Long Term Adverse Reactions to Fluoroquinolone Antibiotic Treatments from DNA-Adduction limits use as a First Line Treatment Protocol
Fluoroquinolone Antibiotics Propensity to Adduct to Human DNA
DNA-Adduct Testing Project Results
By Joseph King, Fluoroquinolone Toxicity Research Coordinator

Research demonstrates the propensity of Fluoroquinolone antibiotics to adduct to human cellular DNA. Fluoroquinolone (FQ) DNA-adducts[1] have been suspected as the catalyst to a host of post–Fluoroquinolone illnesses, including Gulf War Syndrome.

Our testing commenced in August 2013. Individuals were randomly selected from a national data base of reported FQ toxicity victims. Participants were exposed to a Fluoroquinolone antibiotic three years prior, this ensured that circulating levels have dissipated according to manufacturers. Participants were absent of any environmental/industrial toxic exposures.

Participants	Age	Antibiotic	Dosing X Daily	Duration Days
Male	54	Levofloxacin	500mg X 2	28
Male	47	Ciprofloxacin	500mg X 2	42
Male	50	Levofloxacin	500mg X 2	7
Female	51	Ciprofloxacin	500mg X 2	30
Female	38	Levofloxacin	750mg X 1	1
Female	42	Ciprofloxacin	500mg X 2	7

Pre dosing; participants were healthy, physically fit, active, cognitive, and non-drug users. Post dosing; participants demonstrate both extensive genetic damage (genotoxicity - determined by gene-sequencing), and varied multiple chronic and debilitating health conditions.

A High Performance Liquid Chromatography Tandem Mass Spectrometer (HPLC-MS/MS) was utilized. Blood samples were prepared using approved forensic toxicology protocols for DNA extraction and DNA-adduct analysis. All samples analyzed revealed the same markers and fragmentations for Fluoroquinolone antibiotic compounds as DNA-adducted. The control samples were void of the same markers. Fragmentation analysis further revealed that the Fluoroquinolone compound (intact) and its metabolites were adducted to the DNA of the test group.

INITIAL COMPOUNDS IDENTIFIED [HPLC-MS/MS]:

Quinoline/Quinolone; pharma-core of FQs
Ciprofloxacin [Cipro] / Levofloxacin [Levaquin] residue, non-metabolized
Methyl Piperazin
Cyclopropyl
Carboxylic Acid
Quinozoline
Anthraquinone. No other extraneous compounds, toxins, or metals were found in the samples.

Upon investigation there was a singular factor found among the participants, all had been exposed to a Fluoroquinolone antibiotic. This commonality is the only thread linking these individuals to the decay in their health, genotoxicity, genetic mutations, developed antibodies, and the FQ DNA-adducts discovered in the testing.

[1] **DNA-adducts**: covalent adducts between chemical mutagens and DNA. Such couplings activate DNA repair processes and, unless repaired prior to DNA replication, may lead to nucleotide substitutions, deletions, and chromosome rearrangements. (Rieger et al., Glossary of Genetics: Classical and Molecular, 5th ed.).

Fluoroquinolone Research Momentum Builds, Phase-II In Preparation
Fluoroquinolone DNA-Adduct Testing Project Results
By Joseph King, Fluoroquinolone Toxicity Research Coordinator
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Forwarded to the National Institute of Health, Bethesda, MD.

Research published over the last decade demonstrates that Fluoroquinolone antibiotics possess the potential to adduct to human DNA at both the cellular and mitochondria DNA levels. Fluoroquinolone DNA-adducts have been suspected as a potential culprit in post–Fluoroquinolone illness, but no human Fluoroquinolone DNA-adduct clinical studies have been conducted and published until now. Therefore, over the past six months (beginning at the end of August 2013) discrete testing of a diverse sampling of individuals devastated by the Fluoroquinolone antibiotics was conducted. I was asked to direct this research and coordinate the investigation with a team of biochemist, biogenetic engineers, toxicologist, pharmacologist, and microbiologist both nationally and internationally.

