Regular Article

N-Nitrosodimethylamine (NDMA) Formation from Ranitidine Impurities: Possible Root Causes of the Presence of NDMA in Ranitidine Hydrochloride

Hidetomo Yokoo, a,b,# Eiichi Yamamoto,* a,b Sayaka Masada, a Nahoko Uchiyama, a
Genichiro Tsuji, a Takashi Hakamatsuka, a Yosuke Demizu, a Ken-ichi Izutsu, a and Yukihiro Goda a

a National Institute of Health Sciences; 3–25–26 Tonomachi, Kawasaki-ku, Kawasaki 210–9501, Japan; and b Medical Chemistry, Graduate School of Medical Science, Kyoto Prefectural University of Medicine; 1–5 Shimogamohangi-cho, Sakyo-ku, Kyoto 606–0823, Japan.

Received April 6, 2021; accepted May 31, 2021

N-Nitrosodimethylamine (NDMA) is a probable human carcinogen. This study investigated the root cause of the presence of NDMA in ranitidine hydrochloride. Forced thermal degradation studies of ranitidine hydrochloride and its inherent impurities (Imps. A, B, C, D, E, F, G, H, I, J, and K) listed in the European and United States Pharmacopeias revealed that in addition to ranitidine, Imps. A, C, D, E, H, and I produce NDMA at different rates in a solid or an oily liquid state. The rate of NDMA formation from amorphous Imps. A, C, and E was 100 times higher than that from crystalline ranitidine hydrochloride under forced degradation at 110 °C for 1 h. Surprisingly, crystalline Imp. H, bearing neither the N,N-dialkyl-2-nitroethene-1,1-diamine moiety nor a dimethylamino group, also generated NDMA in the solid state, while Imp. I, as an oily liquid, favorably produced NDMA at moderate temperatures (e.g., 50 °C). Therefore, strict control of the aforementioned specific impurities in ranitidine hydrochloride during manufacturing and storage allows appropriate control of NDMA in ranitidine and its pharmaceutical products. Understanding the pathways of the stability related NDMA formation enables improved control of the pharmaceuticals to mitigate this risk.

Key words N-nitrosodimethylamine (NDMA); ranitidine hydrochloride; impurity; stability; degradation

Introduction

N-Nitrosodimethylamine (NDMA) and other nitrosamines are mutagenic impurities in pharmaceutical products. These genotoxic impurities were discovered in multiple valsartan and angiotensin II receptor blocker (ARB) formulations in 2018.3 An investigation by regulatory agencies revealed contamination in the manufacturing process of the active substance as the cause of the safety issues. Following the International Conference on Harmonization (ICH) M7 guideline, the interim criteria for the levels of NDMA were established, based on the acceptable daily exposure limits and the maximum daily dose of the drug.1–4 In addition, varying amounts of NDMA were discovered within ranitidine products, resulting in multiple products being recalled from the market in September 2019.5–9

Formation of NDMA during storage of ranitidine active pharmaceutical ingredients (APIs) and formulations emphasized the importance of clarifying the formation mechanism.10–14 NDMA is formed in ranitidine APIs because of an intermolecular degradation reaction between ranitidine molecules that occurs primarily in the solid state, but the exact reaction mechanism is unknown.15 However, the NDMA formation in storage10–14 suggested that other impurities have the potential to generate NDMA. The European Pharmacopoeia (Ph. Eur.) and the United States Pharmacopeia (USP) list 11 potential impurities, A, B, C, D, E, F, G, H, I, J, and K (Imps. A–K)16,17 (Fig. 1), that may be generated during production and storage of ranitidine hydrochloride. However, to the best of our knowledge, no studies have been reported on NDMA formation from ranitidine impurities. To determine the root cause of the presence or formation of NDMA in ranitidine, forced degradations of ranitidine impurities were performed over short durations under moderate and high temperatures.

