Angiotensin Converting Enzyme Inhibitor Captopril Does Not Prevent Acute Gastrointestinal Radiation Damage in the Rat

John E. Moulder, Ph.D.,* and Brian L. Fish, B.A.
Department of Radiation Oncology, Medical College of Wisconsin, Milwaukee, Wisconsin

SUMMARY Retrospective examination of acute gastrointestinal (GI) lethality in our rat bone marrow transplantation studies showed indication of a protective effect, with a dose modifying factor (DMF) of 1.06 (95% confidence interval: 0.99–1.12). A randomized study, using the same experimental design, was conducted specifically to look for GI protection. Animals were randomized into captopril or non-drug arms and irradiated in a 6-fraction total body irradiation regimen, followed by bone marrow transplantation. Rats received captopril in the drinking water at 500 mg/l (70 mg/kg/day), starting 9 days prior to irradiation and continuing throughout the experiment. The 50% lethal dose at 6 days was 20.8 (20.4–21.7) Gy in the non-drug arm and 20.6 (20.3–20.9) Gy in the captopril arm, for a DMF of 0.99 (0.94–1.04). If the randomized and historical studies are combined the DMF is 1.00 (0.93–1.05). We are unable to find any evidence that the angiotensin converting enzyme (ACE) inhibitor captopril provides protection from acute GI injury in this model. Clearly, it should not be assumed that captopril will modulate radiation reactions in all tissues. *Radiat. Oncol. Invest. 5:50–53, 1997.*

Key words: captopril; ACE inhibitors; radiation; gastrointestinal injury; pharmacologic modification

INTRODUCTION

In 1994, Yoon et al. [1] reported that the angiotensin converting enzyme (ACE) inhibitor captopril protected the jejunal mucosa of mice from acute radiation injury. ACE inhibitors have been reported to ameliorate radiation injury to kidney [2–4], lung [5–7], skin [8], and heart [9], but to have no protective effect for bone marrow [10]. This was an exciting finding, as it was the first report indicating that ACE inhibitors could modify acute radiation injuries, and implied that ACE inhibitors had a more general effect on radiation injury than had previously been suspected.

In previous studies [2,3,10,11] we have shown that captopril is a highly effective modulator of the late renal injury produced in the rats by the total body irradiation (TBI) used as conditioning for bone marrow transplantation (BMT). As part of the radiation dose-response portions of those renal tolerance studies [11], doses high enough to cause acute gastrointestinal (GI) injuries were used in the captopril arm. Similar high radiation doses had been used in animals treated with TBI alone in previous studies. A comparison of acute GI lethality in these two groups suggested that we were seeing a modification of GI injury similar to that reported by Yoon et al. [1]. However, these were non-randomized studies in which the two arms were irradiated at different times, and the studies were not designed to assess GI toxicity. Because of these promising data and the report of Yoon et al. [1], we conducted a randomized study that was spe-

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*Correspondence to: John E. Moulder, Ph.D., Department of Radiation Oncology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. E-mail: jmoulder@its.mcw.edu

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cifically designed to determine whether captopril could modify acute GI toxicity in this rat syngeneic BMT model.

MATERIALS AND METHODS

These studies were performed with 7–8-week-old male WAG/Rij/MCW rats that were bred and housed in a moderate security barrier. Such animals are free of *Mycoplasma pulmonis*, *Pseudomonas*, and common rat viruses. No antibiotics or immunosuppressive agents were used in the animal facility. Animals were maintained in the Animal Care Facilities of the Medical College of Wisconsin, which are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Prior to BMT, rats were given TBI with 18.5–21.5 Gy in 6 fractions over 3 days (4 hr between the two daily fractions) at a dose rate of 0.48 Gy/min, using an orthovoltage X-ray machine [12]. For irradiation, unanesthetized rats were immobilized in a specially constructed plastic jig. Immediately after irradiation the animals received a syngeneic BMT [13].

