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ToxGurukul Foundation Newsletters

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

- Marie Curie

September 2019

Interactive Meet on Insights in Toxicology - 2019

Theme: Pathology for Toxicologists

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Risk Assessment of Nitrosamine Contamination in Angiotensin II Receptor Blockers (ARB) - "SARTAN" Drugs

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A. Preamble

Nitrosamines are a class of chemical compounds with the generic chemical structure R2NN=O. The chemical class of N-nitroso compounds includes the N-nitrosamines, N-nitrosamides and N-nitrosamidines. They are produced under certain conditions (acidic pH, high temperature, presence of certain reducing agents) in a variety of media (products, biological systems, air, etc) when nitrites react with the so-called nitrosatable substances, mainly secondary amines. They have been detected as contaminants in a number of products including foods, drugs, beer, cosmetics, tobacco & rubber products.

On June 2018, FDA and EMA were informed of the presence of an impurity, identified as N-Nitrosodimethylamine (NDMA), from one valsartan API producer. Since then, FDA has determined that other types of nitrosamine compounds, e.g., N-Nitrosodiethylamine (NDEA), are present at unacceptable levels in APIs from multiple API producers of valsartan and other drugs in the Angiotensin II Receptor Blockers (ARB) drug product(DP).

From the toxicological point of view, the genotoxic carcinogenic action of some nitrosamines in animals and their probable link with human carcinogenesis is the most relevant endpoint.

The two most common nitrosamines, N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) are classified by IARC and the EU as category 2 carcinogens(CMR cat 2). Considerable evidence has accumulated that the initiation of the carcinogenic process by this group of carcinogens is linked to the metabolic competence of the target tissues or cells to convert these carcinogens into mutagenic metabolites and to the binding of these metabolites to cellular DNA.

Angiotensin II Receptor Blockers (ARB) are the most important class of drugs for the treatment of hypertension and prevention of a broad range of cardiovascular diseases. Sartans are extensively manufactured in India and exported to regulated countries (the US and Europe). *Contamination of nitrosamines in sartans is of immense economic, political and public health importance to Indian pharma industry. In this article, an effort is made to discuss the current controversy on nitrosamine contamination in sartan drugs and its impact on regulatory and human health and finally on risk assessment.*

B. Angiotensin-II-Receptor Antagonists/Blockers (ARB's)

The angiotensin II receptor blockers (ARBs) represent a newer class of

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antihypertensive agents. Their mechanism of action differs from that of the angiotensin-converting enzyme (ACE) inhibitors, which also affect the renin-angiotens in system. The ARBs were developed to overcome several of the deficiencies of ACE inhibitors. ARBs displace angiotensin II from the angiotensin I receptor and produce their blood pressure lowering effects by antagonizing angiotensin II-induced vasoconstriction, aldosterone release, catecholamine release, arginine vasopressin release, water intake, and hypertrophic response. *ARBs are generally well tolerated, presumably because they are largely cleared in the bile.* No significant drug interactions involving valsartan, irbesartan, or candesartan have been reported.

Valsartan is an orally active antihypertensive drug developed in the 1990s and is a selective angiotensin II receptor blocker (ARB)1 which relaxes the blood vessels and thus reduces blood pressure; it is also used for treating patients with congestive heart failure and post-myocardial infarction. There are eight other ARBs that patients may be switched to if they discontinue their valsartan therapy. Valsartan was patented by Novartis Pharmaceuticals in 1996 on the US market under the name Diovan. The patent was taken off in the USA in 2012 when valsartan was distributed as a generic. Valsartan alone and in combination with other drugs are sold by 30 companies in the US market. They are often prescribed as the first-line anti-hypertensive for patients with diabetes and renal disease.

