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Effects of Some Nonsteroidal Anti-inflammatory Agents on Experimental Radiation Pneumonitis

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Corticosteroids have previously been found to be protective against the mortality of radiation pneumonitis in mice, even when given well after lethal lung irradiation. We explored the possibility that this effect was due to their well-known anti-inflammatory actions by giving various nonsteroidal inhibitors of arachidonate metabolism to groups of mice that had received 19 Gy to the thorax (bilaterally). Treatments of four cyclooxygenase inhibitors, one lipoxygenase inhibitor, and one leukotriene receptor antagonist, given by various routes in various doses, were commenced 10 weeks after irradiation or sham irradiation and continued throughout the period when death from radiation pneumonitis occurs, 11–26 weeks after irradiation. Each of the treatments had the appropriate effect on arachidonate metabolism in the lungs as assessed by LTB4 and PGE2 levels in lung lavage fluid. The principal end point was mortality. The 5-lipoxygenase inhibitor diethylcarbamazine and the LTD4/LTE4 receptor antagonist LY 171883 markedly reduced mortality in dose–response fashion. The effects of cyclooxygenase inhibitors were divergent; piroxicam and ibuprofen were marginally protective, indomethacin in all doses accelerated mortality, and aspirin reduced mortality in a dose–response fashion. These results suggest that the protective effect of corticosteroids in radiation pneumonitis can be tentatively attributed to their anti-inflammatory actions, and that nonsteroidal anti-inflammatory agents, particularly those that affect lipoxygenase products, may offer equal or better protection than corticosteroids against mortality due to radiation pneumonitis.

INTRODUCTION

Radiation pneumonitis, the reaction of the lungs that occurs some weeks or months after large doses of ionizing radiations, is characterized by an exudative inflammation of the lung parenchyma (*1*). Its mechanism is unknown, and there is uncertainty concerning which lung cell type is the target of radiation. Many of its physiological features can be attributed to the leakage of circulatory fluid from the pulmonary microvasculature into alveolar spaces, which results in abnormalities in gas exchange, respiratory rate, and lung compliance (*2*). In many ways, therefore, radiation pneumonitis resembles adult respiratory distress syndrome (ARDS) of any other etiology. One striking difference, however, is that mortality from radiation pneumonitis in experimental animals can be mitigated in part by prophylactic corticosteroid administration (*3–5*), whereas corticosteroids have no beneficial effect on the course of other types of ARDS (*6*).

Corticosteroids have multiple cellular and biochemical effects on lung and other tissues. Previous studies have shown they have beneficial effects on lung mechanics (*4*), microvascular leakage (*4, 7, 8*), alveolar type II cell replication (*9*), and the accumulation of inflammatory cells in the alveoli (*10*) during radiation pneumonitis. They have important effects on the metabolism of arachidonic acid and other aspects of inflammation which could account for their modulating effect on the development and course of radiation pneumonitis. By inhibiting the hydrolysis of membrane phospholipids by phospholipase A2, the first step in the generation of both cyclooxygenase and lipoxygenase products of arachidonic acid (*11*), corticosteroids exert a key role on inflammatory mediators. It is possible, therefore, that their effects in radiation pneumonitis, if due to an anti-inflammatory effect, could be due to inhibition of formation of the products of either or both of these major pathways of metabolism of arachidonic acid. As many inhibitors of both pathways are now available, it may be possible to reproduce the effects of corticosteroids by more selective inhibitors of arachidonic acid metabolism, and even to identify key metabolites whose inhibition might ameliorate the course of radiation pneumonitis.

The present experiments represent an initial screening of the effects of some nonsteroidal anti-inflammatory drugs that affect eicosanoid pathways. Four cyclooxygenase inhibitors, one lipoxygenase inhibitor, and one leukotriene receptor antagonist were given continuously in various dosages and by various routes starting 10 weeks after thoracic exposure to a lethal dose of cobalt irradiation. Mortality between 11 and 26 weeks, the time when death from radiation pneumonitis occurs, was the principal end point ex-

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plored in these experiments. The agents had markedly divergent effects on mortality. Those that affect the lipoxygenase pathway were significantly protective, whereas the cyclooxygenase inhibitors had varied effects.

METHODS

Mice and irradiation. All experiments were performed on CF1 mice (Charles River Farms, Portage, MI) aged 10–12 weeks at the time of irradiation. Bilateral irradiation of the thorax (other body parts being shielded) was performed under light pentobarbital anesthesia with an AECL Theratron 780 teletherapy unit as described in detail elsewhere (*5*). In most cases the mice received 19 Gy; in some experiments a range of radiation doses was used (see Results). Previous dose–response studies from this laboratory show that 19 Gy results in 90–95% mortality between 11–26 weeks later (*5*). Following irradiation animals were maintained under normal laboratory conditions and received food and water *ad libitum.*

Administration of test agents. Mice were assigned randomly to control and test groups, 15–30 mice per group, 10 weeks after irradiation. Three control groups were included in the study of each agent, one that received radiation but no treatment other than the treatment vehicle, a second that received treatment but no irradiation, and a third group that received radiation without treatment or vehicle. Control groups were handled identically to the experimental groups in all other respects. Treatments were commenced 10 weeks after irradiation and continued until 26 weeks after irradiation.