Rats received captopril at 500 mg/l, or no drug, in their drinking water starting 9 days before TBI. Based on water consumption (26–31 ml/day) and body weight (185–220 g), the captopril dose was 71 (64–78) mg/kg/day, within the range (62 and 125 mg/kg/day) used by Yoon et al. [1], and far above the dose required to modify renal radiation injury [11,14].

Historical data consisted of 38 animals given captopril and treated with TBI at 18.5–21.5 Gy, and 19 control animals treated at 18.5–20 Gy. The prospective, randomized study consisted of 55 animals given captopril and treated with TBI at 19.2–21.5 Gy, and 52 control animals treated at 19.2–21.5 Gy.

All animals were followed for a minimum of 30 days after irradiation. A number of animals died 3–6 days after irradiation, or were sacrificed when morbid during this same interval; these deaths were presumed to be due to acute GI injury [12,15,16]. No deaths or sacrifices occurred between 7 and 30 days after irradiation; this was as expected, since the animals had all received BMTs. Fifty percent lethal doses at 6 days ($LD_{50/6}$) and dose modification factors (DMF) were calculated and compared by probit analysis and are given with 95% confidence intervals.

RESULTS

Analysis of the historical, non-randomized data showed that animals not treated with captopril had an $LD_{50/6}$ of 19.4 (18.7–20.3) Gy, while animals treated with captopril had an $LD_{50/6}$ of 20.5 (20.0–20.9) Gy (Fig. 1). This gives a DMF of 1.06 (0.99–1.12). While this DMF was not statistically significant, it supported the observation of Yoon et al. [1].

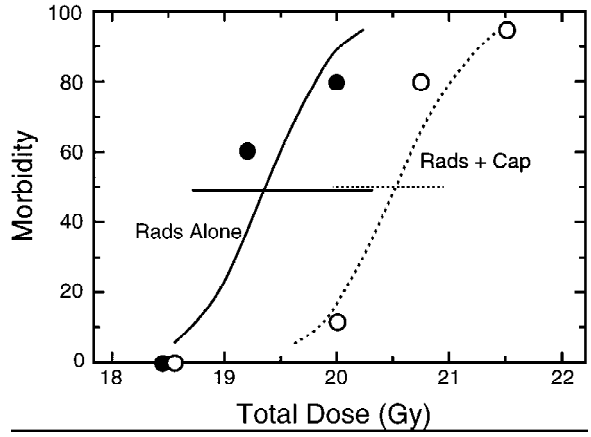

Fig. 1. Radiation dose-response for the development of GI morbidity in rats given captopril (○) or no drug (●), based on historical, non-randomized data. From 5 to 9 animals were treated in each group, except in the 21.5 Gy dose in the captopril arm, which is based on 16 animals. Horizontal bars show the 95% confidence intervals for the $LD_{50/6}$.

In the prospective, randomized study the animals not treated with captopril had an $LD_{50/6}$ of 20.8 (20.4–21.7) Gy, while animals treated with captopril had an $LD_{50/6}$ of 20.6 (20.3–20.9) Gy (Fig. 2). This gives a DMF of 0.99 (0.94–1.04). The $LD_{50/6}$ for the captopril-treated animals was essentially the same as in the historical data, but the $LD_{50/6}$ for the untreated animals was higher ($0.10 > P > 0.05$).

If the two studies are combined, the animals not treated with captopril had an $LD_{50/6}$ of 20.8 (20.0–22.1) Gy, while animals treated with captopril had an $LD_{50/6}$ of 20.5 (20.1–20.9) Gy (Fig. 2). This gives a DMF of 1.00 (0.93–1.05).