Sartans have very high lipophilicity, coupled with a high volume of distribution, indicate that the compound offers the clinically important advantage of good tissue penetration. Sartans have a longer terminal elimination half-life making it suitable for once-daily dosing. Sartans show comparable antihypertensive activity to members of other major antihypertensive classes, such as ACE inhibitors, beta-blockers and calcium antagonists. Clinical trials have confirmed the placebo-like safety and tolerability of sartans in hypertensive patients. Based on these data, sartans offer advantages and represents an important new treatment option for hypertension.

C. Toxicology of Nitrosamines

Most toxicological assessments of nitrosamines focus on their carcinogenic and mutagenic properties. Little is known on other toxicological endpoints (see US EPA website). Some nitrosamines are classified as Category 2 carcinogenic agents, meaning, substances which are to be regarded as carcinogenic to man, because on the basis of sufficient results of long-term animal tests or indications based on animal tests and epidemiological studies it is to be assumed that they make a substantial contribution to the risk of cancer. *Toxicological effects of NDMA and NDEA depend on metabolic activation by CYP2E1 and/or CYP2A6 and DNA-repair capacities for the specific DNA-adducts formed, e.g. MGMT-dependent repair capacity.*

More than 300 N-nitroso compounds have been extensively tested in 40 different animal species and caused cancer in everyone of them, both after respiratory and

oral exposure. Since N-nitrosamines are converted by oxidative enzyme systems into substances that cause DNA mutations, which are thought to initiate carcinogenesis, they are mostly systemically acting genotoxic carcinogens.

NDMA has been tested in rats by oral administration *via* drinking water, gavage and diet and the main target organ upon oral exposure is the liver, but also lung and kidney tumors have been found. A comprehensive oral long-term study of Peto and co-workers in which they studied the dose-response relationship for the effects of NDMA on various types of liver cancer by exposing rats to 16 different doses of NDMA in drinking water, led to an additional lifetime cancer risk of 5.0 x 10-3 per µgNDMA/ kg body weight/day (life span conditions). Inhalation studies resulted in tumors mainly in the nasal cavity, and respiratory tract.

Information on the dose-response association between human nitrosamine exposure and risk of cancer is mainly provided by two types of exposure routes, i.e. occupational exposure through inhalation and exposure in the diet. Occupational exposure led to stomach, esophagus, lip, oral cavity, pharynx and lung cancer. However, the available evidence on occupational exposure and risk for cancer is most convincing for upper gastrointestinal tract cancers.

D. Recall of Sartans from the Marketplace

Normally, I do not use names of pharma companies in my newsletters. However, in this case, I was compelled to mention names of companies (from publicly available information on the internet) to indicate that nitrosamine contamination in sartans is not confined to any one country. It is important to make readers aware that pharma industry worldwide is very complex, in the sense, that not all tables that you see in the marketplace are tantamount to indicate that they are the manufacturers of API. Manufacture of API, formulators, and packager are all can be different for anyone set of marketed tablets.

In June 2018, some lots of valsartan were recalled due to detection of N-nitrosodimethylamine (NDMA), and the problem has just continued since then. A key point is that there are often just a few sources of actual API (active pharmaceutical ingredient). Other companies buy this material and formulate it into capsules, tablets, etc., and then still more companies repackage these.

Most ARBs have a chemical structure that includes a tetrazole group. Tetrazole ring formations, coupled with certain manufacturing conditions using the solvent N, N-Dimethylformamide (DMF), gave rise to this class of impurities in drug substance intermediates used in sartans. Other tetrazole ring formations make up APIs like candesartan, losartan, irbesartan, and olmesartan.

Valsartan recall was originally traced back to a problem with the material from Zhejiang Huahai Pharmaceuticals. That's the Chinese manufacturer who made the API itself. There were originally two generic companies whose tablets included the ZHP material, Teva and Prinston. But the Teva material was sold by

Major Pharmaceuticals and also by Actavis, while the Prinston was sold by Solco Healthcare – there are those three layers of API production, pill manufacturing, and repackaging. Meanwhile, Torrent Pharmaceuticals has emerged as a company who had bought the original valsartan API from Zhejiang Huahai, so their products went on the recall list.

