Cardiac Damage Induced by 2-Amino-3-methylimidazo[4,5-f]quinoline in Nonhuman Primates

Unnur P. Thorgeirsson,1 Andrew Farb,2 Renu Virmani,2 and Richard H. Adamson1

1Division of Cancer Etiology, National Cancer Institute, Bethesda, MD 20892 USA; 2Armed Forces Institute of Pathology, Washington, DC 20306-6000 USA

The heterocyclic aromatic amine 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) is a potent hepatocarcinogen in cynomolgus and rhesus monkeys. The finding of high cardiac IQ–DNA adduct levels prompted a histopathological study of perfusion-fixed hearts from 10 tumor-bearing monkeys chronically dosed with IQ at 10 mg/kg or 20 mg/kg 5 days per week for 48–80 months. Two monkeys dosed only with the vehicle for IQ, hydroxypropylcellulose, served as controls. All the monkeys had normal heart weights, and no abnormalities were observed upon gross inspection of the hearts. Microscopically, focal myocardial lesions were observed in 8 of 10 monkeys dosed with IQ. Light microscopic abnormalities included myocyte necrosis with or without chronic inflammatory infiltrates, interstitial fibrosis with myocyte hypertrophy or atrophy, and vasculitis. Electron microscopic findings included disruption of the mitochondrial architecture (i.e., mitochondrial swelling and clearing of matrix densities), myofibrilar loss, disorganization of the normal alignment of sarcomeres, and occasional myocytes showing nuclear hypertrophy or peripheral clumping of the nuclear chromatin. There was some correlation between the cumulative dose of IQ and the extent of the myocardial abnormalities. These findings suggest that chronic exposure to IQ can lead to myocardial damage in monkeys. Although focal and not associated with clinical evidence of heart failure, these abnormalities may represent the initial stages of IQ-induced toxic cardiomyopathy. Key words: 2-amino-3-methylimidazo[4,5-f]quinoline, cytotoxicity, heterocyclic amines, monkeys, myocardium. Environ Health Perspect 102:194–199(1994)

Highly mutagenic heterocyclic aromatic amines (HAAs) are formed during frying, broiling, and barbecuing of meat (1–3). Metabolic activation, involving N-hydroxylation, is required for HAAs to exert their genotoxic effects (4). Before addition to DNA nucleotides, many carcinogens require metabolic activation to reactive species (5). The N-hydroxy-metabolite of the HAA 2-amino-3-methylimidazole[4,5-f]quinoline (IQ) has been shown to bind directly to DNA at physiological pH (6). IQ is a potent carcinogen in rodents and monkeys, inducing tumors at various sites in rats and mice and hepatocellular carcinomas in cynomolgus monkeys (7–10).

DNA adducts are generally associated with the initiation of the carcinogenic process by HAAs, but less attention has been given to their possible noncarcinogenic effects. The discovery of high IQ-DNA cardiac adducts by 32P-postlabeling analysis prompted us to undertake a histological study on hearts from tumor-bearing monkeys chronically dosed with IQ (11). Preliminary reports of this study have been published in abstract form (12). The myocardial abnormalities described here closely resemble the early pathological lesions associated with Adriamycin (doxorubicin)-induced cardiotoxicity (13). Doxorubicin is an anthracycline antibiotic that binds to DNA and induces DNA strand breaks, which have been shown to correlate with its cytotoxic action (14–16). Recent in vitro studies of doxorubicin analogues have demonstrated that they complex with DNA resulting in transcriptional blockages (17). Considering that IQ has been shown to covalently bind to DNA (11) and induce DNA strand breaks in mammalian cells (18), it is possible that similar mechanisms are involved in the induction of myocardial lesions by IQ and doxorubicin.

Materials and Methods

The two monkey species used in this study were Macaca fascicularis (cynomolgus) and Macaca mulatta (rhesus). They were born in a closed monkey colony located at a contract facility near the National Cancer Institute. As newborns, they remained with their mothers for the first 6 months, until they were weaned. The diet consisted of high protein Purina chow (5045 Standard), vitamin mixture spread on sandwich, and apples (9). Dosing with IQ was started at 1 year of age. The monkeys were housed individually in stainless-steel cages in an AAALAC-accredited facility.

