PRECLINICAL TOXICOLOGY STUDIES WITH THE ANGIOTENSIN-CONVERTING ENZYME INHIBITOR QUINAPRIL HYDROCHLORIDE (ACCUPRIL)


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ABSTRACT — Acute, subacute, and chronic toxicity studies, carcinogenicity bioassays, and reproductive and genetic toxicology studies were performed with quinapril, an ACE inhibitor used in the treatment of hypertension. Acute toxicity is minimal in rodents, and repeated dosing elicits gastric irritation, juxtaglomerular apparatus (JGA) hypertrophy and hyperplasia and tubular degenerative changes in the kidney, and reduced red cell parameters and heart weights in rodents and/or dogs. Other manifestations of toxicity, including hepatic lesions in dogs, reduced offspring weights in rats, marked sensitivity of the rabbit, and clastogenic effects at cytotoxic doses in the in vitro V79 chromosome aberration assay, have been reported with other drugs of this class.

KEY WORDS: Accupril, ACE inhibitor, Toxicity testing

INTRODUCTION

Quinapril hydrochloride is the ethyl ester of a long-acting nonsulfhydryl, angiotensin-converting enzyme (ACE) inhibitor, and is chemically described as [3S-[2-[R*(R*)]-3R*]-2-[2-[(1-ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinoline-carboxylic acid, monohydrochloride (Fig. 1). Quinapril is hydrolyzed to the active diacid form, a potent and selective ACE inhibitor indicated for the treatment of essential hypertension (Kaplan et al., 1989).

Acute, subacute, and chronic toxicity studies in rodents and dogs (Anderson et al., 1984; Andrews et al., 1986; Dominick et al., 1989; Susick et al., 1990; MacDonald et al., 1992), carcinogenicity bioassays in mice and rats (McGuire et al., 1990), and reproductive toxicology studies in rats and rabbits (Dostal et al., 1991) have been performed with quinapril. Genetic toxicology studies were also conducted (Theiss et al., 1989).

This paper summarizes preclinical toxicology findings induced by quinapril and compares these effects with those induced by other ACE inhibitors. Multiples of the quinapril human dose referred to in the text of this paper are based on the maximum recommended daily dose of 1.6 mg/kg or 80 mg.

METHODS AND RESULTS

Acute toxicity studies

In acute toxicity studies with quinapril, oral
median lethal doses (MLDs) were 1440 to 2150 mg/kg in mice and 3541 to 4280 mg/kg in rats. Intravenously, quinapril MLDs were 504 to 523 mg/kg in mice and 107 to 300 mg/kg in rats. Depression or hypoactivity, prostration, and ataxia were nonspecific CNS effects observed in rodents following both routes of administration. Pulmonary congestion in mice, and cecal and liver congestion and pulmonary edema and hemorrhage in rats were observed. Asymptomatic oral doses were 500 mg/kg in mice and 1000 mg/kg in rats; asymptomatic intravenous doses were 200 mg/kg in mice and 50 mg/kg in rats.

Emesis was observed in a male and female dog given escalating oral doses of \( \geq 150 \) mg/kg. Blood pressure in both animals was lower at termination than pretest. At 400 mg/kg, the female had elevated creatinine and blood urea nitrogen (BUN) levels, decreased sodium and chloride levels, and granular casts in the urine. Gastric erosions and ulcers were seen in both animals and mild focal tubular dilatation in the renal cortex was noted in the female.

**Multidose toxicity studies**

Repeated-dose administration in rodents was by gavage since quinapril stability in animal chow was not ensured for the intended feeding interval. Dogs received quinapril in gelatin capsules.

Quinapril, administered orally to mice at 125 to 750 mg/kg/day for two weeks, reduced body weight gain and food intake at all doses and resulted in death of one male at 750 mg/kg. In male and female mice given quinapril orally at 50, 125, 250 and 500 mg/kg in a 13-week study, reduced heart weight was observed in males at all doses and in females at 125 to 500 mg/kg. Renal effects at all doses in both sexes included chronic nephritis and JGA hypertrophy and hyperplasia.

Body weight gain and food consumption were reduced at 125 to 500 mg/kg.