Cyclooxygenase inhibitors. Indomethacin (Sigma Chemical Co., St. Louis, MO) was given in the drinking water at estimated doses of 4–8 mg/kg/day (details under Results). It was dissolved in a small amount of absolute ethanol and diluted with distilled water to a final concentration of 20–40 μg/ml. Solutions were made up fresh with each change of the drinking water, three times per week. Test mice received indomethacin from 10 weeks after irradiation until death or the conclusion of the study 26 weeks after irradiation. The same protocol was followed for each of the other agents mentioned below.

Aspirin (acetylsalicylic acid, Sigma Chemical Co.) was dissolved in tap water and given from 10–26 weeks. Three dose levels were given, 12, 24, and 48 mg/kg/day.

Piroxicam (Sigma Chemical Co.) was given in various doses by one of three routes in separate experiments: (1) dissolved in a small amount of 10% NaOH, which was then diluted 1000 times and given as drinking water at an intake of 8 mg/kg/day; (2) given in the feed with an estimated intake of 4 mg/kg/day; or (3) dissolved in corn oil and injected intraperitoneally three times weekly at doses equivalent to 4 and 8 mg/kg/day.

Ibuprofen (Sigma Chemical Co.) was given in the feed at estimated doses of 4 and 8 mg/kg/day; or dissolved in corn oil and injected intraperitoneally three times weekly at a dose equivalent to 4 mg/kg/day; or given by subcutaneous pellets (Inovative Research of America, Toledo, OH) that were implanted each 3 weeks at a dose equivalent to 10 mg/kg/day.

Lipoxygenase pathway agents. Diethylcarbamazine, (Sigma Chemical Co.) a 5-lipoxygenase inhibitor, was given in the drinking water at various doses from 2.0–8.0 mg/kg/day.

LY 171883 (Eli Lilly, Indianapolis, IN), a leukotriene receptor antagonist, was given in the drinking water at various doses from 8–24 mg/kg/day.

For each of the agents and dosages stated above, control groups were included as described above.

Eicosanoid levels in lung lavage fluid. To confirm that the various treatments had appropriate effects on arachidonic acid metabolism, we assayed leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) in the alveolar lavage fluid of separate groups of sham-irradiated and irradiated mice that had been treated with each agent for 2 weeks. Lungs were quantitatively lavaged five times pertracheally in a standard manner as described previously (*12*). The yield of lung lavage fluid, 6.0–6.5 ml, was centrifuged at 1000*g* for 10 min to sediment cells. The supernatant was applied to solid silicic acid columns (Sep-Pak, Waters Associates, Milford, MA), eluted with methyl formate, and dried under vacuum. Levels of LTB4 and PGE2 in the alveolar lavage fluid were determined by radioimmunoassay (NEN–DuPont, Boston, MA). Recovery of these metabolites was determined to be 94% for LTB4 and 91% for PGE2 from preliminary trials with radioactive LTB4 and PGE2 standards.

Controls for infection. Throughout the course of these studies, the mouse colonies were assayed for infection on a monthly basis. Cultures of blood and lungs and serologic tests were performed for a variety of mouse pathogens as described previously (*13*). When evidence of significant infection was found, the experiment was abandoned.

Analysis of data. Treatment and control groups consisted of 15–30 mice each. Animals that died within 10 weeks of irradiation were excluded. Treatments were administered continuously from 10–26 weeks after irradiation. Deaths were recorded daily from 10–28 weeks and are reported on a weekly basis. Where an agent was found to be protective, deaths were sometimes also recorded up to 34 weeks following irradiation, i.e., up to 8 weeks after discontinuation of treatment.

Statistical tests were performed with commercial software (Statpro, Penton Software, New York, NY). The effects of treatments were assayed by mortality using two parameters, median time to death and proportion of survival at 28 weeks. For each agent, treatment groups were compared to the control group (radiation plus vehicle) that had been irradiated at the same time. We also compared the mortality of the control group (radiation plus vehicle) of each experiment with the mortality of control groups (radiation only) from all experiments using 19 Gy, to ensure that the vehicle or method of administration had not itself affected the results.

Median time to death was calculated by logit analysis (*14*), the significance of the difference between treated and control groups being calculated by t test. The significance of difference in survival at 28 weeks was calculated by χ^2 test using Yates correction. Significance level was taken as $P < 0.05$.

RESULTS

Effect of treatments on LTB4 and PGE2 levels. Table I shows that mice with radiation pneumonitis had increased amounts of both LTB4 (as an index of lipoxygenase activity) and PGE2 (an index of cyclooxygenase activity) in their lung lavage fluid compared with sham-irradiated controls; however, only the PGE2 level was significant elevated. Treatment with each of the cyclooxygenase inhibitors resulted in significant reductions in the cyclooxygenase product PGE2 in both irradiated and sham-irradiated mice, indicating that the treatments had probably had the expected effect on mediators in the lung. Some increase in LTB4 was also usually observed, suggesting that metabolism may have followed the other pathway of arachidonic acid metabolism. Treatment with diethylcarbamazine resulted in significant reductions in leukotriene generation, consistent with its postulated action as an inhibitor of lipoxygenase.