Ten tumor-bearing monkeys used in this histopathological study were part of a larger carcinogenesis study (10). They were dosed with IQ at 10 mg/kg or 20 mg/kg five times per week for a period ranging from 48 to 80 months (Table 1). Several of the animals continued to receive IQ after a tumor was diagnosed to observe other possible effects from IQ. IQ was suspended in hydroxypropylcellulose (HPC) and administered by nasogastric intubation. The two controls received only HPC. The monkeys were euthanized with an overdose of pentobarbital and the hearts were removed immediately and fixed by retrograde perfusion with a Trump-McDowell fixative (4% formaldehyde, 1% glutaraldehyde) for 60 min at 80–100 mm Hg. After the pressure perfusion, 1-mm3 sections were cut from the left (epicardial and endocardial halves) and right ventricles, dehydrated in graded alcohol, and embedded in epon for electron microscopy. One-micron–thick–sections were cut from at least three areas of the left ventricular epicardial and endocardial halves and from the right ventricle. Thin sections for electron microscopy were cut from at least three areas of each sample, stained with uranyl acetate and lead citrate, and examined with a Zeiss 109 microscope. The remainder of the heart was cut transversely from apex to base (five to six slices), immersion-fixed overnight in 10% neutral buffered formalin, and processed for light microscopy. All ventricular slices were embedded in paraffin, cut at 5 μm, and stained with hematoxylin-eosin and Masson trichrome.

Results

Before euthanasia, ECHO-cardiography was carried out on the monkeys dosed with IQ and the HPC treated controls. None of the animals examined exhibited cardiac abnormalities. We performed histopathological analysis on perfusion-fixed hearts from 10 monkeys chronically dosed with IQ and two HPC controls. No abnormalities were seen upon gross inspection of the hearts, and the heart weights of all the animals were within normal limits (Table 1). The average dosing period for the group was 62.2 months (range 48–80 months), the average cumulative dose of IQ was 53.53 g (range 24.58–91.10 g), and the average cumulative dose/kg was 19.15 g (range 12.20–29.44 g; Table 1). For two of the animals, there was correlation between the dosing period and/or the cumulative dose and the extent of myocardial damage (Tables 1–3). For example, the animal (case 1) with the highest cumulative dose (91.10 g) had the largest variety of myocardial lesions. Conversely, the animal (case 5) with the lowest cumulative dose (24.58 g) and the shortest dosing period (48 months) had normal myocardial histology.

Address correspondence to R. H. Adamson, Division of Cancer Etiology, National Cancer Institute, Building 31, Room 11A03, Bethesda, MD 20892 USA.

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um by light microscopy and the only electron microscopy finding was focal myofibrillar loss. For the other animals, the cumulative dose correlated better than the dosing period with the myocardial abnormalities. As stated above, all of the monkeys included in this study had malignant liver tumors. Therefore, the myocardial lesions could possibly be secondary to the liver malignancies rather than IQ-mediated damage. Although it cannot be categorically ruled out at this point, it is unlikely that the liver tumors were responsible for the myocardial lesions because the lesions did not correlate with the number or size of the tumor nodules. Only one of the monkeys in this study (case 1) had numerous primary tumor nodules and metastases (pulmonary), the others had nonmetastatic hepato-cellular carcinoma with well-defined tumor nodules ranging in numbers from two to six. All the monkeys dosed with IQ had one or more abnormal findings by light microscopy, electron microscopy, or both. The two control animals that received only HPC, the vehicle for IQ, had normal cardiac morphology.

We detected focal light microscopic lesions in 8 of 10 hearts examined (Table 2). Multiple histological sections, stained with hematoxylin and eosin or Masson trichrome, were studied from each heart.

### Table 1. Characteristics of monkeys studied for cardiac pathologic changes

| Case no. | Animal no. | Sex | Birth date | IQ (mg/kg) | Dosing period (months) | Cumulative dose (g) | Cumulative dose/kg (g) | Body weight (kg) | Heart weight (g) |
|----------|------------|-----|------------|------------|------------------------|---------------------|---------------------|-----------------|-----------------|
| 1        | 1423CC     | F   | 4/85       | 20         | 61                     | 91.10               | 26.71               | 6.0             | 30.90           |
| 2        | 1427CC     | M   | 5/85       | 20         | 60                     | 70.24               | 24.17               | 5.8             | 18.00           |
| 3        | 1433CC     | M   | 11/86      | 10         | 53                     | 40.06               | 12.20               | 6.5             | 23.95           |
| 4        | 1393BB     | M   | 7/84       | 10         | 70                     | 52.93               | 14.96               | 5.5             | 19.05           |
| 5        | 1428CC     | M   | 6/85       | 10         | 48                     | 24.58               | 14.56               | 5.7             | 22.33           |
| 6        | 1398BB     | F   | 7/84       | 10         | 72                     | 29.20               | 15.94               | 2.9             | 14.75           |
| 7        | 1430CC     | M   | 9/85       | 10         | 61                     | 41.22               | 14.41               | 5.4             | 19.72           |
| 8        | 1454DD     | M   | 6/86       | 10         | 52                     | 75.31               | 24.70               | 6.2             | 26.29           |
| 9        | 1432CC     | M   | 1/85       | 20         | 80                     | 63.25               | 29.44               | 5.2             | 14.84           |
| 10       | 1447DD     | M   | 3/86       | 10         | 65                     | 47.30               | 14.37               | 5.8             | 26.32           |

