Cardiac and Pulmonary Effects of High Doses of Cyclophosphamide and Isophosphamide

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INTRODUCTION

Cyclophosphamide has been used for several years in the treatment of cancer. The usual dosage is 1 to 3 mg/kg daily or 10 to 15 mg/kg weekly, but, since there appear to be advantages in intermittent, massive dosage, as much as 120 to 240 mg/kg have been given over 2- to 4-day periods. In 5 of 30 such courses, electrocardiographic changes were observed (5), and 1 patient died with hemorrhagic myocarditis.

Dogs given isophosphamide died between 4.5 and 18 hr later with evidence of myocardial damage but without pulmonary edema. The absence of pulmonary edema with isophosphamide may be due to the fact that its metabolism differs quantitatively from that of cyclophosphamide or to failure to form a specific edema-producing product.

SUMMARY

It has been reported that patients given 240 mg cyclophosphamide per kg over a 4-day period have shown electrocardiographic abnormalities, and 2 deaths accompanied by myocardial necrosis have been recorded. In view of these reports, dogs were given 500 mg cyclophosphamide per kg (an approximately equivalent dose on a surface area basis) in a single injection. They showed electrocardiographic evidence of myocardial damage (falling ventricular voltage and prolonged Q-T interval) and died in 4 to 6 hr of acute pulmonary edema. Postmortem examination showed early hemorrhagic myocarditis.

Dogs given isophosphamide died between 4.5 and 18 hr after drug administration, with evidence of myocardial damage but without pulmonary edema. These findings indicated that such high doses of cyclophosphamide could cause cardiac damage, we carried out experiments in which dogs were given single doses equivalent on a surface area basis to those given over a 4-day period in these patients, and we monitored the resulting physiological changes in an attempt to analyze the course of events. We also examined the cardiopulmonary effects of isophosphamide, a clinically promising agent that differs in structure from cyclophosphamide.

MATERIALS AND METHODS

Ten mongrel dogs, of either sex, weighing 6 to 12 kg, were anesthetized with a single dose of 30 mg veterinary Nembutal per kg. The right femoral artery and vein were exposed and cannulated. The arterial cannula was connected to a pressure transducer and recorder. The venous cannula was advanced to a central position for monitoring central venous pressure. A catheter was placed in the right ventricle through a right external jugular vein cut down. Another catheter was placed in the left femoral vein for collecting peripheral venous blood, and a Foley catheter was placed in the bladder through a suprapubic cystostomy for collecting urine. About 500 ml of Ringer's lactate were given i.v. during the experiment to keep the catheters open and the animal adequately hydrated. The tendency of the body temperature to fall during the experiment, due presumably to barbiturate anesthesia, was countered by use of thermal blankets, so as to prevent interference with drug metabolism by hypothermia.

The dogs generally survived 4 to 6 hr after drug administration and, in most animals, the following observations were made or appropriate specimens were taken before the drugs were given and at 0.5-hr or 1-hr intervals thereafter: general condition; rectal temperature; respiratory rate; arterial blood pressure; central venous pressure; electrocardiogram (standard lead II); arterial blood serum glutamic oxaloacetic transaminase, hydroxybutyric acid dehydrogenase, and creatine phosphokinase; serum sodium, potassium, calcium, bicarbonate, and chloride; and hemoglobin, total leukocytes, and platelets.

After death of the dog, a general postmortem examination was made and portions of lung, heart, skeletal muscle, spleen, kidney, urinary bladder, and ileum were fixed in formalin and paraffin embedded for histological examination by light microscopy. Three dogs were used as histological controls; 2 were untreated and 1 was kept under Nembutal anesthesia for 5.5 hr before being killed with Nembutal.
Cardiopulmonary Effects of Cyclophosphamide

Pure cyclophosphamide and isophosphamide (Ward, Blenkinsop, Ltd., Wembley, Middlesex, United Kingdom; and Mead-Johnson Research Centre, Evansville, Ind. 47721) were dissolved in 0.9% NaCl solution at 37° immediately before injection to make 4% (w/v) solutions. Seven dogs were given 500 mg cyclophosphamide per kg, and 3 were given 500, 820, and 1500 mg isophosphamide per kg, respectively. All injections were given slowly i.v.