Mortality without treatments. Mortality data following 19 Gy thoracic irradiation (and no other treatment) were pooled from 16 experiments (303 mice) to provide a reference for the expected effects of this dose of radiation. The result is shown in Fig. 1. For each of the individual experiments reported subsequently, when the mortality curve of

TABLE I
Concentrations of Arachidonic Acid Metabolites in Alveolar Lavage Fluid

Treatment	Sham-irradiated (pg/ml ± 1 SD)		Irradiated (pg/ml ± 1 SD)	
	LTB4	PGE2	LTB4	PGE2
None	80 ± 33 (5)[a]	50.9 ± 5.8 (6)	130 ± 67 (6)	61.8 ± 6.7 (6)[b]
Piroxicam	139 ± 43 (3)	30.2 ± 1.2 (3)[c]	115 ± 8 (2)	30.0 ± 0.0 (2)[c]
Indomethacin	161 ± 24 (3)[c]	27.0 ± 13 (3)[c]	167 ± 89 (3)	28.3 ± 14 (3)[c]
Ibuprofen	180 ± 14 (3)[c]	22.8 ± 1.1 (3)[c]	166 ± 34 (3)	29.2 ± 1.5 (2)[b,c]
Aspirin	162 ± 34 (3)[c]	30.0 ± 5.0 (3)[c]	126 ± 25 (3)	22.2 ± 4.4 (3)[c]
Diethylcarbamazine	29 ± 5 (3)[c]	48.0 ± 6.0 (3)	33 ± 18 (3)[c]	52.0 ± 8.0 (3)

Note. Drugs were commenced 16 weeks after irradiation or sham irradiation and continued for 2 weeks, at which time LTB4 and PGE2 levels were assayed. Piroxicam was given at 8 mg/kg/day in the drinking water; indomethacin was given at 4 mg/kg/day in the drinking water; ibuprofen was given at 8 mg/kg/day in the feed; aspirin was given at 48 mg/kg/day in the drinking water; diethylcarbamazine was given at 4 mg/kg/day in the drinking water. Differences not significant if not shown.

[a] Numbers in parentheses are numbers of animals.

[b] Significantly different ($P < 0.05$) from corresponding level in sham-irradiated mice (on same line).

[c] Significantly different ($P < 0.05$) from corresponding level in mice that received no treatment (in same column).

the control (irradiated plus vehicle) group did not fall within the 95% confidence limits of the overall data for control mice irradiated without the vehicle shown in Fig. 1, the data for the experiment in question were discarded. This resulted in the exclusion of five experiments. In four cases exclusion was because the mortality curve was significantly higher than expected, i.e., accelerated. This occurred in all three experiments where the vehicle was corn oil injected intraperitoneally (piroxicam and ibuprofen experiments) and in the one experiment using subcutaneous placebo implants that were replaced each 3 weeks (ibuprofen experiments). We concluded that these two methods of administration are unsuited to the present experiments and their data are not reported further. In one case, mortality of the control group was significantly lower than expected for unknown reasons. This experiment was also discarded because it was felt not to represent a valid test of the effect of the treatment.

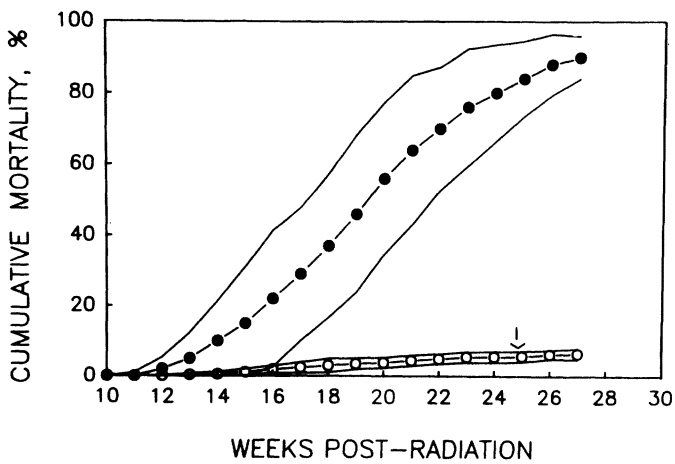

FIG. 1. (●) Mortality with time after 19 Gy bilateral thoracic irradiation in 303 mice that received no treatments. The surrounding lines are 95% confidence limits. (○) Mortality of 163 sham-irradiated mice of the same age that received no treatments and their 95% confidence limits.

Also shown in Fig. 1 is the mortality of 163 control mice that had received sham irradiation and no treatment because the duration of each of the experiments reported here, 6–8 months starting at about 3 months of age, is not negligible by comparison with the normal life expectancy of mice. These data were used to determine if the vehicle or treatment itself resulted in significant mortality.

Piroxicam. Piroxicam in the feed or drinking water had negligible effects on the survival of mice that were sham-irradiated (data not shown).