#### Controls

- 11 1487EE  M  5/87
- 12 1464DD  F  9/86

### Table 2. Light microscope findings

| Case no. | Animal no. | Fibrosis | MN | I/N | I | MH | Vasculitis |
|----------|------------|----------|----|-----|---|----|------------|
| 1        | 1423CC     | +        | +  | +   | - | +  | +          |
| 2        | 1427CC     | -        | +  | -   | - | +  | +          |
| 3        | 1433CC     | +        | +  | -   | + | -  | +          |
| 4        | 1393BB     | -        | -  | -   | - | +  | -          |
| 5        | 1428CC     | -        | -  | -   | - | -  | -          |
| 6        | 1398BB     | -        | -  | -   | - | -  | -          |
| 7        | 1430CC     | +        | +  | +   | + | -  | +          |
| 8        | 1454DD     | +        | +  | +   | + | -  | +          |
| 9        | 1432CC     | -        | -  | -   | - | -  | -          |
| 10       | 1447DD     | +        | +  | +   | + | -  | -          |

#### Controls

- 11 1487EE  -  -  -  -  -
- 12 1464DD  -  -  -  -  -

Abbreviations: MN, myocyte necrosis; I/N, inflammation associated with myocyte necrosis; I, inflammation without myocyte necrosis; MH, myocyte hypertrophy.

### Table 3. Electron microscopic findings

| Case no. | Animal no. | Lipid | ML | MN | MA |
|----------|------------|-------|----|----|----|
| 1        | 1423CC     | -     | +  | +  | +  |
| 2        | 1427CC     | +     | +  | +  | +  |
| 3        | 1433CC     | +     | -  | -  | -  |
| 4        | 1393BB     | -     | -  | -  | -  |
| 5        | 1428CC     | -     | +  | -  | -  |
| 6        | 1398BB     | +     | +  | -  | -  |
| 7        | 1430CC     | -     | +  | +  | +  |
| 8        | 1454DD     | +     | +  | +  | +  |
| 9        | 1432CC     | +     | +  | +  | +  |
| 10       | 1447DD     | -     | -  | -  | -  |

#### Controls

- 11 1487EE  -  -  -  -
- 12 1464DD  -  -  -  -

Abbreviations: ML, myofibrillar loss; MN, myocyte necrosis; MA, mitochondrial abnormalities.

Scattered foci of interstitial fibrosis, with myocyte hypertrophy and atrophy, were present in the hearts from four animals (cases 1, 3, 9, and 10; Fig. 1). The fibrosis was most prominent in the subendocardium. Five animals (cases 1, 3, 7, 9, and 10) had single-cell myocyte necrosis. In four animals (cases 1, 2, 8, and 10), myocyte necrosis was associated with inflammation (lymphocytes and macrophages), but inflammation was also observed without myocyte necrosis (cases 3, 6, 8, and 10), especially adjacent to intramural arterioles (Fig. 2). One heart (case 6) displayed occasional small foci of interstitial chronic inflammation without associated myocyte necrosis or fibrosis. In two hearts (cases 1 and 2), the walls of scattered arterioles, medium-sized muscular arteries, and veins were infiltrated by mixed acute and chronic inflammatory cells. The hearts from two of the test animals (cases 4 and 5) and the two control animals (cases 11 and 12), had normal histology at the light microscopic level.

