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# Original contributions

## Therapy of radiation damage to normal tissues with selective inhibitors of cyclooxygenase-2. l. YM177 tends to reduce mouse mortality from the haemopoietic syndrome

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*Purpose. Non-steroidal anti-inflammatory drugs, i.e, non-selective inhibitors of cyclooxygenase (COX)-1 and – 2, are well known to reduce adverse radiation reactions when given after the exposure. The question remains open as to whether or not newly developed, better-tolerated selective COX-2 inhibitors act likewise.*
*Methods and Materials: A selective COX-2 inhibitor (YM177) was given to whole body X-irradiated mice (5.5-6.5 Gy) starting 24 hr after the exposure until the end of a monthly observation period in doses ranging from 0.17 to 3.4 mg/ kg•day.*
*Results. Administration of ≥0.68 mg YM177/kg•day proved ineffective, whereas two lower dose levels of YM177 reduced the overall mortality from 64% to 54% by making radiation dose-mortality curve of common origin considerably shallower. A non-linear correlation analysis using a Logit plot revealed a significant difference in the mortality curves between placebo group and YM177 treatments (p≤0.023 by one-tailed t-test).*
*Conclusions. Prostanoids synthesized by COX-2 can contribute to death from the haemopoietic syndrome. Similar studies on other organ systems are desirable.*

**Leczenie uszkodzeń popromiennych środkami swoiście hamującymi cyklooksygenazę-2. 1. YM177 może zmniejszać śmiertelność myszy z powodu niewydolności krwiotwórczej**

*Od dawna wiadomo, że niesterydowe leki przeciwzapalne, tj. nieswoiste, inhibitory cyklooksygenaz (COX)-1 i -2 zmniejszają odczyny popromienne, jeśli je stosować po ekspozycji. Nie wiemy natomiast, czy wprowadzane obecnie do kliniki leki, które swoiście hamują COX-2, są też skuteczne w leczeniu popromiennych reakcji tkanek prawidłowych.*
*Po 24 godz. od napromieniania myszy (5,5-6,5 Gy promieni X) rozpoczynano ciągłe podawanie im swoistego inhibitora COX-2 (0,17-3,4 mg YM177/kg dziennie). Stosowanie ≥0,68 mg YM177/kg dziennie było nieskuteczne, ale dwie niższe dawki leku obniżyły śmiertelność z 64% do 54% i znamiennie (p≤0,023) zmniejszyły stromiznę krzywej zależności śmiertelności myszy od dawki promieniowania. Wnioskujemy, że prostanoidy, syntetyzowane z udziałem COX-2, mogą przyczynić się do śmierci, spowodowanej popromienną niewydolnością układu krwiotwórczego. Pożądane są podobne badania innych narządów.*

**Key words:** radiation injuries, therapy, COX-2 inhibitors
**Słowa kluczowe:** uszkodzenia popromienne, leczenie, inhibitory COX-2

### Introduction

Various non-steroidal anti-inflammatory drugs (NSAID) including aspirin and indomethacin have been successfully used in animals and man to alleviate ionizing radiation-induced reactions in normal tissues [1-3]. Although chemically heterogeneous, all NSAID act similarly by inhibiting the biosynthesis of prostanoids (prostaglandins, prostacyclin and thromboxane). They do so by interfering primarily with cyclooxygenase (COX) -1, a constitutive enzyme normally present in most types of cell. NSAID are less effective as inhibitors of COX-2, an inducible enzyme whose occurrence is largely limited to mesenchyme-derived cells involved in inflammatory reactions. For this reason the drugs, when administered repeatedly in high therapeutic doses, e.g. to patients suffering from rheumatic diseases, cause serious side-effects affecting especially the stomach.

A new generation of NSAID is being developed. These compounds selectively inhibit COX-2 while sparing COX-1 and thus exert their anti-inflammatory action without causing damage to the gastro-intestinal tract [4-6]. We have begun studying therapeutic effectiveness of these highly selective COX-2 inhibitors in reducing undesirable radiation reactions.