Piroxicam at an estimated intake of 4 mg/kg/day in the feed had a small but not statistically significant effect on mortality in irradiated mice (Fig. 2). Median time of death was delayed 1.6 weeks (not significant) but mortality at 26 weeks was similar. When delivered in the drinking water at an estimated dose of 8 mg/kg/day (Fig. 3), piroxicam did not delay the median time to death, but deaths at 26 weeks were reduced (58% versus 83%, not statistically significant). However, when compared to the mortality in untreated irradiated mice (shaded area of Fig. 3), the mortality of the piroxicam-treated mice fell mostly below the 95% confidence limit of expected mortality. There was thus a slight trend for piroxicam given by mouth to delay or reduce mortality.

Indomethacin. Preliminary experiments suggested that indomethacin by itself resulted in substantial mortality of control, sham-irradiated mice. A dose of 8 mg/kg/day re-

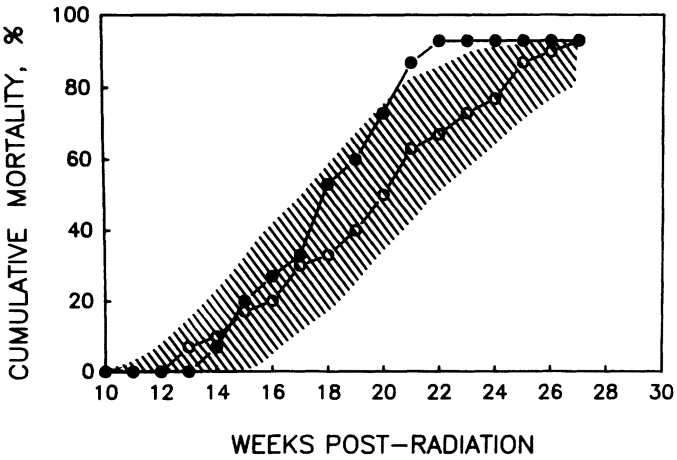

FIG. 2. Effect of piroxicam, a cyclooxygenase inhibitor, on mortality following 19 Gy thoracic irradiation. Piroxicam was administered in the reconstituted feed from 10–26 weeks at an estimated dose of 4 mg/kg/day to 30 mice (○). Fifteen control mice (●) received irradiation and reconstituted feed without piroxicam. The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

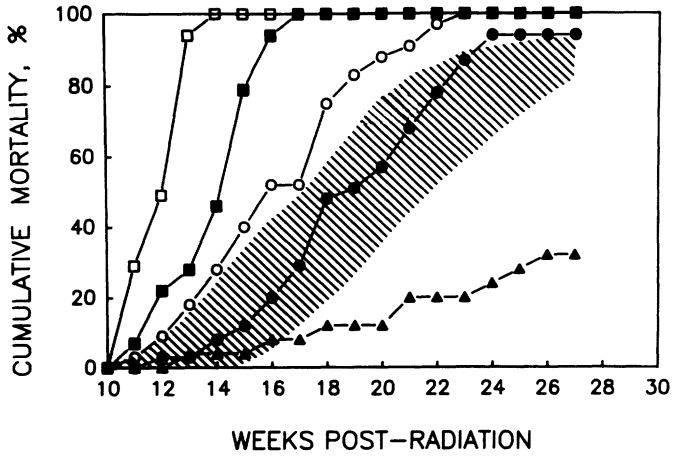

FIG. 4. Effect of indomethacin, a cyclooxygenase inhibitor, administered in the drinking water from 10–26 weeks following 19 Gy at doses of (□) 8, (■) 6, (○) 4, or (●) 0 mg/kg/day (24 mice per group). The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1). (▲) Mice receiving 8 mg/kg/day indomethacin and no radiation.

sulted in mortality of 2% per week in control mice. Moreover, it greatly accelerated deaths in irradiated mice (preliminary data not shown).

We therefore performed a study to determine the dose–response effect of indomethacin following radiation. The results show that in irradiated mice indomethacin did not reduce mortality at any of the doses studied; indeed death was accelerated by indomethacin in a dose-related manner (Fig. 4). The acceleration in deaths was substantially greater than could be accounted for by an additive effect of the two factors, irradiation and indomethacin treatment. Probably, therefore, indomethacin potentiated the deleterious effect of irradiation.

To determine how the apparently deleterious effect of indomethacin was related to the dose of radiation, we gave a consistent dose of indomethacin, 4 mg/kg/day, to groups of mice that received various doses of thoracic irradiation (Fig. 5). Indomethacin had negligible effects on mortality at low doses of radiation, but resulted in accelerated mortality at larger doses of radiation, 16 and 19 Gy ($P < 0.05$ for 19 Gy). Thus the effect of indomethacin in hastening deaths appeared to be related to the dose of radiation. We conclude that indomethacin potentiates the effect of radiation on the lungs.