In rats given quinapril orally at 200 to 1200 mg/kg/day for two weeks, deaths occurred at 400, 800, and 1200 mg/kg. In a second two-week study at 100, 400, and 800 mg/kg/day, the low dose was better tolerated. Liver weights were increased at 400 and 800 mg/kg, without attendant histologic alterations. Heart weights were decreased at all doses. RBC, hemoglobin (Hgb), and hematocrit (Hct) were reduced at 400 and 800 mg/kg. Acute pulmonary edema, bronchopneumonia, and gastric lesions were seen at 800 mg/kg.

When quinapril was given orally to rats at 50, 250, and 500 mg/kg/day for 13 weeks, decreased RBC, Hgb, and Hct levels occurred at all doses in both sexes and increased BUN was noted in males at 250 and 500 mg/kg. Heart weights were decreased in both sexes at all doses and kidney weights were increased in males given 250 mg/kg and in females given 500 mg/kg. Increased JG cell granularity and JGA hypertrophy and hyperplasia occurred at all doses. Gastric ulcers or erosions were seen in both sexes at \( \geq 250 \) mg/kg.

In a 52-week chronic study in rats, quinapril was given at 10, 50, and 100 mg/kg/day. Reduced heart weights in males at all doses and JGA hypertrophy and hyperplasia occurred at all doses in both sexes as in earlier studies. Renal tubular degenerative changes consisting of chronic nephritis were first observed at 50 and 100 mg/kg in males and females from a 26-week interim sacrifice. Increased BUN and plasma renin levels and decreased glucose and Hct also occurred at 100 mg/kg. In the kidney, neither the JGA changes nor the degenerative lesions were reversible after a four-week period of withdrawal from drug.
Dogs were given quinapril at 25, 125, and 250 mg/kg/day for two or 13 weeks. In the two-week study, emesis and increased bone marrow myeloid-to-erythroid ratios were observed at 250 mg/kg. Focal gastric erosions and gastritis, as seen in the rat, occurred in a male and female given 125 mg/kg. In the 13-week study, sporadic emesis, anorexia, and altered fecal consistency were seen in both sexes at all doses. RBC, Hgb, and Hct values were reduced in animals given 250 mg/kg, and BUN was increased at 250 mg/kg at Week 8, and normalized by Week 13. JGA hypertrophy and hyperplasia at 250 mg/kg were detected in this species, as with rodents. Focal gastric erosions were noted at 125 and 250 mg/kg. Hepatic fatty change was detected in a male dog given 125 mg/kg that showed increased aspartate and alanine aminotransferase and alkaline phosphatase. The 25-mg/kg dose was nontoxic. Dogs given 10, 50, and 100 mg/kg/day orally for 52 weeks showed no unexpected toxic effects. Increased plasma renin levels and JGA hypertrophy and hyperplasia were observed at all doses. BUN was slightly increased in one male and one female given 100 mg/kg. Gastric erosions were noted in one female given 50 mg/kg and two males given 100 mg/kg. Additional findings in two males given 100 mg/kg included lymphocytosis, increased alkaline phosphatase and aspartate and alanine aminotransferase levels, and centrilobular congestion, mild fibrosis, and mononuclear cell infiltrates in the liver.

Repeated administration of high doses of quinapril produced a similar spectrum of target organ changes in kidney, heart, stomach, and blood in the three species studied. Hepatic lesions were observed in dogs but the relationship to drug is not clear.

Carcinogenicity studies

In mice dosed with 5, 35, and 75 mg/kg/day by gavage for 104 weeks, there were no significant differences in mortality rates (Table 1). There were two control groups, one received the vehicle and the other was untreated. The high dose selected was the maximum tolerated dose, based on weight gain suppression and organ effects in the preceding 13-week study. In the 104-week study, average body weight gain was approximately 1% to 6% below vehicle control at all doses, except for males given 5 mg/kg. Chronic nephritis at 35 and 75 mg/kg and JGA hypertrophy and hyperplasia at all doses were noted. The neoplasms observed were characteristic of the strain, and no treatment-related increases in tumor incidence or decreases in tumor latency were noted.