But then the FDA found, while testing material from all over, that valsartan manufactured by Hetero Labs of India (and sold as Camber Pharmaceuticals tablets) also had NMDA contamination, so the problem wasn't just that one manufacturing source in China. Another drug in the same angiotensin II antagonist class as valsartan (irbesartan) was found to be contaminated, but this time with the N-nitrosodiethylamine (NDEA) instead of the dimethyl compound. This was made by ScieGen and again was repackaged under still more names. The API manufacturer for the ScieGen material was Aurobindo (of India), and they recalled material. Interestingly, Indian manufacturer Alembic Limited voluntarily withdrew its certificate of suitability (CEP) in September 2018, following the nitroso impurity episode.

Then Mylan pulled several lots of valsartan tablets, using API that they'd made themselves. Teva followed by recalling more lots of their product since they'd also bought some of the Mylan API for manufacturing. Torrent and Aurobindo have expanded their own recall of material because of the second NDEA contaminant.

The common feature, in this case, is the synthesis of the tetrazole ring common to all the sartans. And the change apparently was a solvent switch to dimethylformamide (or presumably diethylformamide, in the cases where NDEA is the contaminant) The dialkylformamides are often contaminated by small amounts of the corresponding dialkylamines, and they are well known to break down to give those (slowly) under heating.

The classic industrial syntheses of these molecules involved reacting an aryl nitrile with tri-n-butyltinazide. ZHP themselves appear to have introduced a cheaper, higher-yielding route using just sodium azide and zinc chloride in an aprotic solvent like DMF. The excess azide is consumed at the end of the process using sodium nitrite – but nitrite under acidic conditions will give some nitrous acid, and nitrous acid will react with secondary amines to give you N-nitrosoamines. That would seem to be the root of the problem.

E. Toxicological Implication of Nitrosamine Contamination

Although, nitrosamines are mutagenic, however, their mutagenicity at lower levels is a matter for debate. It all depends on how the high-dose animal studies can be extrapolated down, how both high- and low-dose animal studies can be extrapolated to humans in general, and how to interpret human observational data (on, for example, the consumption of cured meats, which contain low levels of nitrosamines) in the presence of multiple other factors. Not least among these is the problem that some of the N-nitroso compounds are in foodstuffs

themselves, while others are produced by gut bacteria. Everyone can agree, though, that large amounts of N-nitroso compounds are bad news, for some value of "large". And everyone can agree that exposing yourself to such compounds for no reason at all is senseless.

The FDA's standard is to be below an amount that would be expected (by their dose/response modeling) to cause 1 extra cancer case in 100,000 people who were taking the tablets at a standard dose for 70 straight years. That's pretty stringent, considering the background rates of cancer in people who actually stay alive for 70 years in a row, especially when you factor in that no one goes on valsartan when they're ten years old. Unfortunately, the ZHP material (according to the FDA) would be expected to cause one extra case of cancer with only 8,000 patients taking the highest dose of the drug for only four years, and that's definitely unacceptable. It appears that the other manufacturers' batches had contaminants at lower levels.

This case may benefit some manufacturers that may claim their products are "nitroso impurity-free," as well as analytical laboratories that have validated methods for analyses of nitroso impurities in drug substances.

F. Estimated Exposure Of Nitrosamines To Humans From Sartans

Data that is critical for risk assessment is some index of exposure, granted all exposures are usually best estimates, based on contamination of sartan tablets with nitrosamines.

NDMA and NDEA are above the AI levels defined based on the principles of ICH M7(R1) have been found retrospectively in valsartan batches manufactured by ZH. NDMA or NDEA above the AI level were also found e.g. in valsartan batches manufactured by ZT and Mylan, losartan batches manufactured by Hetero Labs and in irbesartan batches manufactured by Aurobindo. For risk assessment, it is prudent to perform excess risk calculations using the highest mean amounts found in finished products and the levels reported to be found in APIs.