We noted ultrastructural lesions in 9 of 10 hearts examined (Table 3). Examination of the myocardium revealed foci of myofibrillar loss and disorganization of the normal parallel alignment of sarcomeres in seven animals (cases 1, 2, 4, 5, 8, 9, and 10; Fig. 3A–C). In four of these animals (cases 1, 2, 9 and 10), necrotic myocytes were also present. Mitochondrial abnormalities were a frequent finding in seven of the IQ hearts (cases 1, 2, 3, 7, 8, 9, and 10). These varied from mild to moderate mitochondrial swelling, clearing of normal matrix densities, and disruption of mitochondrial cristae (Fig. 3D and 4). Amorphous mitochondrial matrix densities were seen in occasional myocytes in one case. Nuclear changes were seen in occasional cells and consisted of hypertrophy and peripheral clumping of the nuclear chromat. No capillary abnormalities were noted, but increased collagen deposition was present in the interstitium of the subendocardium. In the two animals (cases 4 and 5) with no light microscopic abnormalities, the only electron microscopic findings were myofibrillar loss. Normal ultrastructural architecture was displayed in one of the IQ animals (case 6) and in the two HPC controls (cases 11 and 12; Fig. 5).

### Discussion

The present findings suggest that chronic exposure to mutagenic dietary HAAs such as IQ can result in myocardial damage in nonhuman primates. The myocardial lesions were focal and not associated with ECHO-cardiographic abnormalities or evidence of congestive heart failure. Light microscopic lesions were detected in 80%...
of the perfusion-fixed hearts examined and ultrastructural abnormalities in 90% of the cases. IQ-induced liver pathology has been described elsewhere (9,10), but microscopic evaluation of other organs did not reveal any histopathological findings.

The structural myocardial abnormalities found in these macaques chronically dosed with IQ included focal myocyte necrosis, myocardial interstitial fibrosis, myofibrillar loss, and mitochondrial damage. These findings are consistent with the initial stages of toxic cardiomyopathy. A pathologic hallmark of toxic cardiomyopathy is the simultaneous presence of lesions in various stages of evolution (20). For example, acute myocyte necrosis, inflammation, and myocyte dissolution are seen concurrently with foci of interstitial fibrosis. In five of the monkeys dosed with IQ, myocardial lesions of various stages of development were present, suggestive of a cardiotoxic process. Toxic myocarditis that results in cardiomyopathy is a dose-dependent process and can be expected to be most severe in subjects exposed to the highest dose of the toxic agent (20). There was some correlation between the extent of myocardial damage and the cumulative dose in the monkeys that received the highest and the lowest dose of IQ, but the length of exposure to IQ correlated less favorably with the extent of cardiotoxicity. Why a dose–response relationship was not more apparent is unknown, but with additional animals and additional dose ranges, such a response may become more distinct.

Statistical analysis was not carried out on the data because the number of animals was small (six in the 10 mg/kg group and four in the 20 mg/kg group), and the myocardial lesions were focal and considered at the initial stages of cardiotoxicity. All the monkeys studied had one or more malignant liver tumor nodules, and one animal had evidence of lung metastasis at the time of autopsy. Although it can be argued that the myocardial lesions observed in the IQ monkeys may be secondary to the malignant liver tumors there was no correlation between the number of the liver tumor nodules or the diameters of individual nodules and the extent of myocardial abnormalities. Further evidence that the myocardial lesions were not secondary to tumor formation comes from studies in rats where IQ was shown to produce cardiotoxicity in cultured myocytes and in vivo (21).

Various drugs and chemicals have been associated with toxic cardiomyopathy, including anthracycline antibiotics (doxorubicin, daunomycin) (22–25), cyclophosphamide (26), azidothymidine (27,28), chloroquine (29,30), alcohol (31), amphetamines (32), cocaine (33,34), and cobalt.
The pathogenesis of toxic cardiomyopathy varies among different noxious agents. Cardiotoxic drugs and chemicals may act directly on the myocyte and alter myocyte protein synthesis, interfere with mitochondrial function, and/or disrupt cell membrane permeability. Alternatively, the vascular supply of the heart may be the target of toxic agents; vasoactive compounds such as norepinephrine and amphetamine can cause arteriolar vasoconstriction and capillary endothelial damage, resulting in secondary myocyte ischemia and necrosis. High-dose cyclophosphamide therapy has been associated with microthrombi in intramyocardial capillaries (26).

Figure 3. Electron micrographs from four different IQ hearts. (A) Focal dissolution of myocyte myofibrils and disruption of sarcomeres; 14,400x. (B) Left ventricular myocyte with moderate myofibrillar loss; 11,000x. (C) Three myocytes cut in cross-section demonstrating varying degrees of moderate (arrowheads) to severe (arrows) myofibrillar loss; 17,462x. (D) Abnormal mitochondria demonstrating disruption and dissolution of cristae (arrows) and focal clearing of matrix (arrowheads); 17,400x.