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## Materials and methods

### Animals

Male SPF mice of C57BL/6J strain, 8 to 10 weeks old, were used. The animals were fed pellets for mice (Funabashi Farm Co.) and given HCl-acidified water ad libitum in a facility with 12 hr light-dark cycle at 22±2°C. The experiments were performed under the Guidelines for Animal Welfare and Experimentation of the National Institute of Radiological Sciences.

### Irradiation

Unanaesthetized mice were whole body irradiated using an X--ray machine (Pantak-320S, Shimadzu Seisakusho Co., Kyoto) operated at 200 kVp, 19 mA, with a dose rate of 0.61∼0.66 Gy/min in air and 0.5 mm Al and 0.5 mm Cu filtration. An exposure ratemeter (Ôyo giken AE-1320) was used as a dosimeter. The X-ray doses varied from 5.5 to 6.5 Gy.

### Administration of YM177

YM177 (4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1 H-pyrazole-1-yl] benzensulfonamide from Yamanouchi Pharmaceutical Co.) was dissolved in 100% ethanol, diluted 20-fold with HCl--acidified water and given to mice instead of drinking water, starting 24 hr after irradiation until the end of the monthly observation period. The solvent (5% ethanol in acidified water) was also used to prepare further dilutions of YM177 to be administered, and given alone to control (placebo group) mice. The volume of solutions consumed was measured and the mice were weighed at intervals.

### Statistics

The mortality curves of the mouse groups treated by YM177 or placebo were fitted onto the standard Logistic function ($Y=1 / [ 1+EXP(aX + b)]$) with radiation dose as independent variable using the Quasi-Newton non-linear least square optimization. The statistical differences were tested by Student's t-statistics between the estimated correlation coefficients. Software, Kyplot (v.2.0b6) by Yoshioka K., was used for the statistical analysis.

## Results

Temporal distribution of deaths for all mice which succumbed within the first month of irradiation is shown in Fig. l. The histogram is unimodal and positively skewed, with the median value of 16 days, presenting a pattern compatible with a single cause of death from the haemopoietic failure.

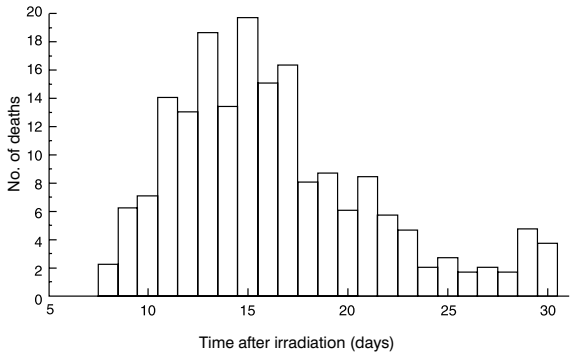


Fig.1. Temporal pattern of mouse mortality after whole body X-irradiation.
Distribution of deaths for all mice which succumbed within the first month of irradiation is unimodal and positively skewed.

In a series of experiments using X-ray doses of 5.75 - 6.50 Gy and three dose levels of YM177 (0.68, 0.34 and 0.17 mg / kg body weight. day), mouse mortality within the first month after irradiation varied from 0 to 100%. By comparison with the placebo group, two lower dose levels of YM177 reduced the overall mortality from 64% to 54% by making radiation dose-mortality curve of common origin considerably shallower (Fig. 2). Correlation coefficients by non-linear regression of the mortality curves to a Logit function were 0.829 and 0.698 for placebo group and YM177 treatments (0.34 and 0.17 mg YM177 / kg body weight •day combined) respectively. The difference between the correlation coefficients was statistically significant ($p \leq 0.023$ by one-tailed t-test). Concomitantly the value of LD50/30 increased from 5.98 to 6.09 Gy. The highest dosage (0.68 mg/kg) did not reduce mortality rate. YM177 at any dose level failed to change the timing of death.