Results and Discussion

Characteristics of Ranitidine Hydrochloride

The X-ray powder diffraction (XRPD) pattern of ranitidine hydrochloride was consistent with the pattern of crystal form 2 in the literature.18 Batch purity exceeded 99.4%, determined using HPLC with UV detection at 235 nm. The sample contained Imps. B and H at 0.24 and 0.06%, respectively, whereas amounts of each Imps. A, C, D, E, F, G, I, J, and K were less than 0.05%.

Forms of Ranitidine Impurities

The forms of the impurities were evaluated using optical and polarized light microscopy. The results were as follows: Imp. A, amorphous (lyophilized); Imp. B, oily liquid; Imp. C, amorphous (lyophilized); Imp. D, crystalline; Imp. E, amorphous; Imp. F, oily liquid; Imp. G, crystalline and amorphous mixture; Imp. H, crystalline; Imp. I, oily liquid (lyophilized); Imp. J, crystalline; and Imp. K, crystalline.

NDMA Formation from Ranitidine Hydrochloride and Its Impurities under Accelerated Stress Conditions

Previous studies have confirmed NDMA formation from ranitidine during storage at 60 °C,14,15 but the reaction mechanism is unknown.15 To investigate NDMA formation from ranitidine impurities, the formation of NDMA from ranitidine hydrochloride and its impurities (Imps. A–K) under stress conditions was evaluated in the solution and intact solid/oily states. NDMA is not detected in the samples prior to heat treatment or from ranitidine hydrochloride or its impurities in solution at 50 °C after 1 h. Conversely, NDMA is generated when several...
Impurity samples are heated in the solid or oily liquid state at 50 or 110°C for 1 h (Fig. 2). Among these, only Imp. I showed slight NDMA formation after storage at 50°C for 1 h (Fig. 2a). However, after heating at 110°C for 1 h, NDMA formation was observed from ranitidine hydrochloride (crystalline) and Imps. A (amorphous), C (amorphous), D (crystalline), E (amorphous), and H (crystalline), in addition to Imp. I (oily liquid) (Fig. 2b). The amounts of NDMA formed after heating at 110°C were 0.09, 13.6, 9.1, <0.01, >14.6, 0.2, and 6.2 µM from ranitidine hydrochloride and Imps. A, C, D, E, H, and I, respectively. The levels of NDMA formation from Imps. A, C, E, and I were remarkably higher than those of crystalline ranitidine hydrochloride and Imps. D and H. The estimated NDMA formation rates from Imps. A, C, E, and I under these conditions were 151, 101, >163, and 69 times higher than that from crystalline ranitidine hydrochloride, respectively. The reaction rates varied depending not only on the impurity structure but also on their forms, i.e., their presence as crystalline or amorphous solids or oils during the reaction. Thus, these results provided qualitative data regarding the formulation of NDMA. The estimated generation ratio (%) of NDMA from ranitidine hydrochloride and its impurities at 50°C and 100°C for 1 h is shown in Table 1.

Hence, the direct formation of NDMA not only from ranitidine but also via impurities risks significant NDMA contamination in ranitidine hydrochloride and its pharmaceuticals. In contrast, Imp. G (mixture of crystal and amorphous) did not produce NDMA (data not shown), as demonstrated by the stability of a ranitidine hydrochloride sample containing 2.7% (area/area) Imp. G. No increased NDMA formation was observed under storage conditions (3d, 60°C, 10% relative humidity). 31

The chemical structures of Imps. A, C, D, E, and I contain dimethylamino and/or N,N-diethyl-2-nitroethene-1,1-diamine groups, and NDMA is believed to form via the reaction of these two functional groups. 15,19 Imp. C, wherein the sulfide moiety of ranitidine is oxidized, generated more NDMA than that generated by ranitidine hydrochloride. The sulfide moiety is thought to act as a nucleophile, generating Imp. G, and thus, other structural transformations may affect the efficiency of NDMA formation.