Details of the individual animals are given in Table 1. Two dogs that died during injections of 500 mg cyclophosphamide per kg and 1000 mg isophosphamide per kg, respectively, are excluded from further consideration.

RESULTS

Clinical Course

In the dogs given cyclophosphamide no significant clinical findings were noted until about 3.5 to 5 hr after drug administration, when rales became audible over the lung fields. These increased in intensity for about 0.5 hr and then all dogs but 1 developed a massive pulmonary edema of dramatic and sudden onset, in which white, frothy fluid poured out of the endotracheal tube and the animal died within 2 to 5 min. The 1 exception (Dog 3) died of respiratory arrest without pulmonary edema 6.25 hr after the drug was given.

None of the 3 dogs given isophosphamide developed pulmonary edema. Dog 8, given 500 mg/kg, showed no clinical changes during an observation period of 6.5 hr and died overnight between 7 and 18 hr after drug administration. The animals given 820 and 1500 mg/kg died of sudden respiratory arrest at 380 and 270 min, respectively.

Respiratory Rate

Dogs given cyclophosphamide generally had steady respiratory activity until 1 to 2 hr before death, when their respiratory rate increased progressively and markedly and their breathing became labored with a prolonged and forced expiratory phase. Wheezing was never heard. Dogs given isophosphamide showed no changes in respiration until they suddenly became apneic and died.

Arterial Blood Pressure

Several dogs showed hypotension while the drug was being given, but this could be reduced by slowing the rate of injection. In most animals, arterial pressure was then maintained for the next 3 to 4 hr, but it fell by 25 to 80 mm Hg in the 1 to 2 hr before death. Other animals, especially those given isophosphamide, showed differing sequences of events. In Dog 3, pressure fell progressively from the outset; Dogs 4, 9, and 10 showed only partial recovery from a marked initial hypotension; and Dog 8 recovered from hypotension and showed no further changes during the observation period of 6.5 hr (Chart 1).

Central Venous Pressure

Central venous pressure rose initially in all dogs, presumably because the intravascular volume increased following administration of the drug solutions (12.5 ml/kg in the case of cyclophosphamide and 12.5 to 37.5 ml/kg in the case of isophosphamide). The pressure then generally returned to control levels and fluctuated around these until 1 to 2 hr before death, when it usually fell. Dog 10 was exceptional in showing a progressive rise in venous pressure during the 2 hr before death.

Electrocardiogram

All dogs developed a transient bradycardia (80 to 120/min) during drug administration. No other significant change in heart rate occurred before death. Normal sinus rhythm was maintained in all but 2 dogs on cyclophosphamide, which showed intermittent episodes of premature ventricular contractions in the hour before death. All dogs except 1 (Dog 9) showed a marked fall in voltage of the QRS (ventricular) complex after the 1st 2 to 3 hr. Death usually occurred 30 to 90 min after the voltage began to fall (Chart 2). The earliest fall was noted at 180 min (Dog 6) and the latest at 330 min (Dog 5). Dog 9 was exceptional in showing no significant change during the observation period but voltage was unusually low (0.6 mV) in this animal from the outset. Q-Tc3 intervals tended to lengthen in animals.

The abbreviation used is: Q-Tc, corrected Q-T.

Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Wt (kg)</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Injection time (min)</th>
<th>Survival (min)</th>
<th>Mode of death</th>
</tr>
</thead>
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<td>240</td>
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<td>10</td>
<td>255</td>
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<tr>
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<td>330</td>
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<tr>
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<tr>
<td>8</td>
<td>9</td>
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<td>10</td>
<td>&gt; 420 &lt; 1080</td>
<td>Pulmonary edema</td>
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<td>380</td>
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<tr>
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<td>Isophosphamide</td>
<td>1500</td>
<td>60</td>
<td>270</td>
<td>Respiratory arrest</td>
</tr>
</tbody>
</table>

1 The abbreviation used is: Q-Tc, corrected Q-T.
This rise may have been due to muscle damage during the initial surgical procedures. Levels of this enzyme did not change further during the experiment.