In the rat carcinogenicity study, conducted at doses of 10, 50, and 100 mg/kg/day by gavage for 104 weeks, a significant dose-related increase in mortality occurred in high-dose males (Tarone, 1975; Tarone and Ware, 1977) (Table 2). Most of these deaths were attributed to gavage dosing errors as a result of struggling during dosing, perhaps due to local irritative effects of the dosing preparation. Sufficient animals remained at study termination to evaluate tumor incidences statistically. The high-dose was selected based on increased BUN, body weight gain suppression, and other organ changes seen in the 13-week study and confirmed in the 52-week study. When compared to the vehicle control groups, average body weight gain in this carcinogenicity study was 3% to 14% lower for the males and 2% to 6% lower for the females in the low-to-high-dose groups. JGA hypertrophy and hyperplasia occurred at all doses in both sexes. Increased renal tubular degenerative changes at 50 and 100 mg/kg were more prominent in males, as in the 52-week study. There were no drug-related increases in tumor incidence, latency, or degree of malignancy. Tumor types were characteristic of the species and strain, and the incidences were within spontaneous background rates. Hemangiomas of the mesenteric lymph node were observed in three high-dose females and in one untreated control female. Peto analysis (Peto et al., 1980) indicated a significant increase (p<0.01) in this tumor type only, approximating the expected frequency of false positive results. Therefore, the occurrence of hemangiomas in females was not considered biologically significant.

Reproductive toxicology studies

A fertility study was conducted in rats orally at 10, 50, and 100 mg/kg/day. Males were treated for 60 days before mating until termination of gestation of the bred females. Females were treated for 14 days before mating until
Table 1. 104-week carcinogen bioassay of quinapril hydrochloride in mice tumor incidence summary.

<table>
<thead>
<tr>
<th>Dose Levels (mg/kg/day)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC</td>
<td>VC</td>
<td>Low Dose</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

No. Animals Starting
- Male: 50
- Female: 50

No. of Autosied at Termination of Experiment
- Male: 47
- Female: 47

No. of Unscheduled Autopsies
- Male: 3
- Female: 9

Mean Time to Unscheduled Autopsies (Weeks)
- Male: 89
- Female: 87

Variation in Time to Unscheduled Autopsies (Weeks)
- Male: 73-102
- Female: 73-102

No. Animals Surviving Through
- 12 months (Week 52): 50
- 15 months (Week 65): 50
- 18 months (Week 78): 49
- 21 months (Week 91): 49
- 24 months (Week 104): 47

Total Number of Tumors
- Male: 37
- Female: 42

Tumor Bearing Animals
- Male: 30
- Female: 30

Incidence/No. Animals Starting
- Male: 0.7
- Female: 0.8

Incidence/Tumor Bearing Animal
- Male: 1.2
- Female: 1.4

Total Number of Malignant Tumors
- Male: 17
- Female: 19

Malignant Tumor Bearing Animals
- Male: 15
- Female: 17

Incident/No. Animals Starting
- Male: 0.3
- Female: 0.4

Incidence/Tumor Bearing Animal
- Male: 1.1
- Female: 1.1

Most Common Tumors
- Lung Adenoma: 5
- Liver Adenoma: 5
- Liver Carcinoma: 10
- Lymphoma: 0
- Pituitary Adenoma: 0

Tumors of Potential Target Organs
- Kidney: Adenoma or Carcinoma: 0
- Lung: Carcinoma: 2
- GI tract (Primary Origin): 0