The worst-case scenario for excess risk calculations is therefore still assumed to be batches finished product manufactured using ZH API containing meanvalues of 75.4 ppm NDMA (24.1 µg in 320 mg valsartan) and of ZH API with 11.53 ppm NDEA (3.7 µg).

The average daily exposure to NDMA due to contaminated beverages and food, air and water pollution is assumed as within an order of magnitude 100 – 1,000 ng/day = 0.1 – 1 µg /day. Thus, NDMA exposure associated with contaminated valsartan tablets (containing API from ZH) containing the mean NDMA levels is approximately 24 to 240 times higher than the daily exposure through beverages and food, air and water pollution.

G. Risk Characterization for Nitrosamines from Sartan

NDMA belongs to N-nitroso compounds, which are part of the so-called "cohort of concern" described in the ICH guideline M7(R1). For such compounds, the generic TTC of 1.5 µg/day as an AI for mutagenic impurities is not considered applicable and a compound specific AI needs to be derived from compound-specific carcinogenicity data.

The generally accepted approach recommended by ICH M7(R1) is to use either the dose giving a 50% tumor incidence (TD_{50}) as the point of departure for the calculation of excess cancer risk.

The TD_{50} listed for NDMA is 0.096 mg/kg/day (in the most sensitive species, the rat). The extrapolation to the excess risk level for cancer is performed by linear back extrapolation to the dose theoretically causing a 1:100,000 risk by dividing the TD_{50} by 50,000 (50% or 0.5 x 100,000). For NDMA this translates into a dose of 1.92 ng/kg/day. For a person with a bodyweight of 50 kg this would result in an AI level of 96 ng/day (50 x 1.92 ng). 96 ng/day correspond to 0.3 ppm in a 320 mg valsartan tablet.

According to the Haber's rule, a fundamental principle in toxicology generally accepted to be used for mutagenic carcinogens and therefore considered appropriate for NDMA, the total dose taken over time (dose x time) produces a fixed level of effect, thus, determining the risk associated with the exposure. Applying this very conservative principle, the cumulated dose acceptable for lifetime is then the AI multiplied by the days of a lifetime (70 years is generally accepted for this) of 25,550 days. Using the AI calculated from TD_{50} i.e. 96 ng/day results in a lifetime acceptable dose associated with a 1:100,000 additional cancer risk of 2453 µg.

As the maximum duration of exposure to NDMA from contaminated valsartan is 6 years or 2190 days the AI for 6 years can be calculated by dividing by 2190 resulting in: 1.12 µg/day as AI over 6 years.

This calculation resulted in a theoretical excess lifetime cancer risk of 1:5000 (0.02%) for taking daily 320 mg valsartan contaminated with 24.1 µg NDMA (the highest amount tested in valsartan FP).The lowest calculated TD_{50} is 0.00725 mg/kg/day for liver tumors in the cynomolgus monkey.

The TD_{50} for NDEA obtained in the rat is 0.0265 mg/kg/day. In rat besides liver tumors also other tumors were reported e.g. esophagus, kidney, and vasculature in the male rat.

The extrapolation to the excess risk level for cancer is then done the same way as for NDMA by linear back extrapolation to the dose theoretically causing a 1:100,000 risk by dividing the TD50 by 50.000 (50% or 0.5 x 100,000). For NDEA this translates into a dose of 0.144 ng/kg/day extrapolated from cynomolgus monkeys and 0.53 ng/kg/day extrapolated from rat. For a person

with a bodyweight of 50 kg this would result in an AI level of 7.2 ng/day (50 x 0.144 ng) or 26.5 ng/day (50 x 0.53 ng). 7.2 ng is equal to 0.0225 ppm and 26.5 ng is equal to 0.083 ppm in a 320 mg valsartan tablet.