Serum Electrolytes

Preinjection values of serum sodium, potassium, calcium, chloride, and bicarbonate were normal and no significant changes occurred during the experiment.

Hemoglobin, White Cells, and Platelets

These were measured in the 1st 4 dogs given cyclophosphamide and the 1st 2 given isophosphamide. Changes in hemoglobin levels were unimportant except in Dogs 1 and 2, which showed terminal rises from 14.6 to 15.5 g/100 ml and from 15.2 to 18.2 g/100 ml, respectively, presumably due to loss of fluid into the lungs. The white cell count generally tended to rise slightly, the mean preinjection count being 11,100 ± 1,350/cu mm and the mean at 5 hr being 14,000 ± 2,400/cu mm. The platelet count showed no significant change in any dog.

Postmortem Examination

In all dogs given cyclophosphamide except Dog 3, the hearts were dilated and flabby and their cut surfaces appeared edematous. In several, there were small subepicardial and subendocardial ecchymoses. The lungs were distended with edema fluid and failed to collapse when the chest was opened. White, frothy fluid poured from the bronchi and the cut lung surface. The urinary bladders were edematous and their mucosae were hemorrhagic.

Dogs given isophosphamide showed similar bladder lesions but in only 1 of the 3 (Dog 8) were cardiac ecchymoses seen. None of these dogs showed any evident lung abnor-

Arterial Blood Gases

Preinjection and some of the early postinjection arterial blood samples occasionally showed mild and transient hypoxia (pO₂ = 80 mm Hg) and respiratory acidosis (pCO₂ = 50 mm Hg, pH = 7.3), but there were no significant alterations in blood gases during the rest of the experiment. This early respiratory depression was probably due to barbiturate anesthesia.

Serum Enzymes

Serum glutamic oxaloacetic transaminase and hydroxybutyric acid dehydrogenase levels were normal in the preinjection specimens of blood and remained so during the period of observation. Creatine phosphokinase levels were raised in the preinjection specimens (67 to 180, mean 88 units/ml). This rise may have been due to muscle damage during the initial surgical procedures. Levels of this enzyme did not change further during the experiment.

Chart 1. Changes in arterial pressure in dogs given cyclophosphamide (●) or isophosphamide (○). The numbers refer to the individual animals in Table 1.

given cyclophosphamide from 0.277 ± 0.025 sec initially to 0.338 ± 0.016 sec at 4 hr. However, this change was not uniform; in some animals (Dogs 1 and 4) the Q-Tₚ interval did not change during the period of observation whereas in others (Dogs 2 and 3) it nearly doubled in 4 to 5 hr. Two dogs given isophosphamide showed small and inconsistent changes in the Q-Tₚ interval and, in the 3rd, it could not be determined because of indistinctness of the T-waves.

The length of the P-R interval did not change significantly. S-T segments generally remained isoelectric but almost all dogs showed minor elevations or depressions terminally. Fig. 1 shows a representative series of recordings (Dog 6).

Chart 2. Changes in voltage of the QRS complex in dogs given cyclophosphamide (●) or isophosphamide (○).
mality. No other organ in either group of treated animals appeared to be abnormal.

**Histology**

**Heart.** Of the animals given cyclophosphamide, Dogs 1 and 4 showed intramural and subendocardial hemorrhages in the left ventricular papillary muscles, with extravasation of polymorphonuclear leukocytes (Fig. 2). Dogs 5 and 6 showed only congestion of capillaries in the left ventricular muscle and Dogs 2 and 3 showed no obvious abnormality. Dog 8, which was found dead 18 hr after being given isophosphamide, showed severe changes (Fig. 3). There was widespread patchy eosinophil change in ventricular muscle fibers, with loss of striation and sometimes fragmentation. Hemorrhage had occurred beneath the endocardium and intramurally and, in the areas of muscle degeneration, the capillaries were congested and crowded with polymorphonuclear leukocytes. These changes were most pronounced in the left ventricle but were also seen to a lesser extent in the right ventricle. The 2 other dogs given isophosphamide showed only congestion of the left ventricular intramural capillaries.