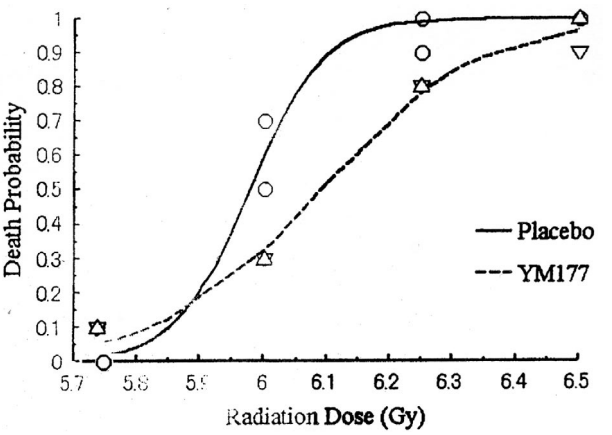


Fig. 2. Dose-dependent death probability of placebo and YM177-treated mice.
Mortality curve for two lower dose levels of YM177 (Δ 0.34 and ∇ 0.17 mg/kg body weight•day) is less steep when compared with the placebo group (O). The difference between correlation coefficients of the standard Logistic functions is significant ($p \leq 0.023$ by one-tailed *t*-test).

In another series of experiments using X-ray doses varying from 5.5 to 6.5 Gy, placebo group was compared with mice given daily 0.68 mg YM177/kg. As in the previous series, at this dosage the compound failed to either lower the mortality rate (20/50 vs. 19/50) or prolong survival time of those mice which succumbed during the monthly observation period.

Finally, still higher amount of YM177 (3.4, 1.7 and 0.85 mg/kg) was given to X-irradiated mice (6.25 or 6.50 Gy). Neither mortality rate nor survival time was significantly affected by YM177.

## Discussion

It has been argued that irradiation not only reduces the number of clonogenic cells but also elicits disturbances in the level of mediators involved in intercellular communication (humoral component of radiation reactions) [1]. The abnormal level of the mediators may substantially

contribute to the severity of normal tissue damage caused by radiation. The adverse humoral reactions to irradiation are amenable to treatment with drugs. which either prevent excessive production of the mediators or block their cell receptors [3].

Several mediators of normal cell-to-cell communication cause inflammatory reactions when present in excess in the extracellular fluid compartment. Radiation exposure in vivo leads to abnormally high levels of prostanoids (prostaglandins, prostacyclin, thromboxane), which are involved in a variety of inflammatory conditions and diseases caused by agents other than radiation. Alleviation of radiation reactions with NSAID which all act by inhibiting biosynthesis of prostanoids shows that overproduction of this class of mediators substantially contributes to these reactions [1-3].

NSAID which ameliorate radiation damage have so far been non-selective inhibitors of both cyclooxygenase isoenzymes (COX-1 and COX-2). Recently well tolerated, selective inhibitors of COX-2 have become available [4-6]. We have used one such inhibitor (YM177) to find out whether or not it can influence the course of the haemopoietic syndrome in whole-body irradiated mice when given continuously after radiation exposure.

YM177 was administered to irradiated mice in their drinking water to achieve constant level of the inhibitor while measuring its intake. The treatment was aimed at a specific humoral component of radiation reaction only. Accordingly, it was begun 24 hours after whole-body irradiation to avoid interference with instantaneous reduction in number of clonogenic cells and rapid intracellular repair processes. YM177 was given until the end of the monthly observation period following radiation exposure. Mouse survival rate (LD 50/30) and duration of survival of those animals which succumbed due to haemopoietic failure within the first post-irradiation month served as criteria of therapeutic effectiveness of YM177 administered in doses ranging from 0.17 to 3.4 mg/kg·day. Only two lowest doses of YM177 significantly reduced mouse mortality (Fig. 2) suggesting that in whole body-irradiated mice endogenous prostanoids synthesized with the involvement of COX-2 can contribute to death from the haemopoetic syndrome.

Similar studies on partial-body irradiated animals using non-clonogenic end-points for assessment of radiation damage are called for to find out whether or not selective inhibitors of COX-2 ameliorate the damage to organs other than the haemopoetic tissue.

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