of action of FAA appears to be indirect and different from that of conventional agents [4]. FAA acts as a biological response modifier, inducing natural killer (NK) activity in mouse spleen [5] and other organs [6], as well as in human peripheral blood [7]. It induces cytokine synthesis in the mouse [8–10], and it is likely that FAA activates pleiotropic host cell mechanisms, which include tumour cell lysis, cytokine production and NK induction. To determine whether the induction of NK activity and of tumour necrosis are part of the same pleiotropic response, it would be useful to correlate the two effects with structurally similar FAA analogues differing from highly active to inactive. Such a series is provided by derivatives of xanthenone-4-acetic acid (XAA, Fig. 1), a drug containing a fused tricyclic pharmacophore which has similar effects to those of FAA against the murine colon 38 tumour [11]. Our studies on XAA derivatives have identified 5-methyl-XAA, a drug with similar activity to FAA at approximately one-eighth of the dose, and other XAA derivatives which vary from

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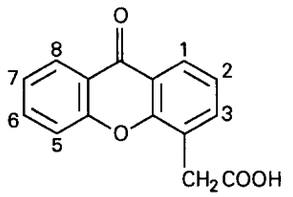

Fig. 1. Structure of XAA.

highly active to inactive [11, 12]. In the present study we have measured splenic NK activity and tumour necrosis after treatment with 15 of these XAA derivatives.

MATERIALS AND METHODS

Drugs

FAA, obtained from the National Cancer Institute, Bethesda, through the courtesy of Dr K. Paull, was dissolved in 5% sodium bicarbonate. Sodium salts of XAA and analogues were synthesised as described [11, 12] and were dissolved in water.

Measurement of NK activity

BDF_1 hybrid mice (C57BL/6J × DBA/2J) were bred under constant temperature and humidity with sterile bedding and food and following institutional animal ethical guidelines. Mice were killed by cervical dislocation and spleen cells were collected and assayed for NK activity in a standard 4 h ^{51}Cr-release assay [5] in α-MEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 50 μmol/l 2-mercaptoethanol. The target cell used was the YAC-1 lymphoma cell line obtained through the courtesy of Dr J. Marbrook, Department of Molecular Medicine, Auckland Medical School, and maintained in culture in RPMI 1640 (Gibco) supplemented with 10% FBS, 50 μmol/l 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin sulphate. Quadruplicate wells in V-bottom 96-well plates (Linbro, Flowlabs) containing ^{51}Cr-labelled target cells (5×10^3 cells per well), and spleen cells at effector to target cell (E:T) ratios of 400:1, 200:1 and 100:1 in 0.2 ml were incubated for 4 h at 37°C. Supernatant (0.1 ml) was removed and the amount of radioactivity was measured in a gamma counter (LKB Wallac 1270 Rackgamma II). Percent lysis was calculated as ([experimental release–spontaneous release] × 100)/total–spontaneous release.

Histological assessment of tumour necrosis

BDF_1 mice with subcutaneous tumours 4–12 mm in diameter (8–10 days after tumour implantation) were injected with a single intraperitoneal drug dose. Mice were killed by cervical dislocation 24 h later and tumours were removed, cut into 2 mm slices and fixed in 10% formalin. Fixed tumours were embedded in paraffin wax and sections through the maximum diameter of the tumour were stained with haematoxylin and eosin. Slides were coded and assessed [13] without the identity of the drug used being known. A grid marked at 0.4 mm intervals was placed over the slide and the intersections were scored as either undamaged or necrotic. Grid intersections on blood vessels or other non-tumour elements were ignored. The percentage necrosis was the number of grid intersections showing necrotic tumour divided by the total number of intersections counted. A mean of five treated tumours was used for each dose of drug and the results were averaged.