Figure 4. Electron micrograph of myocyte necrosis. Case 1: Low (A) (2700x) and high (B) (30,000x) power micrograph of a necrotic myocyte demonstrating mitochondrial flocculent densities in (B) (arrowheads).

Figure 5. Electron micrographs of hydroxypropylcellulose controls. (A,B)—cases 11 and 12, respectively: normal myocardial ultrastructure with sarcomeres (arrows) separated by normal mitochondria (arrowheads). (A) 7200x, (B) 18,000x.
Perhaps the best-characterized example of drug-related toxic cardiomyopathy is anthracycline-induced cardiomyopathy (22–24). The most striking pathologic features seen in myocytes is that of mitochondrial damage, vacuolar degeneration (swelling of sarcoplasmic reticulum and T-tubules), and myofibrillar dissolution (20,23). The mechanism of anthracycline-induced cardiomyopathy is uncertain, but may primarily involve direct toxic damage of mitochondria. In ultrastructural studies, mitochondrial swelling, clearing of normal matrix densities, mitochondrial cristae disruption, and mitochondrial degeneration have been described (19). As a result of mitochondrial dysfunction, impaired oxidative phosphorylation may precipitate loss of energy-requiring membrane permeability function. Thus, explosive swelling of sarcoplasmic reticulum may be the consequence of abnormal permeability. Additionally, anthracyclines may be directly toxic to myofilaments. The effectiveness of anthracyclines as chemotherapeutic agents is related to their ability to react with DNA. The principal action of doxorubicin is DNA strand breakage (36). We are not aware of any studies where covalent binding of doxorubicin to myocardial DNA has been assessed. However, the anthracycline cyanomorpholino doxorubicin (MRA-CN) has been shown to form adducts and cross-links with DNA in vitro, and it has been suggested that adducts to a single strand of DNA precede the formation of the interstrand cross-links by MRA-CN (14). Sequence-specific complexes of MRA-CN with DNA have also been observed as blocked transcripts in an in vitro transcription assay (17). In comparison to IQ-induced DNA adducts, the possibility must be considered that cardiotoxic anthracyclines may act in the nucleus and interfere with DNA transcription, resulting in impairment of protein synthesis necessary for the turnover of contractile and membrane-bound proteins.

Anthracycline-induced cardiotoxicity has been studied in a variety of animal models, including rabbits, rats, mice, pigs, dogs, and monkeys (37,38). The light microscopic and ultrastructural cardiac findings in the monkeys dosed with IQ are similar to those described for anthracyclines, including myocyte degeneration, focal interstitial fibrosis, myofibrillar loss, swelling of mitochondria, and clearing of normal matrix densities. However, none of these findings is specific for drug-induced cardiomyopathy, nor do they indicate the precise mechanism of potential myocyte damage by IQ. An active metabolite of IQ, N-hydroxy-IQ, and its reactive esters, including N-acetoxy-IQ, are known to form adducts with DNA, and high levels of IQ–DNA adducts have been observed in the myocardium of monkeys chronically dosed with IQ (17). Further studies to analyze the possible role of IQ–DNA adducts in the etiology of the IQ-associated cardiotoxicity are warranted. One can speculate that a potential mechanism of the IQ toxicity to myocytes may be interference with protein synthesis essential for the maintenance of normal contractile elements. Alternatively, the abnormalities seen in the mitochondria may suggest interference with the transcription of mitochondrial DNA, resulting in impairment of mitochondrial protein synthesis and/or oxidative phosphorylation. There is evidence that IQ forms adducts with mitochondrial DNA in rat myocardium (21). It will be important to find out if IQ–DNA adducts are present in the mitochondria of the monkey myocardium and if they are responsible for the mitochondrial damage observed in the majority of the IQ cases.

This report describes the pathological findings consistent with cardiotoxic effects in nonhuman primates after chronic IQ exposure. The myocardial lesions were focal and none of the animals had evidence of overt heart failure. It is possible that a more prolonged exposure to IQ may be necessary before clinical evidence of cardiac dysfunction occurs. However, one must now consider the possibility that in addition to being carcinogenic, IQ and other heterocyclic aromatic amines may produce adverse effects on the cardiovascular system. Further studies to quantify the role that heterocyclic aromatic amines may play in the etiology of cardiovascular diseases are in progress.

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