UC-untreated control; VC-vehicle control
Table 2. 104-week carcinogen bioassay of quinapril hydrochloride in rats tumor incidence summary.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td></td>
<td>UC</td>
<td>VC</td>
</tr>
<tr>
<td>Dose Levels (mg/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. Animals Starting</td>
<td>65</td>
<td>65</td>
</tr>
<tr>
<td>No. of Autosied at Termination of Experiment</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>No. of Unscheduled Autopsies</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Mean Time to Unscheduled Autopsies (Weeks)</td>
<td>83</td>
<td>67</td>
</tr>
<tr>
<td>Variation in Time to Unscheduled Autopsy (Weeks)</td>
<td>47-104</td>
<td>9-104</td>
</tr>
<tr>
<td>No. Animals Surviving Through 12 months (Week 52)</td>
<td>64</td>
<td>57</td>
</tr>
<tr>
<td>15 months (Week 65)</td>
<td>62</td>
<td>56</td>
</tr>
<tr>
<td>18 months (Week 78)</td>
<td>55</td>
<td>54</td>
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<tr>
<td>21 months (Week 91)</td>
<td>48</td>
<td>50</td>
</tr>
<tr>
<td>24 months (Week 104)</td>
<td>39</td>
<td>42</td>
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<tr>
<td>Total Number Tumors</td>
<td>60</td>
<td>67</td>
</tr>
<tr>
<td>Tumor Bearing Animals</td>
<td>44</td>
<td>43</td>
</tr>
<tr>
<td>Incidence/No. Animals Starting</td>
<td>0.9</td>
<td>1.0</td>
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<tr>
<td>Incidence/Tumor Bearing Animal</td>
<td>1.4</td>
<td>1.6</td>
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<tr>
<td>Total Number Malignant Tumors</td>
<td>20</td>
<td>21</td>
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<tr>
<td>Malignant Tumor Bearing Animals</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Incidence/No. Animals Starting</td>
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</tr>
<tr>
<td>Incidence/Tumor Bearing Animal</td>
<td>1.1</td>
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Most Common Tumors

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<tr>
<td>Pituitary Adenoma</td>
<td>17</td>
<td>22</td>
<td>19</td>
<td>17</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>Subcutaneous Fibroma</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>0</td>
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<tr>
<td>Islet Adenoma</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>4</td>
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<tr>
<td>Lymphoma</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
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<td>3</td>
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<tr>
<td>Astrocytoma</td>
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<td>2</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Mammary Fibroadenoma</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
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<tr>
<td>Mammary Carcinoma</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Uterine Polyp</td>
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<td>0</td>
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<td>0</td>
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Tumors of Potential Target Organs

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<tbody>
<tr>
<td>Kidney : Adenoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Kidney : Carcinoma</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lung : Adenoma</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GI tract (Primary Origin)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Liver : Adenoma</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Liver : Carcinoma</td>
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<td>1</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>Liver : Cholangioma</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Lymph Node Hemangioma</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

UC-untreated control ; VC-vehicle control
* Statistically significant (Tarone, 1975 ; Tarone and Ware, 1975)
* Statistically significant in peto Test (IARC Monographs, 1980).
weaning of their offspring. No adverse effects on fertility or general reproductive performance of the parental generation or the progeny were noted, and no external abnormalities of the second generation fetuses were observed.

Teratology studies of quinapril were conducted in pregnant rats and rabbits. No fetotoxicity or teratogenicity was demonstrated in pregnant rats dosed orally with 50, 150, and 300 mg/kg/day during the critical period of organogenesis. Reduced maternal weight gain was seen at 150 and 300 mg/kg but this finding did not affect the fetuses.

In contrast with rats, rabbits were exquisitely sensitive. Severe maternal and fetal toxicity were observed in an initial dose-range finding study with quinapril given orally 10 to 400 mg/kg/day. In an additional dose-range finding study, abortions and maternal death occurred at 4, 6, and 8 mg/kg/day, and maternal and fetal toxicity occurred at ≥ 1 mg/kg. In the definitive study, rabbits dosed orally with 0.5, 1.0, and 1.5 mg/kg/day showed no teratogenicity. Maternal weight loss and postimplantation loss (embryotoxicity) occurred at 1 and 1.5 mg/kg. Maternal toxicity observed in rabbits with quinapril has also been noted with other ACE inhibitors and has been attributed to marked hypotension and electrolyte depletion (United States Food and Drug Administration, 1981, 1983, 1986; Overturf et al., 1985; Broughton Pipkin et al., 1980, 1982). With captopril, placental blood flow was reduced (Ferris and Weir, 1983). Enalapril also demonstrated maternal and embryotoxicity, which was reduced by salt administration (United States Food and Drug Administration, 1986). In general, effects in rabbits with ACE inhibitors occur at low doses, comparable to those administered to human.