RESULTS

NK-stimulating activity of XAA analogues

XAA and XAA analogues with a methyl substituent at each of the 7 possible positions available for substitution were evaluated for their ability to stimulate NK activity *in vivo*. Since NK levels are elevated within 4 h after FAA treatment and remain elevated to the same extent for 24 h [5], mice were treated with the drugs at varying concentrations up to the maximum tolerated dose (MTD) and splenic NK activity against YAC-1 tumour targets was measured 16–18 h later. Each analogue was examined in a single assay, and activity was determined in quadruplicate at three E:T ratios with spleen cells pooled from two mice for each dose. Analogues were tested at least twice and representative dose-response activities at 200:1 E:T are compiled in Fig. 2. Analogues with the methyl group at positions 2, 7 and 8 did not enhance NK activity at any dose tested, while analogues with methyl groups at 1, 3, 5 or 6, as well as the parent XAA, all stimulated NK activity significantly above that of control cultures ($P < 0.05$). Above an optimal dose, NK activity decreased with increasing drug dose, and at the maximally tolerated (non-lethal) dose, NK levels were generally lower than those of untreated controls. 5-methyl XAA was the most potent analogue with an optimal NK-stimulating dose of 44 mg/kg,

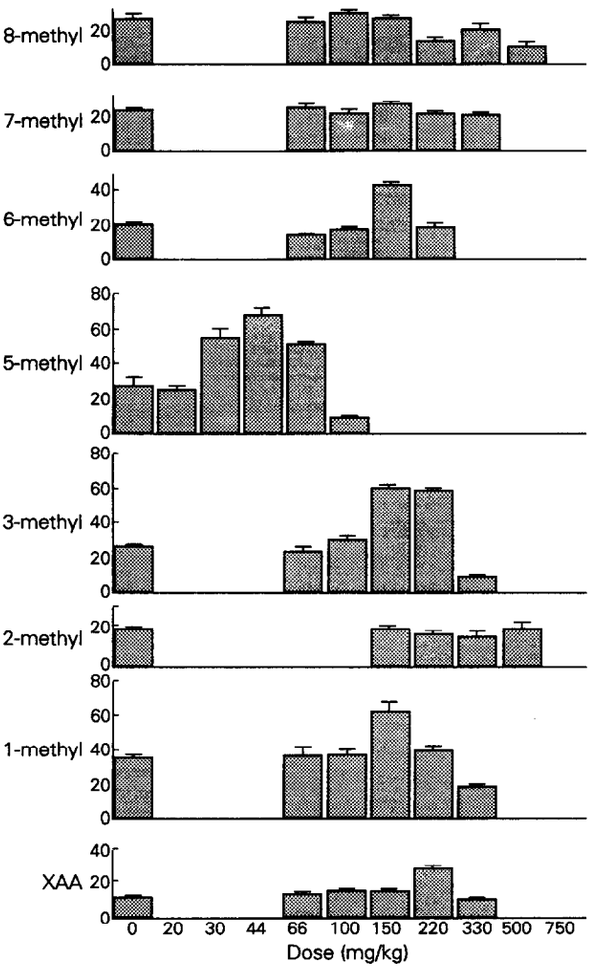

Fig. 2. Enhancement of NK activity by methyl-substituted analogues of XAA. Mean percent lysis at 200:1 E:T are shown on ordinate; error bars = S.E. of quadruplicate cultures.

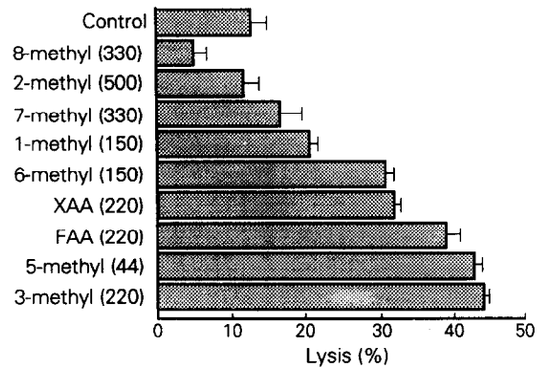

Fig. 3. Rank order of methyl-substituted XAA derivatives elevating NK activity at their respective optimal dose. Dose shown in brackets (mg/kg). Percent lysis at 200:1 E:T ratio (mean and S.D of quadruplicate wells).

compared with 150 mg/kg for 1-methyl, 3-methyl and 6-methyl XAA, and 220 mg/kg for the parent XAA. The percent lysis values from ^{51}Cr-release assays, while giving a relative measure of NK activity in the effector population, were also dependent on the index of ^{51}Cr-labelling and the rate of ^{51}Cr-release by the target cells, which varied from assay to assay. Thus, comparison of values could be made only for groups within the same assay and against controls for that assay. We therefore ranked the active analogues for their NK-enhancing ability at their respective optimal doses, as determined from Fig. 2 in a single assay. Analogues that did not show NK-enhancing activity were tested at an arbitrary dose slightly lower than the MTD, since NK levels tended to be suppressed by active compounds at the MTD. The results of one such experiment is shown in Fig. 3. The 5-methyl and 3-methyl derivatives of XAA stimulated NK activity to a higher degree ($P < 0.05$) than the parent XAA and the degree of lysis at the optimal dose was approximately three times that of untreated controls.