Notably, Imp. H is a different type of NDMA-forming compound that contains neither the N,N-diethyl-2-nitroethene-1,1-diamine moiety nor the dimethylamino group. To elucidate the efficiency of NDMA formation from Imp. H (crystalline), the storage of the solid samples at 60°C was investigated. Whereas NDMA formation is not detected after heating for 9 h, it is detected after 18 and 24 h, with the amount increasing with time (Fig. 3). Although the amount of NDMA generated from Imp. H (crystalline) is lower than those generated from Imps. A, C, and I in the amorphous or oily liquid states, Imp. H may be another pathway for NDMA formation in ranitidine samples. This highlighted the necessity of additional research on NDMA formation and the degradation pathways of ranitidine and its impurities for controlling potential risk.

The nitrosation of dimethylamine or the dimethylamino group of ranitidine generates NDMA, whereas Imps. A, C, D, E, and I contain dimethylamino groups in their structures, Imp. H does not contain this group. We hypothesize that the generation of the alkyl amine, methylene, by hydrolysis of the N-methyl group of Imp. H and subsequent mono-methylation with nitromethane, which is generated by hydrolysis and decarboxylation of Imp. H, leads to the formation of dimethylamine and nitrous acid. Subsequently, the dimethylamine and nitrous acid react, forming NDMA (Chart 1). However, the formation of NDMA from methylene and nitromethane is merely a theory. To confirm it, a mixture of nitromethane and dimethylamine was heated at 110°C for 1 h, and the reaction mixture was analyzed, indicating that NDMA was formed, supporting our proposed mechanism of NDMA.
According to Ph. Eur. and USP, the upper limit of the concentration of Imp. A in ranitidine hydrochloride drug substance is 0.3% (w/w). To control NDMA generation in ranitidine hydrochloride during storage, impurity formation must be strictly controlled.

Accelerated stability studies for ranitidine hydrochloride showed an increase in the NDMA formation rate as the temperature and relative humidity increased. 14,15)

Table 1. Estimated Generation Ratio (%) of NDMA from Ranitidine Hydrochloride and Its Impurities at 50°C and 100°C for 1 h

| Substance          | State form | Generation ratio (%) |
|--------------------|------------|----------------------|
|                    |            | 50°C | 110°C      |
| Ranitidine hydrochloride | Crystal (form 2) | ND   | 0.01       |
| Imp. A             | Amorphous  | ND   | 1.36       |
| Imp. C             | Amorphous  | ND   | 0.91       |
| Imp. D             | Crystalline| ND   | <0.001     |
| Imp. E             | Amorphous  | ND   | >1.46      |
| Imp. H             | Crystalline| ND   | 0.02       |
| Imp. I             | Oily liquid| ND   | <0.00001   |
|                    |            |      | 0.62       |

ND: Not determined.

Fig. 2. Liquid Chromatography-Tandem Mass Spectrometry Chromatograms of N-Nitrosodimethylamine (NDMA) in the Solid or Oily Liquid Phase Samples (Ranitidine Hydrochloride and Impurities (Imps.) A, C, D, E, H, and I) after Heating at (a) 50 or (b) 110°C for 1 h

Fig. 3. Amount of N-Nitrosodimethylamine (NDMA) Produced by Heating Impurity (Imp.) H at 60°C for 0, 9, 18, and 24 h in the Solid State (n = 2)
process impurity that is generated in the manufacturing of the drug substance\(^{20}\) and is formed, along with Imps. E and I, by the hydrolysis of ranitidine.\(^{20,21}\) Imp. C is formed by the oxidative degradation of ranitidine,\(^{22}\) while Imps. D and H are formed from ranitidine under acidic or basic conditions.\(^{15,19}\) Therefore, the formation of NDMA as a secondary degradation product of ranitidine, in addition to direct NDMA formation from ranitidine during storage, is reasonable.