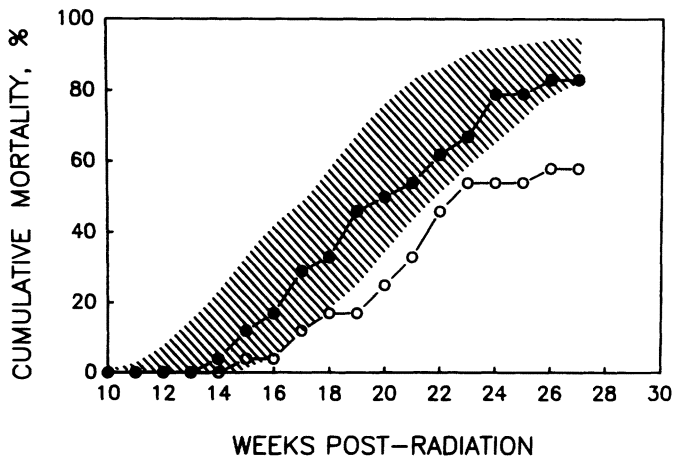

FIG. 3. Effect of piroxicam administered in the drinking water from 10–26 weeks at an estimated dose of 8 mg/kg/day to 24 mice following 19 Gy (○). The control group, 24 mice (●), received radiation but no piroxicam. The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

Ibuprofen. Ibuprofen administered in the feed at an estimated dose of 8 mg/kg/day did not affect the survival of mice between Weeks 10 and 28 following sham irradiation (data not shown).

Ibuprofen given in the feed at estimated doses of 4 and 8 mg/kg/day resulted in some apparent trend to delay of deaths at each of these doses, the trend being more marked for the higher dose (Fig. 6). However, all three mortality curves fell mostly within the 95% confidence interval of control mice, and neither dose of ibuprofen statistically delayed mean mortality or reduced mortality at 26 weeks after irradiation. Thus any effect of ibuprofen on radiation pneumonitis in these doses was slight at best.

Aspirin. Aspirin administered in the drinking water at estimated doses up to 48 mg/kg/day had no effect on the survival of control mice from 10–26 weeks after sham irradiation (data not shown).

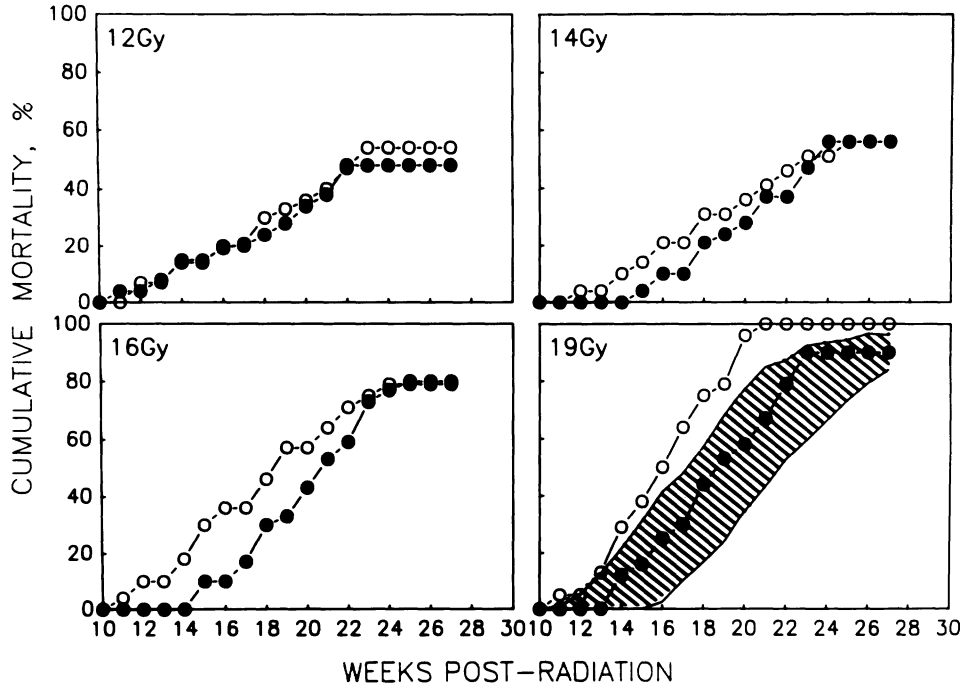

FIG. 5. Effect of indomethacin administered at 4 mg/kg/day from 10–26 weeks following indicated doses of thoracic irradiation, 28–30 mice per group. (●) Control irradiated mice; (○) indomethacin-treated irradiated mice. The shaded area in the 19 Gy panel represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

In irradiated mice, aspirin reduced the mortality at 26 weeks after irradiation. Mortality was significantly reduced at doses of 24 mg/kg/day ($P < 0.01$), and 48 mg/kg/day ($P < 0.001$). There was also a clear dose–response effect. The experiment was repeated to obtain data from each dose of aspirin with essentially identical data; the results shown in Fig. 7 are the means of all data for each treatment.

Aspirin was thus the only cyclooxygenase inhibitor that clearly resulted in significant reduction in the mortality of thoracic irradiation.

