

# In this issue

## Matters of the Heart

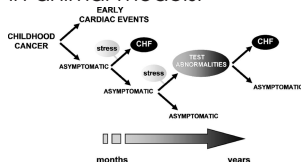
Cardiotoxicity is emerging as an important side effect for many different classes of drugs. This issue contains a series of complementary perspectives on this timely topic, organized by Giorgio Minotti. These perspectives provide new insight into mechanisms of damage to cardiomyocytes by clinically used pharmaceuticals.

Minotti et al. (p 978) launch the series with an exploration of the cardiotoxicity of antitumor drugs, of which the anthracyclines are the best-known examples. Anthracycline-mediated cardiac damage is believed to follow one-electron reduction, to a quinone capable of redox cycling that yields reactive oxygen species. Alternatively, two-electron reduction forms a less redox-active alcohol that accumulates in the cell, disrupting iron and calcium homeostasis.

A key feature of anthracycline toxicity is its exacerbation by the concomitant administration of the taxane paclitaxel. This can now be explained by the ability of taxanes to allosterically facilitate the reductases that form the anthracycline alcohol metabolites. Anthracycline toxicity is also exacerbated by trastuzumab (Herceptin), a monoclonal antibody directed against the epidermal growth factor receptor family member HER2, which is often overexpressed in cancer. This highly targeted drug was expected to have low toxicity, so its facilitation of anthracycline-mediated cardiotoxicity was a surprise. We now know that the HER2/HER4/neuregulin-1 signaling machinery aids in the resistance of cardiomyocytes

to anthracycline-induced damage. Blocking this machinery with Trastuzumab, therefore, introduces a second hit that accelerates cardiac damage.

A recurring theme in this perspective is the importance of evaluating toxicity in human heart tissue. For example, anthracycline-metabolizing enzymes vary among species, and the effects of paclitaxel on anthracycline metabolism have only been detected in a human heart model. Similarly, because trastuzumab is directed against the human HER2 receptor, its toxicity cannot be detected in animal models.



Kohler et al. (p 990) focus on the cardiotoxicity of nucleoside reverse transcriptase inhibitors (NTRIs), which are the mainstays of treatment for HIV/AIDs. Cardiomyopathy is a complication of HIV infection, and data strongly indicate that it is exacerbated by NTRIs. The cardiotoxicity of NTRIs is characterized by defects in mitochondrial function. Indeed, in many ways, NTRI toxicity resembles the sequelae of genetic disorders of mitochondrial DNA replication. Kinetic studies show that NTRIs inhibit DNA polymerase- $\gamma$ , the polymerase responsible for mitochondrial DNA replication, more efficiently than any other mammalian polymerase. This finding alone may explain the cardiotoxicity of NTRIs, but studies using a transgenic mouse model of HIV/AIDs suggest that the deoxynucleoside carrier and thymidine kinase 2, which

transport NTRIs into the mitochondrion and phosphorylate them, respectively, may also contribute to NTRI toxicity.

Tagliatela et al. (p 997) move the discussion to a consideration of the cardiotoxicity of antihistamines. These drugs have been used for decades for the treatment of allergic symptoms, but it was not until the advent of the second generation antihistamines, noted for reduced sedative properties, that cardiotoxicity was first noted. This came in the form of a polymorphic ventricular arrhythmia, known as torsade de pointes (TdPs), which is characterized by a prolonged QT segment on the electrocardiogram. The QT segment corresponds to ventricular repolarization. We now know, as a result of genetic disorders known as long QT syndrome, that repolarization is dominated by the function of as many as five ion channels, three of which conduct potassium ions. Further studies showed that one of these potassium channels hERG1 is blocked by antihistamines that cause TdPs arrhythmias.

Mitcheson goes on to reveal that many classes of drugs other than antihistamines block hERG1 channels. He explains this on the basis of the proposed hERG1 structure, comprised of four subunits, which unite to form an ion channel with a narrow filter region at the top, which widens into an inner cavity. The relatively large size of this cavity allows for binding and entrapment of a wide variety of molecules, explaining why so many different drugs block this channel. Further structural studies aim to better understand the mechanism of binding so that this toxicity may be avoided early in drug development.

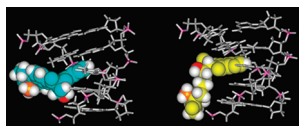
The recognition of the role of hERG1 blockade in TdPs generation has provided the framework for screening existing and new agents for potential cardiotoxicity. It has also contributed to the understanding of the role of drug metabolism and clearance in the cardiotoxicity of some second generation antihistamines. This is clearly an example where mechanistic understanding can be directly used to avoid drug toxicity in the future.