**Lungs.** In animals given cyclophosphamide, patches of intraalveolar edema were irregularly distributed throughout the lung, as shown by amorphous eosinophilic material in the alveoli and bronchioles (Fig. 4). These appearances were conspicuous in Dogs 4, 5, and 6. No other significant abnormalities were seen.

Dog 8, which received isophosphamide, showed a marked patchy congestion of alveolar capillaries and early postmortem changes in the bronchial mucosa. Otherwise, no abnormalities were noted in isophosphamide-treated animals.

**Urinary Bladder.** All dogs given cyclophosphamide or isophosphamide showed cystitis, with submucosal edema and congestion and a little hemorrhage and polymorphonuclear leukocyte extravasation. In the most severely affected bladders, much of the epithelium had disappeared.

**Kidney.** In most dogs the only abnormalities in the kidney proper consisted of congestion of the glomerular capillaries with a slight excess of polymorphonuclear leukocytes. Dog 8 showed necrosis of tubules in the junctional zone (at least some of this may have occurred post mortem). Dogs 2, 4, and 8 showed acute inflammation of the renal pelvic mucosa, but this was conspicuous only in Dog 8.

**Other Organs.** Active germinal centers in the spleen and intestine showed necrotic changes. Otherwise, no material abnormality was seen in the other organs examined. No significant lesions were seen in the control dogs.

**DISCUSSION**

We have shown that dogs given single large doses of cyclophosphamide die within 4 to 6 hr from acute pulmonary edema. The question as to whether the causal lesion is cardiac, pulmonary, or both cannot be answered with confidence at present. In favor of a cardiac origin there is, first, the fact that myocardial lesions were shown histologically and electrocardiographically, and their nature suggests that they were unlikely to be secondary to pulmonary changes. Second, the fact that the cyclophosphamide analog, isophosphamide, caused myocardial lesions without evident pulmonary changes also favors the hypothesis that the myocardial damage produced by this class of agents is primary. Third, there were no alterations in blood gases to suggest failure of pulmonary function before the onset of edema. In favor of a pulmonary origin, we observed that, in most animals in which a clear sequence of events could be made out, a significant rise in respiratory rate generally preceded changes in the electrocardiogram or in arterial pressure. Another finding suggesting a primarily pulmonary origin for the edema was the bloodless nature of the edema fluid. In lung edema due to acute cardiac failure in man, the fluid generally contains red cells. In these dogs the fluid was white and resembled that produced in pulmonary edema caused by exposure to toxic gases. Finally, once the drug injection had been completed, there was no rise in central venous pressure to suggest cardiac failure, except in 1 dog. However, if failure had been mainly left ventricular, it would not necessarily have been reflected in the central venous pressure.

On balance, it seems that the pulmonary edema caused by massive doses of cyclophosphamide can be explained by a combination of acute heart failure due to myocardial damage and increased permeability of damaged lung capillaries, although our evidence for pulmonary damage is inconclusive.

It has been shown that large doses of cyclophosphamide have an antidiuretic effect and cause a transient water intolerance in rats, dogs, and man (7, 14, 15). If this had played a significant role in the production of pulmonary edema in our dogs, we would have expected evidence of hemodilution and hypervolemia whereas, in fact, serum sodium levels were unchanged, the hematocrit remained constant or rose, the blood pressure fell, and the central venous pressure fell after its initial rise.

The nature of the myocardial lesion is obscure. The electrocardiographic changes (fall in ventricular voltage, lengthening of the Q-T interval and T-wave changes) cannot be attributed to disturbances of plasma electrolytes, for these remained normal throughout. They resemble those found in myocarditis and quinidine toxicity. The fact that we found no changes in serum cardiac enzymes can be explained by death occurring before substantial leakage of enzymes from the muscle cell could take place. Alternatively, the lesion might have been similar to that seen in quinidine toxicity, in which the electrical and mechanical properties of the myocardial cell membrane are altered, although its structure appears to be unimpaired. Levels of creatine phosphokinase were raised from the outset in these dogs, presumably because of damage to skeletal muscle during the initial surgical preparation, and this may have obscured later rises from a myocardial source.