BACKGROUND

DNA adducts are chemical compounds that are bound to one or more of the amino acid protein molecules constructing the DNA of living organisms. Once this event occurs, that section of the organisms DNA and the section's representative gene is fractured. The source of the chemical compounds that bind and fracture DNA can be either exogenous or endogenous and can also include metabolites (fragments) of chemical molecular structures. Other words, the entire compound, a fragment of the compound, or a metabolite formed from the compound can adduct to the DNA amino acid structure if there is a molecular attraction between any of the two. The Fluoroquinolones demonstrate such a molecular attraction. A DNA-adduct will subsequently block that corresponding gene's expression and ability to replicate correctly. This hinders that gene's ability to encode and correctly oversee the production of the protein synthesis necessary for its associated cell and tissue regeneration, replication, and repair. This is better known as genotoxicity

Along the entire DNA helix, there are sections that represent the genes that makeup that living organism. The Human Genome Project has revealed that there are approximately 20,000-25,000 genes sectionalized along our DNA. Genes are responsible for every aspect of maintaining that organism by, amongst other functions, orchestrating the reproduction and repair of cells. Cells that makeup the liver, brain, nerves, muscles, connective tissue, bones, and all other organs of the human body. One major role that genes play is the encoding of protein, taking ingested protein and manufacturing new cells, like muscle cells. But if that gene is damaged through a DNA-adduct, the bio-chemical formula held by that gene (encoding) for mixing the protein correctly in the manufacturing of new cells (for that organ) is flawed, mutated. If this occurs, that organ of the body does not repair or replenish itself correctly or timely, and begins to manifest a variety of symptoms, pain, fatigue, wasting, and others. In numerous cases the body will respond to these flawed cells as alien, and produce antibodies to attack these cells as an autoimmune response. This autoimmune response can produce inflammation, pain, debilitation, and additional health and physical challenges.

Today the most efficient and exacting testing for compounds adducting to DNA is the use of High Performance Liquid Chromatography–Tandem Mass Spectrometry (HPLC-MS/MS). A tandem mass spectrometer is a specialized instrument that detects chemical compounds by measuring their molecular weight (mass). Mass spectrometers determines and quantifies the weight of the molecules (Hydrogen, Oxygen, Carbon, etc.) comprising a chemical compound electronically, and produces a spectrogram displaying the exact weights of each segment of the compound, this is known as a mass

spectrum-spectrogram. This is a highly accurate and specific analysis since each chemical structure retains its own unique molecular weight formed from its unique atomic structure. Additional verification is provided through the instrument by providing the individual molecular mass (weights) of the segments (fragments) of molecules (atoms) arranged to produce the chemical compound. In essence, Tandem Mass Spectrometry reveals the compound, the group of molecules arranged to make the compound, and structural information at the molecular level surrounding the compound under investigation. Think of it as revealing the forest, the arrangement of the trees, the grouping of the trees, and the type of trees in each of the groups producing the forest.

TESTING RESULTS

This testing commenced in late August 2013. Analysis has been ongoing to date, and will continue throughout 2014.

Five individuals were selected that had been dosed with a Fluoroquinolone (FQ) antibiotics; primarily Ciprofloxacin (Cipro) or Levofloxacin (Levaquin) . Each participant was selected based upon duration of dosing and currently reported medical conditions that did not pre-exist prior to administering the antibiotic. To be included in the study, participants had to be exposed to a fluoroquinolone at least three years prior. This time frame was established to ensure that serum circulating levels of FQs would be dissipated from blood samples as indicated by the drug manufacturers. All individuals were voluntary participants in this study.

Participants (samples) were selected at random from a date base of >1000 individuals reporting post treatment Debilitating Adverse Drug Reactions (DADR) from a Fluoroquinolone antibiotic. This represents a subset population from the 1.5 million individuals within the United States debilitated from a Fluoroquinlone antibiotic regiment.

Three females and two males where selected. Demographics of participants varied from national and international locations. Occupational backgrounds greatly varied, but where limited to professional employment and thereby absent of any hazardous or industrial materials exposure or chemical toxins.