It was reported that morphology of ranitidine hydrochloride crystal would affect the rate of NDMA formation. It could be related to differences in surface properties which promote interactions with water, resulting in increased rates of NDMA formation as the change from a crystalline solid to oil occurred.\(^{15}\) Thus, in the case of the amorphous substance, the significant water sorption in comparison with the crystalline solid caused by the large surface area would lead the faster formation of NDMA.

**Conclusion**

To evaluate the potential for NDMA formation from the ranitidine impurities (Imps. A–K) to control the total risk, the amount of NDMA in solution or the solid/oil samples under stress conditions was elucidated. NDMA was not generated from ranitidine or its impurities in solution at 50 °C for 1 h. Meanwhile, in the solid/oil states at 50 °C for 1 h, NDMA was only generated from the oily liquid Imp. I. In addition to Imp. I, NDMA was generated from ranitidine hydrochloride and Imps. A, C, D, E, and H at 110 °C for 1 h. The generation of NDMA from Imp. I occurred more easily than from ranitidine hydrochloride and the other impurities. Thus, solid storage of ranitidine hydrochloride may increase the potential NDMA risk; moreover, the risk of NDMA generation from impurities in ranitidine API should also be considered. Certain chemical moieties within ranitidine, including the dimethylamino and \(N,N\)-dialkyl-2-nitroethene-1,1-diamine groups, have been considered necessary for the formation of NDMA.\(^{15,19}\) However, NDMA formation from Imp. H, which contains neither the \(N,N\)-dialkyl-2-nitroethene-1,1-diamine group nor the dimethylamino group, indicated that there may be a similar risk of NDMA formation from any compound containing the \(N,N\)-dialkyl-2-nitroethene-1,1-diamine moiety and/or the dimethylamino group. Further studies are necessary to achieve a deeper understanding of the process, as the complexities in NDMA generation are inherently related to several other factors. This study elucidated the risk of NDMA generation from ranitidine impurities/degradants as a second-generation degradant under certain conditions. Combining these results with abundant data regarding the quality and stability of ranitidine hydrochloride, the safe delivery of ranitidine medication to patients may be achieved.

**Experimental**

**Chemicals and Reagents** The NDMA standard (>99.0% purity), acetonitrile (ACN, LC-MS grade), ammonium acetate, trifluoroacetic acid (TFA), and methanol were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), and deuterium-labeled NDMA (NDMA-\(d_6\)), the internal standard, was purchased from AccuStandard (New Haven, CT, U.S.A.). Ranitidine hydrochloride and Imp. B (oily liquid) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Imps. A and E were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.), while Imps. C, G, H, and I (oily liquid) were purchased from Carbosynth (Compton, U.K.) and Imps. D, F (oily liquid), J, and K were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Water was deionized and purified using a Milli-Q\textsuperscript{®} TOC purification system (MilliporeSigma, Burlington, MA, U.S.A.). Imps. A, C, and I that contained NDMA were purified using HPLC equipped with a Supelco Discovery\textsuperscript{®} BIO Wide Pore C18 column (250 × 21.2 mm I.D., 10 μm particle size) (Bellefonte, PA, U.S.A.). Mobile phase A was water and mobile phase B was ACN. The flow rate and column temperature were 10.0 mL/min and 35 °C, respectively. A linear gradient was used for elution, consisting of mobile phase B 0–100% (v/v) in 40 min. The detection wavelength was 220 nm. Collected sample fractions were lyophilized for further purification, and the absence of NDMA in the samples was confirmed using LC-tandem mass spectrometry (LC-MS/MS).