Since 5-methyl XAA was the most potent of the monomethyl derivatives, we examined the NK-stimulating activity of other 5-substituted analogues. The optimal dose was first determined in individual experiments as with the methyl analogues, and the compounds were then ranked at their optimal dose (Fig. 4). The 5-aza derivative was found not to stimulate NK activity while the 5-chloro, 5-ethoxy, 5-ethyl and 5-methoxy derivatives, like the 5-methyl derivative, showed significant ($P < 0.05$) activity

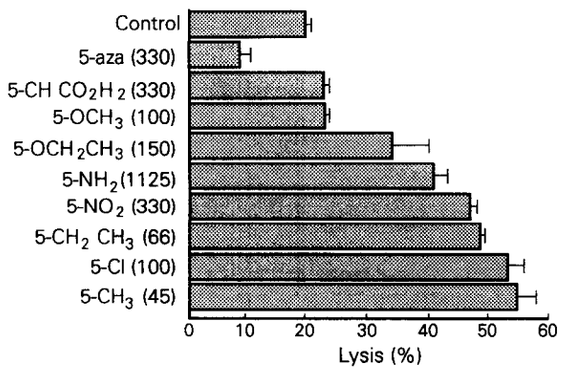

Fig. 4. Rank order of 5-substituted XAA derivatives in elevating NK activity at their respective optimal doses. NK activity was measured under same conditions as in Fig 3.

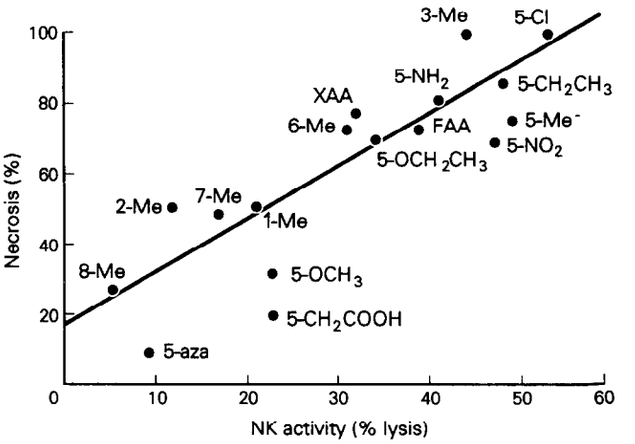

Fig. 5. Relation between induction of haemorrhagic necrosis in subcutaneous colon 38 tumours and induction of NK in mice for FAA and a series of XAA derivatives administered at the optimal NK-stimulating dose (compare with Figs 2 and 6). Average percentage necrosis in histological sections of colon 38 tumours treated 24 h previously with drug are plotted against average NK activity of spleen cells determined after 18 h at 200:1 E:T against YAC-1 targets.

at lower doses than that for XAA. The ethyl and chloro derivatives showed high activity while other compounds showed significant activity at doses the same as or higher than the optimal dose for XAA (Fig. 4).

Correlation between NK-stimulating and antitumour activity

Since XAA derivatives vary considerably in their antitumour activity as measured either by histological assessment of tumours at 24 h or by growth delay [11, 12], as well as in their ability to enhance NK activity, we tested for correlation between the two activities. For each of the analogues, existing data for the degree of tumour necrosis scored 24 h after drug treatment (in some cases updated by the inclusion of further results) were compared with those for splenic NK activity (Figs 3 and 4). Data for the degree of necrosis were averaged from at least five mice since the variation between animals was high. A significant coefficient of linear regression ($r = 0.85$; $P < 0.001$) between the two variables was obtained (Fig. 5).