Participant	Age	Antibiotic	Dosing	Duration	Year	Occupation
Male*	54	LVX	500mg x 2	28 days	2010	Professional
Male	47	CPX	500mg x 2	42 days	2010	Professional
Male	50	LVX	500mg x 2	7 days	2010	Professional
Female	51	CPX/LVX	500mg x 2	30 days	2009	Researcher
Female	38	LVX	750mg x 1	1 day	2010	Professional
Female	42	CPX	500mg x 2	7 days	2010	Consultancy

[Ciprofloxacin (CPX), Levofloxacin (LVX)]
[* GC-MS/MS utilized]

All six participants reported good health prior to ingesting FQs, were physically fit, active, cognitive, non-sedentary, and non-recreational drug users. All six demonstrated extensive genetic damage as determined by gene-sequencing analysis and varied chronic health conditions that did not exist prior to dosing.

Diagnosed Health Conditions of participants after Fluoroquinolone treatment

Participant-Age-Chronic Health Conditions reported and medically diagnosed:

1). Male (54) Neuropathy, chronic body pain, joint pain, fatigue, gastro-intestinal intolerances, diminished cognition, chronic and severe muscle wasting, and connective tissue destruction-wasting

2). Male (47) Neuropathy, cognitive disturbances, sensory disturbances, gastrointestinal issues, cardiovascular disturbances and skin reactions, body pain, and fatigue

3). Male (50) Neuropathy, chronic body pain, joint pain, fatigue, gastro-intestinal intolerances, and diminished cognition

4). Female (51) Neuropathy, connective tissue destruction, muscle wasting, chronic body pain, joint and muscle pain, chronic fatigue, diminished cognition, electro-magnetic radiation intolerance, chronic respiratory problems, food allergies, and environmental allergies

5). Female (38) Neuropathy, chronic body pain, joint and muscle pain, fatigue, diminished cognition, electro-magnetic radiation intolerance, chronic respiratory problems, food allergies, and environmental allergies

6). Female (42) Neuropathy, chronic body pain, diminished cognition, electro-magnetic radiation intolerance, fatigue, food allergies, and environmental allergies

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The control group were recruited from internal laboratory sources and included two individuals who had never been exposed to any type, class, or subclass of antibiotics, drugs, environmental compounds, or toxins, as verified by self-report and prescreened using Gas Chromotography analysis for any toxins (toxicity screening), medications, and illegal drugs in blood serum, and hair samples using standardized forensic toxicology protocols.

10 milliliters of whole blood was extracted via venipuncture into a Vacu-Vile containing a standard anticoagulant, and delivered by overnight air-express to the testing laboratory for processing. Standardized shipping and handling methods for human biological fluids was implemented utilizing a United Nations approved UN-3373 Biological Substance Box and transport requirements, the chain of custody was maintained during all stages of collection, transport, and delivery.

The lab processed the blood samples by separating the plasma from the red blood cells (platelets) by centrifuging the blood. The red blood cells where extracted and the protein membrane of the red blood cells was removed utilizing a widely approved and recognized chemical chelating procedure adopted by toxicology laboratories worldwide. This exposed the cell DNA and Mitochondria DNA, both located in human blood cells. The remains where centrifuged again separating the two sources of blood cell DNA from the protein members, producing approximately 100 micrograms of DNA per sample to be extracted for testing.

All procedures were conducted using approved and recognized toxicology methods for DNA extraction from human cells, blood sample preparation, and instrument analysis. Methodologies utilized were standardized protocols by criminal forensic toxicology procedures for evidence handling, detection, and analysis.

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A High Performance Liquid Chromatography Tandem Mass Spectrometer (HPLC-MS/MS) was utilized based upon prior publications touting and supporting the role of HPLC-MS/MS as the future wave in detecting DNA adducts in toxicology and genetic studies.[2]

Upon completion of the analysis of the six individual blood DNA samples, two control samples where prepared and also analyzed. Results where cross-compared to the six exposed blood DNA samples.