**HPLC-UV Analysis of Ranitidine Hydrochloride** To evaluate the chemical purity profile of the ranitidine hydrochloride, HPLC-UV analysis was performed. HPLC was performed using an LC-20A HPLC system (Shimadzu, Kyoto, Japan) with an InertSustain AQ-C18 column (4.6 × 100 mm, 3 μm particle size, 12 mm pore size) (GL Sciences, Tokyo, Japan). Mobile phase A was water containing 0.1% (v/v) TFA and mobile phase B was ACN containing 0.1% (v/v) TFA. The flow rate and column temperature were 0.5 mL/min and 35 °C, respectively, and the injection volume was 10 μL. A linear gradient was used for elution, consisting of mobile phase B at 5% (v/v) for 1.0 min, 5–10% (v/v) in 13 min, 10–90% (v/v) in 2.0 min, and maintained for 3 min. The column was then equilibrated for 9 min with 5% (v/v) mobile phase B. The detection wavelength was 235 nm, and the performance of the method was validated in terms of specificity, linearity, and reproducibility. Specificity and impurity separation was confirmed using authentically identified impurities (A, B, C, D, E,
The purity (%) of ranitidine hydrochloride was evaluated as follows: the peak areas for ranitidine and all impurity peaks not less than 0.05 area% were determined via integration, and ranitidine purity (%) was calculated as (ranitidine area + total impurity area)−total impurity area%.

**XRPD** XRPD was performed using a Miniflex X-ray powder diffractometer (Rigaku, Tokyo, Japan) equipped with Cu Ka radiation (\(\lambda = 1.54\,\text{Å}\)) and a D/teX Ultra2 detector. The scan range (2\(\theta\)) was 5.00°−40.00° with a data collection time of 1 s and a step size of 0.01°. The tube voltage and current were 40 kV and 15 mA, respectively, and the scanning speed was 10.00°/min.

**Optical and Polarized Light Microscopy** Optical and polarized light microscopy was performed using an Axio Scope A1 (Carl Zeiss AG, Oberkochen, Germany) equipped with a VHX-970F digital camera (KEYENCE, Osaka, Japan). The powders or oils of the ranitidine impurities (approximately 2 mg) were placed in glass vials with hermetic caps and heated at 50 or 110 °C in an oil bath for 1 h. The temperature of 110 °C was selected based on the forced degradation of ranitidine in solution in our previous study and a forced degradation study of an API.\(^{23}\)

**NDMA Formation from Imp. H** A mixture of nitromethane (10 mM) and methylamine (10 mM) in 10% (v/v) aqueous ACN in a glass vial with a hermetic cap was heated at 110 °C in an oil bath for 1 h.

**LC-MS/MS** The powder or oily forms of the ranitidine impurities before and after heat treatment were dissolved in 10% (v/v) ACN to obtain a concentration of 1 mM. The sample solutions were then analyzed using LC-MS/MS. The limits of quantitation and detection were 0.03 and 0.01 ppm, respectively.

**Acknowledgments** The authors would like to express their deepest appreciation to Prof. Osamu Onomura (Nagasaki University) for his help in elucidating the proposed mechanism of NDMA formation, Dr. Hiroko Tokumoto for her help with polarized light microscopy, and Hitomi Kan-no and Naomi Tomita for their help with HPLC. This study was partially supported by the Japan Agency for Medical Research and Development (AMED) under Grant Numbers JP20mk010171 and JP21mk0101208.