Both compounds are assumed to be toxic mainly by the mutagenic action of highly reactive metabolites which alkylate DNA and form highly mutagenic DNA-adducts. It is assumed that DNA adducts add up in linear kinetics. Adduct formation follows a linear kinetic and the risk is also considered to be additive.

For patients exposed for 6 years to NDMA-contaminated valsartan assuming a 1:1 transfer of the impurity from API to finished product and a mean NDMA content of 24.1 µg in a 320 mg tablet, the theoretical excess risk of cancer during lifetime is calculated to be 21.5 in 100,000. This is approximately 0.02%. For patients exposed for 4 years to NDEA-contaminated valsartan assuming a 1:1 transfer of the impurity from API to finished product and a mean NDEA content of 3.7 µg in a 320 mg tablet, this theoretical excess risk is calculated to be 8 in 100,000 (0.08%). The excess risk in patients co-exposed to both NDMA and NDEA for 4 years and NDMA alone for 2 years is calculated to be 29.5 in 100,000 or 0.03%.

Compared to the overall background risk of cancer during a lifetime in the Western world, the theoretical additional risk due to the highest levels reported of NDMA/NDEA in some valsartan batches is considered very low.

H. Risk Management

In order to manage minimal to unknown risk of exposure of nitrosamines through contamination of sartan API and tablets, as of 25thJune 2019 the European Medical Agency (EMA) has issued a directive for temporarily interim limits for nitrosamines based on the TD_{50} values in rat carcinogenicity studies have been set for NDMA and NDEA: acceptable intakes (AI) of 96.0 ng for NDMA and 26.5 ng for NDEA.

I. Conclusions

Sartan-containing medicinal products are important treatment options for hypertension or certain heart or kidney diseases. Efficacy and safety of sartan-containing medicines, in general, is very good. The key issue of this newsletter concerns the detection of N-nitrosamine (esp. NDMA and/or NDEA) contaminations in sartans, the resulting potential long-term risk to patients and measures to minimize as much as possible these contaminations.

Nitrosamines are chemically simple molecules and can be formed in pharmaceutical manufacturing steps whenever there is a presence of a secondary (or tertiary) amines and nitrites, usually in acidic conditions. NDMA and NDEA are two of the most potent mutagenic carcinogens known. As soon as the problem of nitrosamine-contamination became known, immediate, precautionary measures were taken by competent authorities across the EU and US FDA such as recalls of affected batches from pharmacies. Initially, this was only necessary

for valsartan-containing APIs from few manufacturers but later also for some other sartans with a tetrazole ring.

The impact of NDMA and NDEA on human health is currently only extrapolated from animal studies. It is prudent to assume that effects seen in animals may also occur in humans after exposure to sufficiently large amounts of these nitrosamines.

Risk assessment for patients previously exposed to NDMA and/or NDEA impurities in sartans, especially valsartan which was found to contain the highest nitrosamine contamination, is not possible as the real extent of exposure of patients is unknown. The risk assessment is based on a potential worst-case scenario, which would be a partially combined exposure to the highest levels of NDEA for 4 years (2011 – 2015) and to NDMA for 6 years (2012 – 2018) reported from a sartan, resulting in a cumulative theoretical excess cancer risk of 29.5:100,000 or 1:3390 (0.029%) when extrapolated from rat studies according to ICH M7(R1). Compared to the lifetime cancer risk in the population of approximately 50%, this additional risk is considered to be very low. Even though estimated cancer risk is low, it is prudent to have a goal to achieve for negligible nitrosamines to zero sartans.

As a final step to managing minimal to unknown risk of exposure of nitrosamines through contamination of sartan API and tablets, as of 25thJune 201 the European Medical Agency (EMA) has issued a directive for temporarily interim limits for nitrosamines based on the TD50 values in rat carcinogenicity studies have been set for NDMA and NDEA: acceptable intakes (AI) of 96.0 ng for NDMA and 26.5 ng for NDEA.

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