All six of the Fluoroquinolone exposed blood-DNA samples revealed the same identical markers for DNA-adducts originating from Fluoroquinolone dosing. While the control samples where void of the markers found in the Fluoroquinolone group. The control samples remained flat across the spectral spread with no significant markers revealing any toxins or Fluoroquinolones as DNA-adducts.

However, significant Fluoroquinolone markers were found within the test group as DNA-adducts. Identification of Fluoroquinolone metabolites and compound fragments where also found at elevated levels as DNA-adducts. It was revealed that both the Fluoroquinolone compound itself and metabolites from the Fluoroquinolone compound where adducted to the DNA of the entire test group.

COMPOUNDS IDENTIFIED AS DNA-adducts in TEST GROUP

Fluoroquinolone and Quinolone Metabolites found in test group using HPLC-Tandem MS/MS Ranking by prevalence: Compound Revealed as DNA-adducts as Compound Fragments or Metabolites of Interest Revealed as DNA-adducts from analysis [Ciprofloxacin (CPX) and Levofloxacin (LVX)]

1). Quinoline; pharma-core of all Quinolone based antibiotics (heterocyclic core fragment of CPX and LVX)

2). CPX and LVX Residue, non-metabolized accumulated residue of the entire compound, intact

3). Methyl Piperazin; Piperazin compound attached to Quinoline core compound of CPX and LVX

4). 1-Cyclopropyl; Cyclopropyl attached to Quinoline core compound of CPX

5). 3-Carboxylic Acid; Carboxylic Acid attached to Quinoline core compound of CPX

6). Carboxylic Acid; Carboxylic Acid attached to Quinoline core compound of LVX

7). Quinozoline; Metabolite of Quinolone and Quinoline

8). Anthraquinone; Metabolite of Quinolone

9). N-alkylated (alkylating agent) [N-alkylated 3-carboxypyrid-4-one-fused with substituted aromatic ring.

10). other DNA-Destabilizing Agents, Acyl-Glucucoronide metabolite from Quinoline core

11). Nalidixic Acid – metabolite, intact

[2] Protocols, and instrument initiation and analysis protocols are withheld at this time (copyright and patent pending).

The probability of a statistical anomaly: 0.0012687 from samples tested and random methodology utilized in this research exclusively and independently.

The probability of a statistical anomaly when cross referenced to external research of similar nature and sample size: 0.00011256.[3]

Probability of a statistical anomaly (fluke) occurring simultaneously in all five samples tested is: 0.0001287 - statistical error.

Probability of cross-contamination occurring in five samples tested: 0.00000012

Statistical Correlation with other FQ research conducted: 98.8755 +/- 1.2

Statistical Conclusion: Cause and effect established.

Gene Sequencing

The following gene mutations and fractures where found in the following six individuals as results from the Fluoroquinolone DNA-adducts:

CYP - Cytochrome P450 family and subgroups.[4] Responsible for the metabolism of many drugs and environmental chemicals that it oxidizes and neutralizes

NAT - N-acetyltransferase NAT1, NAT2. The NAT acetylation polymorphism is important because of its primary role in the activation and/or deactivation of many chemicals in the body's environment, including those produced by cigarettes as well as aromatic amine and hydrazine drugs used medicinally. In turn, this can affect an individual'cancer risk.

GAD - Glutamic Acid Decarboxylase, GAD1, Neuropathy and Stiff-Persons-Syndrome

MTHFR, Protein-C deficiency resulting in later-onset neurodegenerative disorders

PRTN, PRTN3, PR-3 Proteinase/Proteinase-3Polymorphonuclear - leukocyte serine protease that degrades elastin, fibronectin, laminin, vitronectin, and collagen types I, III, and IV [connective tissue - muscular/skeletal complications]

COMT - Catechol-O-methyltransferase, Catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines, including the neurotransmitters dopamine, epinephrine, and norepinephrine.