We also determined the NK response of a subset of these compounds (the methyl derivatives) in tumour-bearing mice. Mice bearing subcutaneous colon 38 tumours implanted 10 days earlier were treated intraperitoneally with these derivatives at the same doses as those used in Fig. 3. Mice were killed after 18 h, and both splenic NK activity and tumour haemorrhagic necrosis were assessed. NK activity in tumour-bearing mice was significantly correlated with NK activity in normal mice ($r = 0.90$; $P < 0.001$). Moreover, even though some differences were found in the degrees of NK activity and tumour necrosis in comparison with the previous data (compare Figs 5 and 6), the two measurements were significantly correlated ($r = 0.87$; $P < 0.01$).

DISCUSSION

Derivatives of XAA, previously shown to have FAA-like activity against advanced solid tumours [11, 12], have been shown here to induce NK activity. As with antitumour activity, small changes in drug structure result in large changes in biological activity. Such a series of compounds is of great value in evaluating the relation between different biological effects. Substitution with a methyl group at positions 2, 7 or 8 renders

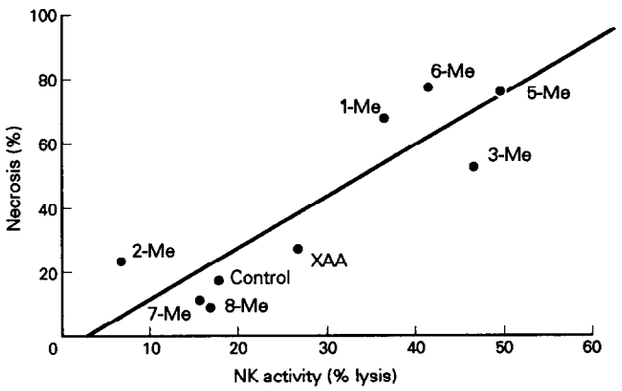

Fig. 6. Comparison of NK response and tumour response to methyl-substituted analogues in the same mice. Mice (4 per group) bearing colon 38 tumours were treated with XAA analogues at doses used for experiment in Fig. 3, and splenic NK activity and tumour necrosis were measured after 18 h.

the compound inactive (Fig. 2), whereas substitution with a methyl group at positions 3 or 5 results in analogues more active than the parent XAA and which enhance NK activity to three times that of the control level (Fig. 3). 5-methyl-XAA was the most potent of all the mono-substituted analogues tested with an optimal NK-stimulating dose of 44 mg/kg compared with 220 mg/kg for FAA or XAA.

Using a grid counting technique to quantitate tumour histology [13], we have obtained a high degree of correlation between the induction of tumour necrosis and the induction of splenic NK activity. The correlation was obtained with NK activity measured from pooled spleen cells and averaged necrosis data (Fig. 5) or from tumour-bearing mice in a single experiment (Fig. 6). One possible explanation for such correlation is that tumour necrosis is mediated directly by NK lymphocytes. However, as discussed elsewhere [14, 15], this mechanism is highly unlikely, at least for FAA. An alternative explanation, and our current working hypothesis, is that these compounds can stimulate macrophages to release various cytokines and other products which then mediate tumour necrosis [4, 13], immune-modulation [5, 6, 8, 14, 16] and vascular effects [17, 18]. Data consistent with this hypothesis are available for FAA, where it has been shown that FAA stimulates macrophage responses directly [16, 19, 20] and that after FAA treatment levels of interferons [8, 9] and tumour necrosis factor α are elevated in the serum of mice [10]. Further, elevation of NK activity *in vivo* after FAA treatment is mediated by the production of interferons [8]. Some of the active XAA analogues, shown here to enhance NK activity, have also been shown to induce the synthesis of messenger RNA for tumour necrosis factor α and the interferons (Dr R.H. Wiltrout, Laboratory of Experimental Immunology, Frederick Cancer Research Facility). Thus, the antitumour potential of these compounds may depend on their ability to modulate macrophage function. Assays involving the inhibition of tumour cell proliferation or tumour colony growth after *in vitro* exposure to drugs have been used successfully to determine the activity of conventional directly cytotoxic antitumour agents [21]. However, when such *in vitro* assays are applied to FAA, the data obtained do not correlate with *in vivo* antitumour effects [4, 22]. Thus, alternative *in vitro* systems need to be developed for compounds with an indirect mechanism of antitumour activity. The correlation between NK elevation and tumour necrosis (Figs 5 and 6) suggests that an assay for immune modulation might predict antitumour activity. As mentioned above, the elevation of NK activity by FAA *in vivo* is mediated by the interferons [8] and the synthesis of mRNA for a number of cytokines can be induced *in vitro* by FAA [10]. Thus, assays of cytokine induction could be used for the *in vitro* screening of compounds which depend on host biological response modification for their antitumour activity. We are developing *in vitro* systems based on mouse models [16, 20], which will permit the examination of the response of human macrophages to these drugs. These systems should provide an *in vitro* assay useful for the selection of analogues worthy of clinical evalution.