Diethylcarbamazine. Preliminary experiments suggested that diethylcarbamazine itself resulted in slight but significant mortality in unirradiated mice. Dose–response

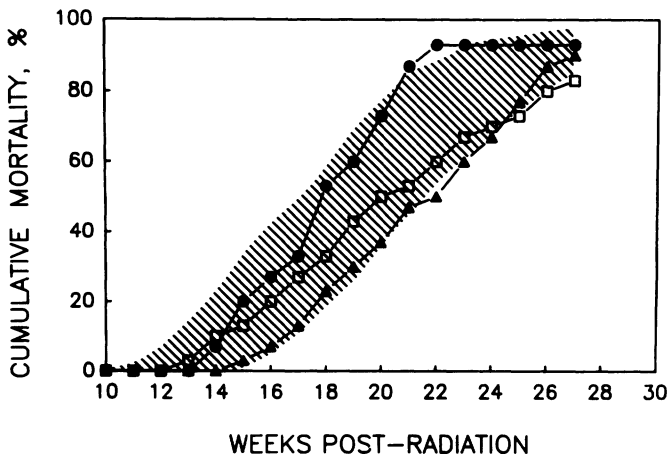

FIG. 6. Effect of ibuprofen, a cyclooxygenase inhibitor, administered in the feed from 10–26 weeks following 19 Gy. (●) Control mice ($N = 15$), (□) ibuprofen 4 mg/kg/day ($N = 30$), (▲) ibuprofen 8 mg/kg/day ($N = 30$). The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

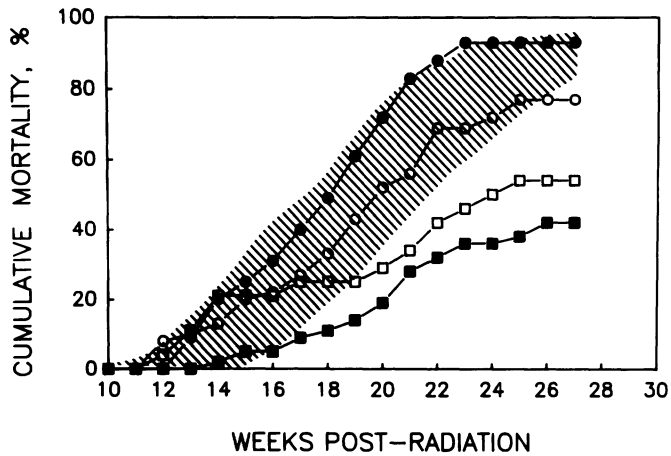

FIG. 7. Effect of aspirin, a cyclooxygenase inhibitor, administered in the drinking water at indicated doses from 10–26 weeks following 19 Gy. (●) Control irradiated mice ($N = 63$), aspirin (○) 12 mg/kg/day ($N = 30$), (□) 24 mg/kg/day ($N = 24$), (■) 48 mg/kg/day ($N = 48$). The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

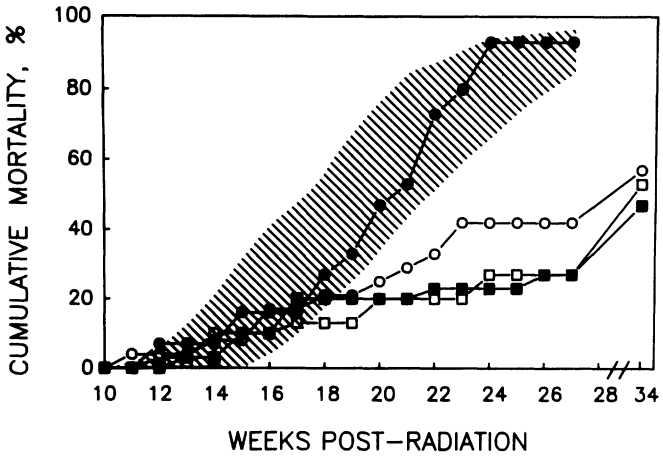

FIG. 8. Effect of diethylcarbamazine, a 5-lipoxygenase inhibitor, administered in the indicated doses from 10–26 weeks following 19 Gy. (●) Control mice (N = 39), (○) 2 mg/kg/day (N = 45), (□) 4 mg/kg/day (N = 30), (■) 8 mg/kg/day (N = 30). The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

experiments to study this effect suggested that mortality due to this agent alone could be limited to about 2% per week by doses of 8.0 mg/kg/day (data not shown). Subsequent experiments did not exceed this dosage.

Two experiments were performed with doses from 2.0–8.0 mg/kg/day; the pooled data are shown in Fig. 8. A dose of 2.0 mg/kg/day reduced mortality at 26 weeks after irradiation by at least one-half (χ^2, $P < 0.01$). Doses of 4.0 and 8.0 mg/kg/day further reduced mortality of irradiated mice at 26 weeks (both $P < 0.001$) to levels that were not significantly different from those seen with the drug alone in sham-irradiated mice. Following withdrawal of the drug at 26 weeks, additional mortality occurred by Week 34, which also suggests that mortality had been inhibited by administration of this agent.

Thus diethylcarbamazine itself resulted in some mortality, but the drug reduced the mortality due to thoracic irradiation in a dose–response fashion.

LY 171883 (LY). LY in the drinking water of unirradiated mice in doses up to 48 mg/kg/day also resulted in some mortality, about 2% per week without a clear dose–response effect (data not shown).