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[3] **Genotoxic and cytotoxic effects of antibacterial drug, ciprofloxacin, on human lymphocytes in vitro**
PS Ambulkar,1 SK Ghosh,2 IV Ingole3 and AK Pal3
1 Department of Biotechnology,Agnihotri College of Science, Wardha-442001, India, 2 Department of Anatomy, Nepal Medical College, Jorpati, Kathmandu, Nepal
3 Human Cytogenetics Unit, Department of Anatomy, Mahatma Gandhi Institute of Medical Sciences, Sewagram, Wardha – 442102, India.

[4] Seven subgroups from the CYP-P450 gene family were identified.

MAO - Catalyzes the oxidative deamination of biogenic and xenobiotic amines and has important functions in the metabolism of neuroactive and vasoactive amines in the central nervous system and peripheral tissues

Other genes associated with connective tissue protein synthesis, muscle, nerve fiber/cells, and Epithelial Cells synthesis[5].

General Discussion

With an escalating number of individuals that have been dosed with an Fluoroquinolone (FQ) reporting debilitating reactions to the treatment, we must consider that a serious biochemistry alteration is manifesting within these victims. A biochemical change that has resulted in genetic damage revealed through genome sequencing testing by FQ victims post treatment. Ongoing testing has shown specific gene fractures and mutations repeatedly amongst thousands of participants dosed with an FQ. Such genetic damage can only result from one of four sources;

1). Hereditary-Inherited - a gene mutation passed from parent to child. This category would generally show symptoms within the early years of the child's growth and development. This has not been the case in FQ respondents, no prior or pre-existing conditions of this origin have been reported, and certainly not by the test group

2). Radiation exposure, primarily from nuclear fallout. Again, this has not been the situation involving the FQ respondents or the test group as their source of genetic damage

3). Environmental toxins could be considered. However, 97% of the FQ victims showing genetic damage have reported that they have never been exposed or worked in an environment conducive to toxins of any category. This was certainly true of the six participants in the testing, and was validated by background checks. Furthermore, the blood samples taken from the six participants in this research were scanned for environmental toxins, their metabolites, and other pharmaceutical compounds (prescription and illegal). No such compounds were found.

4). Directly ingested toxins of a concentration and duration significant enough to alter the individual's DNA permanently in the form of a DNA-adduct.

There has been only one common factor reported and found among the Fluoroquinolone victims and the study group, a Fluoroquinolone antibiotic. This singular compound and its associated metabolites were the only thread linking these individuals to the decay in their health, genotoxicity, genetic mutations, developed antibodies, and the DNA-adducts discovered in the test group.

DNA-adducts are nothing new. The process has been researched and tracked for decades. A DNA-adduct is any compound that molecularly arranges itself to cleave to one or more of the four amino acids that construct the DNA of living organisms. Once this event occurs that section of the organisms DNA and the section's representative gene is fractured. This hinders that specific gene's ability to encode and correctly oversee the production of the protein synthesis necessary for corresponding tissue regeneration and replication. This is better known as genotoxicity.

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[5] Comprehensive list of genes identified from Fluoroquinolone genotoxicity exceeded one hundred associations. The above genes referenced is only a partial list found and cross-linked to analysis.

Prior to this project it was of general consensus that DNA-adducts originated primarily from environmental toxins (posions), such as Benzopyrenes, heavy metals, DDT and compounds of similar molecular structures. However, on going research surrounding the compound 2-Amino-3,8-dimethylimidazo 4,5-f Ëquinoxaline better known as MelQx and its sister MelQ as a DNA-adduct caught our attention when investigating the Fluoroquinolones do to similar molecular structures and DNA-adduct pathways.

MelQx and MelQ are compounds that form when meat, especially red meat is cooked at high temperatures, and adducts to mammalian DNA. These compounds have a similar quinoline-quinolone heterocyclic core structure to the FQs. This opened a door of investigation surrounding the receptiveness of a quinoline-quinolone based compound to position itself as a potential DNA-adduct. This could also be the case with chemotherapy agents that are based upon a quinoline-quinolone core constituent and heterocyclic molecular arrangements.[6]

There is no doubt that the quinolone molecular core is a very manipulative structure that permits the construction of powerful chemical compounds both industrial and pharmaceutical. Unfortunately it also a very resilient compound that resists fragmentation and reversal.