Cyclophosphamide is activated by microsomal mixed function oxidases, mainly in the liver, which convert it to an as yet imperfectly defined series of toxic alkylating metabolites (2, 6, 10, 12, 13). Oxidation first produces 4-hydroxycyc-
cyclophosphamide and aldoephosphamide, in equilibrium with each other. These highly toxic metabolites are converted by aldehyde oxidase or aldehyde dehydrogenase to the considerably less toxic carboxyphosphamide. The 900 x g supernatant of lung homogenate has about 20% of the cyclophosphamide-activating potential of a similar preparation from liver but, unlike liver, does not convert the early highly toxic metabolites to carboxyphosphamide (10).

Isoephosphamide is also metabolized by the hepatic mixed-function oxidase system to produce active alkylating substances that have not so far been identified with certainty (1, 4, 9). Extrahepatic metabolism has not yet been reported. This drug did not cause pulmonary edema in the experiments on dogs described here. In mice also, lethal doses of cyclophosphamide cause lung edema but equally toxic doses of isophosphamide do not (3). Isoephosphamide differs from cyclophosphamide in that 1 of the chloroethyl side chains is attached to the ring nitrogen. Therefore, the alkylating materials that are produced when it is metabolized must differ from those produced during the metabolism of cyclophosphamide and, in particular, they cannot include aldoephosphamide, which is believed to be the main toxic metabolite of cyclophosphamide (6, 10, 12, 13) although production of the analogous aldoephosphamide has been suggested (9). Possibly, therefore, pulmonary edema caused by cyclophosphamide is due to local production of aldoephosphamide, which is not detoxified in the lung as it is in other tissues (10), or to some other metabolite not produced during the metabolism of isophosphamide. On the other hand, it is possible that a lung-damaging agent, perhaps aldoephosphamide, is formed during the metabolism of isophosphamide but that, in the doses used here, it does not reach the level required to cause acute pulmonary edema. It should be noted that, dose for dose, isophosphamide gives rise to only one-half as much nitrobenzylpyridine-reactive material as cyclophosphamide (4) and that its $K_m$ for microsomal oxidation is greater (1, 9). These quantitative differences may be sufficient to explain our failure to produce pulmonary edema with isophosphamide.

With the exception of Dog 8, given isophosphamide, all animals died within a relatively restricted time span (240 to 395 min), so that a correlation between time of survival and the morphological changes found can hardly be expected. The severity of the lesions seen in Dog 8 must be attributed to about 250 mg/kg in an adult man (8). It is not surprising, therefore, that many patients given 240 mg cyclophosphamide per kg within 4 days have shown electrocardiographic changes and that some have died with myocardial necrosis. In view of the risk of cardiac damage, dose regimens of this intensity would appear to be unjustified. Our findings suggest that patients considered for intensive therapy with cyclophosphamide or its analogs should be carefully examined for preexisting cardiac or pulmonary disease and that cardiopulmonary function should be closely monitored in those who receive such treatment. It appears that isophosphamide may be less liable than cyclophosphamide to produce acute pulmonary lesions, but further investigations are needed on this point.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Electrocardiogram (standard lead II) in Dog 6, given 500 mg cyclophosphamide per kg, showing falling QRS voltage and changes in S-T segment. Recordings made before injection (C) and at 0.5-hr intervals until death at 5.5 hr.

Fig. 2. Tip of a left ventricular papillary muscle of Dog 1, which died 240 min after receiving 500 mg cyclophosphamide per kg, showing degenerative changes in muscle and infiltration with polymorphonuclear leukocytes H & E, x 450.

Fig. 3. Subendocardial region of left ventricle of Dog 8, which died between 7 and 18 hr after receiving 500 mg isophosphamide per kg, showing hemorrhages. H & E, x 450.

Fig. 4. Lung of Dog 4, which died 310 min after receiving 500 mg cyclophosphamide per kg, showing intraalveolar edema. H & E, x 280.
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