1. Plowman J, Narayanan VA, Dykes D, *et al.* Flavone aetic acid: a novel agent with preclinical antitumor activity against colon adenocarcinoma 38 in mice. *Cancer Treat Rep* 1986, **70**, 631–638.
2. O'Dwyer PJ, Shoemaker D, Zaharko DS, *et al.* Flavone acetic acid (LM 975, NSC 347512), a novel antitumor agent. *Cancer Chemother Pharmacol* 1987, **19**, 6–10.
3. Kerr DJ, Maughan T, Newlands E, *et al.* Phase II trials of flavone acetic acid in advanced malignant melanoma and colorectal cancer. *Br J Cancer* 1989, **60**, 104–106.
4. Finlay GJ, Smith GP, Fray LM, Baguley BC. Effect of flavone acetic acid (NSC 347512) on Lewis lung carcinoma, evidence for an indirect effect. *J Natl Cancer Inst* 1988, **80**, 241–245.
5. Ching L-M, Baguley BC. Induction of natural killer cell activity by the antitumour compound flavone acetic acid (NSC 347512). *Eur J Cancer Clin Oncol* 1987, **23**, 1047–1050.
6. Wiltrout RH, Boyd MR, Back TC, Salup RR, Arthur JA, Hornung RL. Flavone-8-acetic acid augments systemic natural killer cell activity and synergizes with IL-2 for treatment of murine renal cancer. *J Immunol* 1988, **140**, 3261–3265.
7. Urba WJ, Longo DL, Lombardo FA, Weiss RB. Enhancement of natural killer activity in human peripheral blood by flavone acetic acid. *J Natl Cancer Inst* 1988, **80**, 521–525.
8. Hornung RL, Young HA, Urba WJ, Wiltrout RH. Immunomodulation of natural killer cell activity by flavone acetic acid, occurrence via induction of interferon α/β. *J Natl Cancer Inst* 1988, **80**, 1226–31.
9. Hornung RL, Back TC, Zaharto DS, Urba WJ, Longo DL, Wiltrout RH. Augmentation of natural killer (NK) activity, induction of interferon and development of tumor immunity during the successful treatment of established murine renal cancer using flavone acetic acid (FAA) and interleukin 2. *J Immunol* 1988, **141**, 3671–3679.
10. Mace KF, Hornung RL, Wiltrout RH, Young HA. Induction of cytokine expression *in vivo* by flavone acetic acid: strict dose dependency and correlation with therapeutic efficacy against murine renal cancer. *Cancer Res* 1990, **50**, 1742–1747.
11. Rewcastle GW, Atwell GJ, Baguley BC, Calveley SB, Denny WA. Potential antitumor agents. 58. Synthesis and structure–activity relationships of substituted xanthenone-4-acetic acids active against the colon 38 tumor *in vivo*. *J Med Chem* 1988, **32**, 793–799.
12. Atwell GJ, Rewcastle GW, Baguley BC, Denny WA. Potential antitumor agents. 60. Relationships between structure and *in vivo* colon 38 activity for 5-substituted xanthenone-4-acetic acids. *J Med Chem* 1989, **33**, 1375–1379.
13. Baguley BC, Calveley SB, Crowe KK, Fray LM, O'Rourke SA, Smith GP. Comparison of the effects of flavone acetic acid, fostriecin and homoharringtonine and tumour necrosis factor α on colon 38 tumors in mice. *Eur J Cancer Clin Oncol* 1989, **25**, 263–269.
14. Ching L-M, Baguley BC. Effect of flavone acetic acid (NSC 347512) on splenic cytotoxic effector cells and their role in tumour necrosis. *Eur J Cancer Clin Oncol* 1989, **25**, 821–828.
15. Ching L-M, Baguley BC. Reduction of cytotoxic effector cell activity in Colon 38 tumours following treatment with flavone acetic acid. *Eur J Cancer Clin Oncol* 1989, **25**, 1061–1065.
16. Ching L-M, Baguley BC. Enhancement of *in vitro* toxicity of mouse peritoneal exudate cells by flavone acetic acid (NSC 347512). *Eur J Cancer Clin Oncol* 1988, **24**, 1514–1526.
17. Bibby MC, Double JA. Loadman PM. Reduction of tumor blood