It also worth noting that Fluoroquinolone DNA-adducts or the associated metabolites as DNA-adducts further enhance the development and positive testing of varied antibodies against altered cellular activity. Such antibodies as the Ganglioside GM1, GD1b, and GQ1b that are associated with neurological symptoms, neuropathies, and neuro-degeneration. Also antibodies such as Proteinase-PR3 that are linked to various degenerative connective tissue disease. Other antibodies include Sjogren, Rheumatoid factors, Lupus, Epstein-Barr and various anti-nuclear antibodies. In the event that the Fluoroquinolone compound has inflected extensive genetic damage from widespread DNA-adducts across the DNA helix, various hybrid health conditions may appear such as; non-inherited Marfan's Syndrome, or Nakajo-Niskimura Syndrome, both of which are on the increase globally, and are classified as muscle-skeletal alterations with connective tissue destruction. These two syndromes are difficult to diagnose and treat using current diagnostic-treatment regiments as byproducts of Fluoroquinolone DNA adduction.

Fluoroquinolones and Genotoxicity
Has a Monster Been Created?

Has this class of antibiotics created a long term genotoxicity with future generational problems? Can the exponential up-tick of neurological, muscular-skeletal, and phantom health problems be associated to these antibiotics? Yes, it is highly suspected, and current data and results are pointing an accusing finger at the Fluoroquinolones. It is time for the initiation of an international research response to this rapidly growing problem.

It is time for Doctors to rethink their approach to FQ victims. Doctors must understand that a patient with a FQ DNA-adduct is not having an ADR (adverse drug reaction) to the FQs. The patient has been poisoned, cellular damage has occurred in response to a toxic reaction. The patient's original genetic profile has been altered-mutated, and their symptoms and health complaints are not psychosomatic. But are in fact legitimate health concerns requiring treatments that will not acerbate the condition or propagate the genetic damage. It is going to be the physician's responsibility to reconstruct the biological crime as a medical Sherlock Holmes to determine the extent of the genotoxicity and cellular damage caused by the FQs in their patients. The investigation by the physician can easily begin by simply asking the patient if they have ever been prescribed a FQ antibiotic.

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[6] Heterocyclic, and Heterocyclic amines (HCAs).

An Urgent Call for Phase-II Testing and Funding

While there is always a place for "soft" science consisting of surveys and patient tracking, the time is now for intensive testing of those suffering from these antibiotics.