In irradiated mice, administration of LY in all doses studied markedly reduced mortality (Fig. 9) to levels that were significantly below those of irradiated, untreated mice (all $P < 0.001$). Observations up to 34 weeks after irradiation, 8 weeks after discontinuation of LY, showed that some deaths occurred after discontinuation of LY (Fig. 9), again suggesting that LY had mitigated mortality. No clear dose–response effect was seen.

We conclude that LY, although resulting in some mortality itself, provided considerable protection against mortality due to thoracic irradiation.

DISCUSSION

A number of agents have been examined previously for possible protective effects on radiation-induced sequelae in the lungs, but many of these focused on the development of radiation fibrosis (e.g., Ref. (*15*)). So far as we are aware, no studies have explored the effects of nonsteroidal anti-inflammatory drugs on radiation pneumonitis. A recent report by Graham and co-workers (*8*) examined the effect of 13 agents with a variety of actions, including two nonsteroidal anti-inflammatory drugs and dexamethasone, on the lungs 3 weeks after irradiation. Their data are discussed below. Conversely, there is a large and growing body of literature on the effect of nonsteroidal anti-inflammatory drugs on other models of lung damage. However, the results of these studies are quite divergent from each other, from which one must conclude that each model of lung damage has a unique pathophysiology. Their relevance to radiation-induced damage is thus questionable.

The only anti-inflammatory agents that have previously been shown clearly to be beneficial to the clinical course of radiation pneumonitis are corticosteroids, a finding first reported by Phillips and co-workers (*3*). We have confirmed and extended their observations (*4, 5*). Corticosteroids have multiple biological effects in addition to their anti-inflammatory action, however. We reasoned that if the anti-inflammatory action of corticosteroids were relevant to their beneficial effect in radiation pneumonitis, it might be possible to reproduce this effect by the administration of agents with more specific actions on arachidonic acid metabolism. As an initial screening of such agents we examined the effects of inhibitors of the cyclooxygenase and lipoxygenase pathways and one leukotriene receptor antagonist.

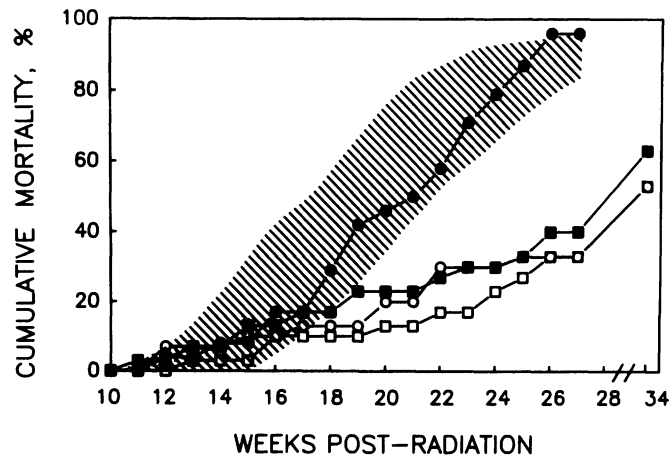

FIG. 9. Effect of LY 171883, a leukotriene receptor antagonist, administered from 10–26 weeks following 19 Gy. (●) Control mice (N = 40), (■) 16 mg/kg/day (N = 30), (○) 24 mg/kg/day (N = 60), (□) 48 mg/kg/day (N = 30). The shaded area represents the 95% confidence limits of 303 mice that received the same dose of radiation and no further treatments (data from Fig. 1).

The end points for these assays were based on mortality between 11 and 26 weeks after bilateral thoracic irradiation as mortality during this period has been shown to be due to radiation pneumonitis (*16*). As the risk of radiation pneumonitis is the dose-limiting factor in clinical radiotherapy, it was felt that death due to radiation pneumonitis was the most clinically relevant outcome to examine.

Treatments were not begun until 10 weeks after thoracic irradiation because we wished to determine whether effects on metabolism of arachidonic acid could account for the protective effect reported previously for corticosteroids which were similarly given at this late stage. Protection at this stage, well after exposure to lethal irradiation, would also be particularly relevant to clinical situations such as following radiation therapy and accidental radiation exposure resulting from nuclear events. Such pharmacological methods of limiting the effects of ionizing radiations could possibly be employed after radiation exposure to rescue otherwise doomed subjects. In the present experiments, treatments were started only about 1–2 weeks before deaths due to radiation pneumonitis normally commence.

The radiation dose used for most experiments, 19 Gy in a single fraction, was selected because previous radiation dose–response experiments in mice of the same strain using the same radiation system showed that 19 Gy resulted in 90–95% mortality between 11–26 weeks (*5*). This dose would therefore be sufficiently large to optimize detection of small protective effects of the agents that were administered but not be so large as to obscure even large protective effects.