DISCUSSION

Captopril, and to a lesser extent other ACE inhibitors, have been shown to be effective in the modification of a wide range of late radiation-induced tissue injuries [14]. However, data on the efficacy of these agents for modification of acute radiation-induced tissue injury have been limited to a single positive report regarding radiation-induced GI injury [1] and a single negative report regarding radiation-induced bone marrow injury [10]. A more detailed knowledge of the range of tissues in which modification of radiation injury by captopril (or ACE inhibitors in general) is possible would be of considerable importance for determining both the

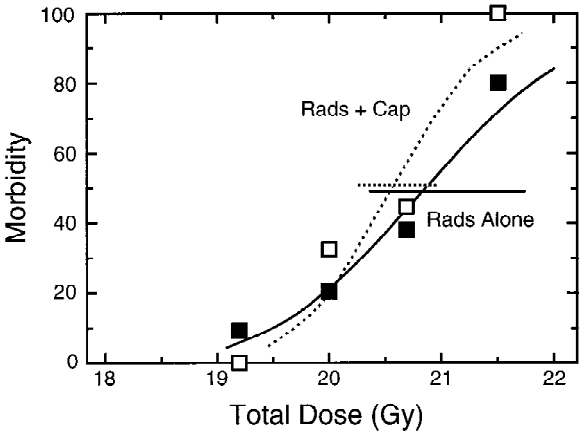

Fig. 2. Radiation dose-response for the development of GI morbidity in rats given captopril (□) or no drug (■) in a prospective, randomized trial. From 10 to 20 animals were treated in each group. Horizontal bars show the 95% confidence intervals for the $LD_{50/6}$.

clinical utility of these agents and the mechanism of their action. The broader the range of tissues in which modification of radiation-induced injury is effective, the more likely it is that a clinical trial of a modifier would be practical [14]. A broad range of efficacy would also indicate that the mechanism of action involved a fundamental step in the development of radiation injury, whereas a narrow range of efficacy would provide clues as to the mechanisms whereby captopril is effective in modifying tissue injury [14].

In the rat syngeneic BMT model, we found no evidence that captopril can modify acute radiation-induced GI morbidity. In fact, as the upper 95% confidence interval on the DMF for modification of GI injury is less than 1.05, the data presented here would appear to rule out biologically significant modification. These studies were designed to extend, rather than replicate, the observation of Yoon et al. [1], and as such, there are a number of differences between the two studies (Table 1). Yoon et al. [1] used two doses of radiation and two doses of drug, whereas we used a single dose of drug and a range of radiation doses. While both experimental approaches are valid, they make a direct comparison of the results impossible. Another major difference is that Yoon et al. [1] used histopathological endpoints, whereas we used morbidity. This raises the possibility that captopril treatment might produce the small reduction in jejunal crypt cell killing seen by Yoon et al. [1] without significantly decreasing morbidity. Such an interpretation is compatible with the dose-response curve for jejunal cell killing [15,16]. It is also worth noting that Yoon et al. [1] reported better protection at 9 Gy

Table 1. Comparison of the Captopril Studies of GI Protection

Item	Current study	Yoon et al. [1]
Species	Rat	Mouse
TBI schedule	6 fractions in 3 days	Single dose
Dose rate	0.5 Gy/min	2 Gy/min
Total radiation dose	18.5–21.5 Gy	9 or 15 Gy
Equivalent single dose[a]	10.6–13.8 Gy	—
Radiation	250 kV	6 MV
BMT	Yes	No
Captopril dose	~70 mg/kg/day	62 or 125 mg/kg/day
Endpoint	Morbidity	Histopathology
Dose-response	Radiation	None

[a]Assuming an α/β ratio of 7–15 Gy [12,17,18].

than at 15 Gy, whereas we find the absence of protection in a fractionated radiation course at doses roughly equivalent to an 11–14 Gy single dose (Table 1). Thus a second possible explanation for the difference in outcome is that the captopril protective effect found by Yoon et al. [1] is confined to relatively low radiation doses. Of the remaining differences between the studies (Table 1), none would appear to be able to explain the differences in outcome.

CONCLUSIONS

Using acute lethality in a rat BMT model, we have been unable to confirm the report by Yoon et al. [1] that captopril is effective in the modification of GI radiation injury. The most likely explanation of the difference is that the reduction in jejunal damage seen by Yoon et al. [1] in captopril-treated mice is insufficient to cause a significant reduction in radiation-induced morbidity. Clearly, it should not be assumed that captopril will modulate radiation reactions in all tissues or even all reactions in a single tissue [14].

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