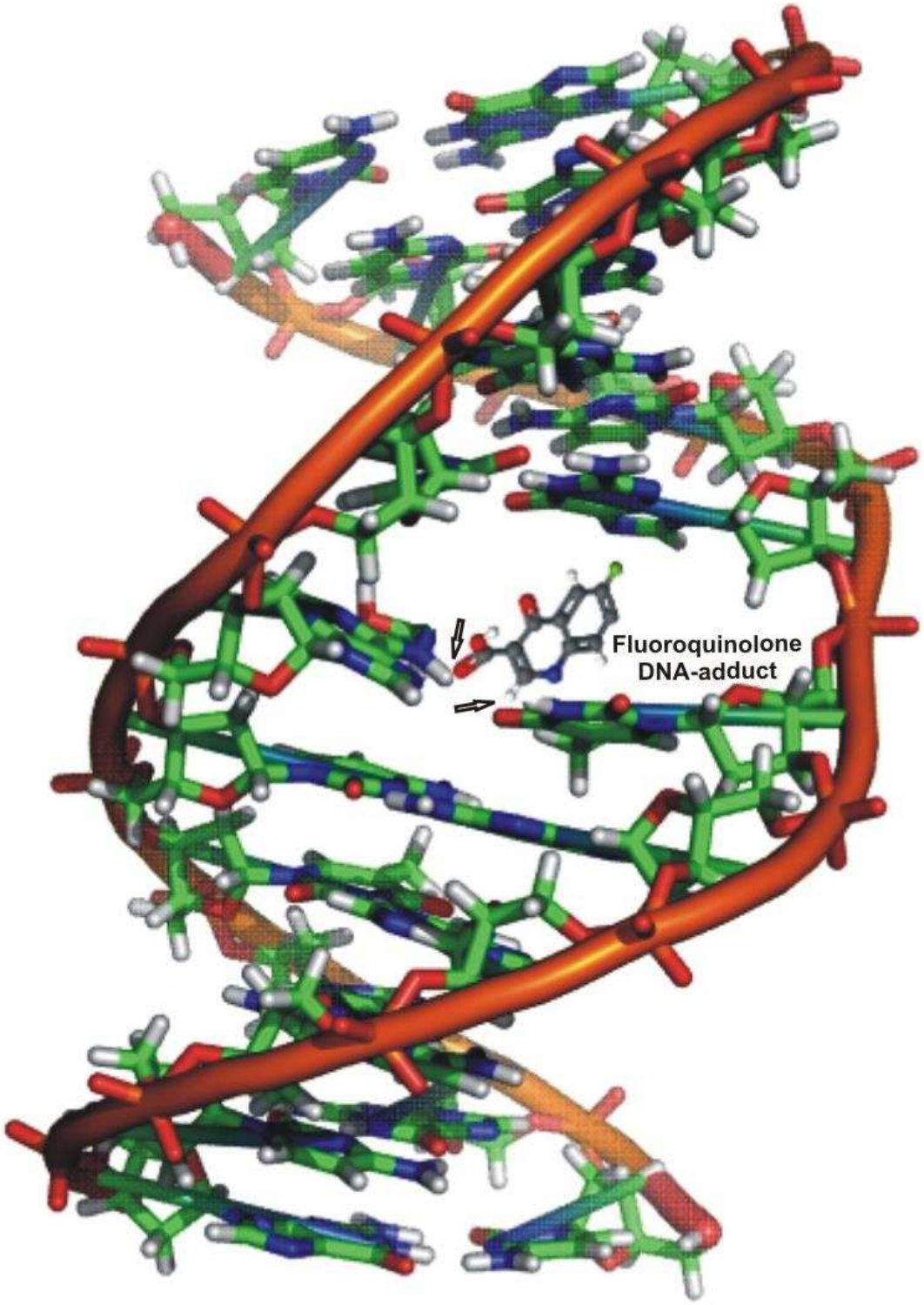
Based upon the evidence that the six individuals tested only had one factor in common, dosed with a Fluoroquinolone antibiotic, and identical markers for DNA-adduction by these antibiotics where forthcoming from the analysis, we are prompted to call for a Phase-II series of testing. Phase-II funding and testing, whether from public or private funding, would escalate the research to include individuals effected by the Fluoroquinolones in the early 2000 and 1990's era. This would certainly include military personnel diagnosed with Gulf War Syndrome from the 1990 to 1993 time frame. Knowing now that soldiers deployed to the Gulf War where administered Ciprofloxacin as a prophylactic to biological weapons, they should be included in the next study. It is disturbing that DNA-adduct testing on these individuals has never been conducted. We must also consider all the government and private workers that where debilitated after being dosed with Ciprofloxacin in 2001 and 2002 in response to Anthrax tainted letters. Private funding for Phase-II would be ideal since oversight could be better and more rigidly implemented through independent non-drug manufacturer related moderators with knowledge in Fluoroquinolone toxicity.

Final Word

Let me extend my deepest and heartfelt appreciations and respect to the team and financial backers that launched this research. They sacrificed a lot to engage this research. Their dedication and motivation to expose the damage from the Fluoroquinolone antibiotics deserves all our gratitude and respect. Also, a great appreciation to the scientist that guided the process and protocols. We are indebted to all of these individuals.

Correspondence to:
Joseph King, Fluoroquinolone Toxicity Research Coordinator, Human DNA-adduct research
jk33168@aol.com
Miami, Florida - USA

Declaration of Conflict of Interest:
The author and Research Coordinator has no affiliation with any pharmaceutical entity, governmental agency, or research facility. Only private funding was used for testing, research, and analysis. No conflict of interest is declared.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