Regarding routes of administration, we planned to study the effects of each agent given by several routes, including parenteral administration. We found, however, when these agents were dissolved in corn oil for intraperitoneal injection, that the corn oil vehicle itself resulted in very substantial mortality, 3–4% per week. We found also that administration of the agents or placebo by means of subcutaneous implants (given every three weeks) also resulted in substantial mortality. These data are not shown. The baseline mortality after administration by both of these routes was therefore so high that the effect of the agents administered by these routes would be uninterpretable. Consequently, all the data reported here were derived from experiments in which the agents were given by mouth, either in the drinking water or in reconstituted feed. Where effects suggestive of slight protection were obtained, e.g., with piroxicam as shown in Figs. 2 and 3, we cannot be certain that larger doses would not have achieved significantly greater protection. The dosage in the experiments was sometimes limited either by solubility of the agent in the drinking water or by acceptance in the water or feed as indicated by daily intake. However, intake was not studied exhaustively and it could well be that, in some cases, a larger dose of the agent than was employed could have been administered, parenterally if not by mouth.

Table I indicates that administration of the 5-lipoxygenase inhibitor for 2 weeks was associated with a substantial decline in levels of LTB4 in lung lavage fluid in both groups of mice. Similarly, each of the cyclooxygenase inhibitors resulted in reductions of PGE2 levels. Some "substrate shunting" may also have occurred as the LTB4 levels tended to be higher in cyclooxygenase-treated mice. These two metabolites were used as representative markers of general effects of the agents on the two major metabolic pathways of arachidonic acid; they are both the most abundant metabolites and the easiest to measure. These data can only be taken as evidence that the treatments had achieved significant effects on lung arachidonic acid metabolism, and not that the observed clinical effects of any agent were directly due to specific reductions of PGE2 or LTB4 levels per se. The clinical effects may have been due to alterations in the many other important inflammatory products of arachidonic acid, levels of which were not measured. Moreover, it has been shown that levels of eicosanoids in lung lavage fluid do not necessarily reflect their tissue levels (*17*).

With these reservations in mind, the results show that drugs which impact the 5-lipoxygenase pathway enhanced the survival of lethally irradiated mice (Figs. 8 and 9). Diethylcarbamazine is an inhibitor of the lipoxygenase pathway (*18*), and LY 171883 is a specific receptor antagonist of LTD4 and LTE4 (*19*). There is abundant evidence that these sulfidopeptide leukotrienes promote vascular leakage and other manifestations of inflammation in other varieties of lung damage, and that both of the above two agents can ameliorate these effects in part (*20, 21*). In a recent report, diethylcarbamazine was found to reduce the increase in lung vascular permeability that occurs 3 weeks after thoracic irradiation (*8*). Our findings, therefore, are consistent with previous reports and suggest that leukotrienes may well play a proinflammatory role in radiation pneumonitis. Moreover, the protection afforded by either of these agents was at least as good as that due to corticosteroid administration in previous studies (*5*).

One notes that with both 5-lipoxygenase pathway agents, additional mortality occurred between 28 weeks (when the drug was withdrawn) and 34 weeks. No histological evaluation of this additional mortality was obtained. It might conceivably be due to either intermediate phase lesions or to "catch-up" of mortality following withdrawal of the anti-inflammatory agent, as was seen previously when corticosteroids were withdrawn (*5*).

In contrast, the effects of various cyclooxygenase inhibitors were quite divergent. Aspirin was significantly protective against mortality in a dose–response fashion (Fig. 7). Piroxicam and ibuprofen possibly provided minor protection in the doses studied (Figs. 3 and 5), but indomethacin appeared to be harmful in a dose–response fashion (Fig. 4).

The last finding is at variance with a recent study of lung vascular permeability following thoracic irradiation (*8*). Graham and co-workers found that indomethacin was almost as potent as dexamethasone in limiting vascular permeability 3 weeks after irradiation. Apart from the obvious difference in end points between our study and theirs, and the larger dose in our study (4–8 mg/kg/day here vs 0.8–1.6 mg/kg/day in theirs), the reason for the discrepancy is unclear.

The multiplicity of cycloxygenase products and their widely divergent effects in different varieties of lung damage preclude any simple explanation of the effects observed here with cyclooxygenase inhibitors. In any case, the effect of each of the putative cyclooxygenase inhibitors is not entirely specific, most also having some inhibitory effects on the lipoxygenase system (*22, 23*). The data on cyclooxygenase inhibitors reported here can thus be taken only as guidelines that may be useful in selecting agents for future experiments of this sort.

In conclusion, nonsteroidal anti-inflammatory drugs have divergent and often apparently large effects on the mortality due to thoracic irradiation even when administered well after irradiation but just before deaths occur. In general, 5-lipoxygenase inhibitors and leukotriene antagonists appear to offer the most fruitful field for future studies, mortality at 28 weeks being reduced from 96 to about 30% with each. Both such agents afforded protection that was at least as good as that which we previously found with corticosteroids. Whether they are better tolerated over the long term has not been shown. Promising results were also obtained with some cyclooxygenase inhibitors, e.g., aspirin, where the highest dose resulted in a reduction of mortality at 28 weeks from 96 to 42%, raising the possibility that combinations of agents with different actions on arachidonate metabolism, perhaps in smaller doses than used here, may be as protective against the mortality of radiation pneumonitis.

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