## Special Features

*This month, Philip Burcham continues our global analysis of the state of toxicology from his point of view in Australia. Don't miss this great opportunity to learn about aboriginal toxicologists and their modern descendants! Also, in May, we have four perspectives on the mechanisms of cardiotoxicity of widely used pharmaceuticals. We thank Giorgio Minotti for organizing this series and all of the authors for their valuable insights. Highlights of the series are provided above, but check out the full perspectives to learn the latest on this important topic.*

**DNA Adduct Diversity**

The treatment of symptoms of menopause by using hormone replacement therapy often employs the drug Premarin, a combination of equine estrogens. Premarin has been shown to increase the risk of breast cancer. Its primary components are equilin and equilenin, both of which are metabolized to 4-hydroxy-equilenin (4-OHEN), a reactive catechol that forms adducts with the cytosine, adenine, and guanine bases of DNA. These adducts have been detected in animals and humans exposed to equine estrogens, suggesting that they may play a role in mammary carcinogenesis.



The reaction of 4-OHEN with each of the bases yields four stereoisomers. Thus, 12 different adduct structures are possible. Now, Ding et al. (p 1064) use a molecular modeling approach to explore the structure of each of these adducts within duplex DNA to gain insight into how variations in structure may affect their mutagenicity and potential for repair.

Structural analysis of each of the 12 base adducts reveals that the connecting ring systems of the stereoisomeric cytosine and adenine adducts are rigid, whereas there is some flexibility in the connecting ring of the guanine adducts. In all cases, however, the adducts occupy the hydrogen-bonding edge of the base, and the equilenin ring is nearly perpendicular to the base.

Within the DNA duplex, the cytosine and adenine adducts may position themselves with the equilenin ring in the major or minor grooves, whereas the guanine adducts are always placed within the major groove. Positioning the adduct within the major groove results in less distortion of the DNA helix but greater solvent accessibility of the equilenin ring system. Positioning within the minor groove produces a high level of helix distortion but maximizes favorable hydrophobic interactions. For each adduct, there is a unique combination of favorable and unfavorable factors dictating the final structure that is assumed.

Clearly, this high degree of structural diversity lends insight into the wide range of effects observed with these adducts. Indeed, certain stereoisomer and base-dependent differences in their mutagenic properties and repair efficiencies have already been observed experimentally. We look forward to learning more about how specific functional outcomes are ascribed to individual structural features in the future.

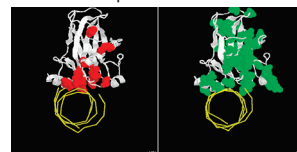
**p53 Mutations in the Lung**

The *p53* tumor suppressor is one of the most commonly mutated genes in human cancers. Characterization of the mutations found in large numbers of lung tumors reveals a signature unique to lung cancer. The first part of the signature is the predominance of G to T transversions over other types of base changes. The second part of the signature is a strand bias for G to T transversions over the complementary C to A transversions indicating that tran-

scription-coupled repair preferentially repairs the transcribed strand. The third part of the signature is the preferential location of mutations such that 50% occur in 20 or so hotspot codons. It has long been believed that this mutation signature provides clues to the mechanism of DNA damage. Because 85–90% of lung cancers can be associated with smoking, it is reasonable to attempt to explain *p53* mutations in lung cancer on the basis of carcinogens found in tobacco smoke. Among these are the polycyclic aromatic hydrocarbons (PAHs). These compounds can be activated by cytochromes P450 to generate reactive diol epoxides that form bulky DNA adducts. Alternatively, PAHs may be activated by aldo-keto reductases to generate redox active *o*-quinones. These compounds lead to the formation of reactive oxygen species and, ultimately, formation of 8-oxo-dGuo (oxidized deoxyguanosine). Now, Park et al. (p 1039) explore which of these DNA-damaging mechanisms can best describe the pattern and spectrum of *p53* mutations in lung cancer.

The investigators first incubated human *p53* DNA in vitro with three different PAH *o*-quinones under redox cycling conditions or with a PAH diol epoxide. The DNA was then analyzed to assess the types of DNA damage and for mutations using a yeast reporter system. The PAH *o*-quinones induced mutations in a concentration-dependent manner. The mutations were predominantly G to T transversions, and they correlated

directly with the amount of 8-oxo-dGuo detected in the treated DNA. In contrast, although the diol epoxide also produced mutations, they were predominantly G to C transversions and correlated with the formation of diol epoxide-dGuo adducts rather than with the formation of 8-oxo-dGuo. The PAH *o*-quinones were approximately 20-fold more potent as mutagens than the diol epoxide.



When the mutated DNA was analyzed for the site of alteration, a random spectrum was found among codons. However, when the mutated *p53* genes were coexpressed with the wild-type gene and assessed for dominant-negative potential, the dominant mutants reflected a distribution pattern similar to the hotspots found in the *p53* gene from lung cancers.

Together, these results suggest that PAH *o*-quinones, through oxidative stress, can cause G to T transversions observed in lung cancer. However, the pattern of distribution of the mutation hotspots found in vivo is most likely the result of selection for functionally dominant mutations rather than the action of the specific targeting of mutagens to hotspot codons. These results provide a plausible alternative to the widely studied diol epoxide pathway, an alternative that may be relevant to the role of oxidative stress in carcinogenesis.

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