**Conflict of Interest** The authors declare no conflict of interest.

**References**
1) U.S. Food and Drug Administration, “Statement on the agency’s ongoing efforts to resolve safety issue with ARB medications.”: <https://www.fda.gov/news-events/press-announcements/ongoing-efforts-resolve-safety-issueARBmedications>, cited 31 January, 2021.
2) ICH, “ICH HARMONISED GUIDELINE ASSESSMENT AND CONTROL OF DNA REACTIVE (MUTAGENIC) IMPURITIES IN PHARMACEUTICALS TO LIMIT POTENTIAL CARCINOGENIC RISK M7(R1).” cited 11 February, 2021.
3) European Medicines Agency, “Sartan medicines: companies to review manufacturing processes to avoid presence of nitrosamine impurities.”: <https://www.ema.europa.eu/en/news/sartan-medicines-companies-review-manufacturing-processes-avoid-presence-nitrosamine-impurities>, cited 11 February, 2021.
4) Ministry of Health Labour and Welfare of Japan, “Notification: setting of interim limits for NDMA and NDEA in Sartan drugs.”: <https://www.pmda.go.jp/files/000226684.pdf>, cited 11 February, 2021.
5) U.S. Food and Drug Administration, “Statement on new testing results, including low levels of impurities in ranitidine drugs.”: <https://www.fda.gov/news-events/press-announcements/statement-new-testing-results-including-low-levels-impurities-ranitidine-drugs>, cited 11 February, 2021.
6) Australian Government Department of Health, “Contamination of ranitidine medicines with the nitrosamine NDMA.”: <https://www.iga.gov.au/sites/default/files/iga-laboratories-testing-ranitidine-medicines.pdf>, cited 11 February, 2021.
7) European Medicines Agency, “EMA to review ranitidine medicines following detection of NDMA.”: <https://www.ema.europa.eu/en/documents/press-release/ema-review-ranitidine-medicines-following-detection-ndma_en.pdf>, cited 11 February, 2021.
8) Ministry of Health Labour and Welfare of Japan, “Notification: Analysis of carcinogenic substances in ranitidine hydrochloride.”: <https://www.pmda.go.jp/files/0002231528.pdf>, cited 11 February, 2021.
9) Food and Drug Administration, “Questions and Answers: NDMA impurities in ranitidine (commonly known as Zantac).”: <https://www.fda.gov/drugs/drug-safety-and-availability/questions-and-answers-ndma-impurities-ranitidine-commonly-known-zantac>, cited 29 March, 2021.
10) Jamróziewicz M., Wielgomas B., J. Pharm. Biomed. Anal., 76, 177–182 (2013).
11) Parr M. K., Joseph J. F., J. Pharm. Biomed. Anal., 164, 536–549 (2019).
12) Scherf-Clavel O., Kinzig M., Besa A., Schreiber A., Bidmon C., Abdel-Tawab M., Wohlfart J., Sörgel F., Holzgrabe U., J. Pharm. Biomed. Anal., 172, 278–284 (2019).
13) Sörgel F., Kinzig M., Abdel-Tawab M., Bidmon C., Schreiber A., Ermel S., Wohlfart J., Besa A., Scherf-Clavel O., Holzgrabe U., J. Pharm. Biomed. Anal., 172, 395–405 (2019).
14) Abe Y., Yamamoto E., Yoshida H., Usui A., Tomita N., Kanno H., Masada S., Yokoo H., Tsuji G., Uchiyama N., Hakamatsuka T., Demizu Y., Izutsu K.-S., Goda Y., Okuda H., Chem. Pharm. Bull., 68, 1008–1012 (2020).
15) King F. J., Searie A. D., Uruhart W. M., Org. Process Res. Dev., 24, 2915–2926 (2020).
16) European Directorate for the Quality of Medicines, “European Pharmacopoeia 9.2 Supplement. Council of Europe,” Strasbourg, France, pp. 4603–4604, 2017.
17) United States Pharmacopeial Convention, “United States Pharmacopeia and National Formulary (USP 42-NF 37).” Rockville, MD, U.S.A., pp. 3811–3812, 2019.
18) Chieng N., Zajovic Z., Bowmaker G., Rades T., Saville D., Int. J. Pharm., 327, 36–44 (2006).
19) Brittain R. T., Harris D. M., Martin L. E., Poynter D., Price B. J., Lancet, 318, 1119 (1981).
20) GlaxoSmithKline, “Interview form of Zantac Tablets, 11th edition.”: <http://www.pmda.go.jp/p>, cited 11 February, 2021.
21) Haywood P. A., Martin-Smith M., Cholerton T. J., Evans M. B., J. Chem. Soc. [Perkin I], 951–954 (1987).
22) Sharma N., Rao S. S., Kumar N. D., Reddy P. S., Reddy A. M., Sci. Pharm., 79, 309–322 (2011).
23) Narasimhan B., Abida K., Srinivas K., Chem. Pharm. Bull., 56, 431–447 (2008).