flow by flavone acetic acid: a possible component of therapy. *J Natl Cancer Inst* 1989, **81**, 216–220.
18. Zwi LJ, Baguley BC, Gavin JB, *et al.* Blood flow failure as a major determinant in the antitumor action of flavone acetic acid (NSC 347512). *J Natl Cancer Inst* 1989, **81**, 1005–1013.
19. Ching L-M, Baguley BC. Hyporesponsiveness of macrophages from C3H/HeJ (endotoxin-resistant) mice to the antitumour drug flavone acetic acid (NCS 347512). *Eur J Cancer Clin Oncol* 1989, **25**, 1513–1515.
20. Thomsen LL, Ching L-M, Baguley BC. Evidence for the production of nitric oxide by activated macrophages treated with the antitumour agents flavone-8-acetic acid and xanthenone-4-acetic acid. *Cancer Res* 1990, **50**, 6966–6970.
21. Finlay GJ, Baguley BC, Wilson WR. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. *Anal Biochem* 1984, **139**, 272–277.
22. Schroyens WA, Dodion PF, Sanders C, *et al. In vitro* chemosensitivity testing of flavone acetic acid (LM 975; NSC 347512) and its diethylaminoethyl ester derivative (LM 985; NSC 293015). *Eur J Cancer Clin Oncol* 1987, **23**, 1135–1139.

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Incidence of Cancer of the Respiratory and Upper Digestive Tract in Urban and Rural Eastern Austria

Herwig Swoboda and Hans-P. Friedl

The incidence of head and neck, oesophagus and lung cancer between 1981 and 1985 was studied in Eastern Austria for an urban–rural division. In males, rural rates of oral cavity, oropharynx and oesophagus tumours were higher than urban rates. For lung tumours, urban rates slightly exceeded rural rates. In females, the incidence of oral cavity, oropharynx, larynx, hypopharynx, oesophagus and lung cancer showed an urban predominance, steepest for head and neck and oesophagus cancers. Cancer of the oral cavity, pharynx, larynx, oesophagus and lung had a high male preponderance.
Eur J Cancer, Vol. 27, No. 1, pp. 83–85, 1991.

INTRODUCTION

NEOPLASMS IN the ear, nose and throat represent a minor part of all malignant tumours. Because of exogenous risk factors acting on some sites, such neoplasms deserve epidemiological analysis. We report regional patterns of incidence of cancer of the respiratory and digestive tract above the diaphragm in Eastern Austria (Vienna, Lower Austria and Burgenland, about 3.2 million inhabitants) for 1981–1985.

METHODS

Incidence values were calculated from data collected by the Austrian Cancer Registry [1–3]. The Austrian census of 1981 served for regional allocation, classifying communities with more than 2000 inhabitants as "urban" [1]. Although the mortality/incidence ratios [M/I] are high—because it was not possible to include death certificate only cases in the registry until 1985—the differences between the subparts of Eastern Austria are insignificant. This means that the coverage can be estimated as uniform for the whole region (Table 1).

Oral cavity and oropharynx (ICD/9 141, 143–146, 149), and larynx and hypopharynx (ICD 161, 148) were grouped into two major sites. The other sites analysed were oesophagus (ICD 150) and trachea, bronchi and lungs (ICD 162).

Incidences were calculated as crude, age-standardised and truncated standardised rates with the standard population

Table 1. Regional comparison of population data (1981 *census*) *and indices of cancer registry quality. New cases* 1981–1985 (*ICD*/9 140–149, 150, 161, 162)

	Austrian Eastern Region (1+2+3)	Provinces		
		Burgenland (1)	Lower Austria (2)	Vienna (3)
Population				
Absolute	3228 966	269 771	1427 849	1531 346
% of urban	74.3	32.6	54.6	100.0
New cases				
Absolute	8944	709	3283	4952
HV (%)	88.3	85.2	86.2	90.2
M/I (%)	110.9	108.7	111.3	110.9

HV = histologically verified and M/I = mortality/incidence ratio.

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