Quinapril was administered to rats by gavage at 25, 75, and 150 mg/kg from gestation Day 15 through parturition and lactation. Mean pup body weight was decreased in all drug-treated groups at each interval of the postnatal period, except in the 25 mg/kg group at birth. This effect on pup weight gain is observed with other ACE inhibitors and is not considered of toxicologic importance, since no developmental abnormalities were noted following quinapril administration in any reproductive study in the rat.

Quinapril was not teratogenic and had no effects on fertility, mating capacity, or reproductive potential. Perinatal effects consisting of body weight reduction were noted but had no associated developmental effects.

**Mutagenicity studies**

The mutagenic potential of quinapril was evaluated in a number of in vitro assays in bacterial and mammalian cells. The in vivo mouse micronucleus assay and rat bone marrow structural chromosomal aberration assays were negative. The following in vitro assays also were negative: Salmonella typhimurium mutation test, mammalian cell point mutation assay, and mammalian cell sister chromatid exchange assay. A clastogenic effect of quinapril at cytotoxic doses of 1.4 and 1.6 mg/mL with metabolic activation was noted in the in vitro V79 chromosome aberration assay. This test result was not considered biologically significant in view of the high doses needed to induce an increase in aberrations relative to peak drug levels obtained in vivo; the small magnitude of the increase; and the lack of clastogenic effects in the mouse and rat in vivo assays.

**DISCUSSION**

The toxicity profile of quinapril is similar to that of other ACE inhibitors. Renal changes are prominent (United States Food and Drug Administration, 1981, 1983, 1986). Increased plasma renin and increased size and granularity of juxtaglomerular cells in the kidney were noted with quinapril in all species tested. Renal tubular dilatation was observed at a high dose in an acute study in dogs, and renal tubular degenerative changes were observed in chronic rodent studies at approximately 20 to 30 times the human dose. In dogs, slight to mild increases in BUN at doses in excess of 60 times the human dose were not associated with increases in creatinine or histologic evidence of renal toxicity and were attributed to prerenal causes.

Severe effects consisting of anemia and thrombocytopenia or leukopenia with bone marrow hypocellularity, observed in dogs given captopril, were not observed with quinapril (Ohtaki et al., 1981). Reduced heart weight, probably
due to lower cardiac workload, were observed in preclinical studies with quinapril and other ACE inhibitors, but were without pathologic myocardial changes (Bagdon et al., 1985).

Emesis was the dose-limiting clinical manifestation in dogs given quinapril. As noted with other ACE inhibitors (United States Food and Drug Administration, 1981, 1983, 1986), gastric erosions and ulcers were noted with quinapril in dogs and rats, with the gastrointestinal irritation attributed to local effects. Hepatotoxic potential of quinapril is very low; minor hepatic changes were noted in three dogs treated for 13 to 52 weeks with quinapril at doses 60 to 80 times the human dose.

Quinapril was not teratogenic or fetotoxic and had no effect on fertility, mating capacity, or reproductive potential in rat studies. Maternal toxicity observed with quinapril in the rabbit has also been noted with other ACE inhibitors and has been attributed to marked hypotension and electrolyte depletion. (United States Food and Drug Administration, 1981, 1983, 1986; Broughton et al., 1982). Offspring weights were reduced in the rat perinatal-postnatal study with quinapril. However, this finding was not noted with dosing throughout gestation and lactation or during the critical period of organogenesis. Therefore, reduced offspring weight observed in this study occurred only when the compound was administered late in gestation.

Quinapril is neither carcinogenic nor mutagenic, and a positive result in an in vitro chromosome aberration assay observed only at cytotoxic doses was not considered biologically significant.

CONCLUSION

With the exception of effects on the hematopoietic system noted in dogs given high doses of captopril, the toxicity profile of ACE inhibitors is similar. The acute toxicity of quinapril is low in rodents. Repeated dosing elicits gastric irritation, renal effects involving JGA hypertrophy and hyperplasia and tubular degenerative changes, and reduced red cell parameters and heart weight in rodents and/or dogs. These manifestations of toxicity, as well as hepatic lesions in dogs, reduced offspring weight in rats, marked sensitivity of the rabbit, and clastogenic effects at cytotoxic doses in the in vitro chromosome aberration assay, have been reported with drugs of this class.

REFERENCES


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