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Review

Synthesis, Characterization, and Application of 2-((3-(Chloromethyl)-benzoyl)oxy)benzoic Acid: A Review

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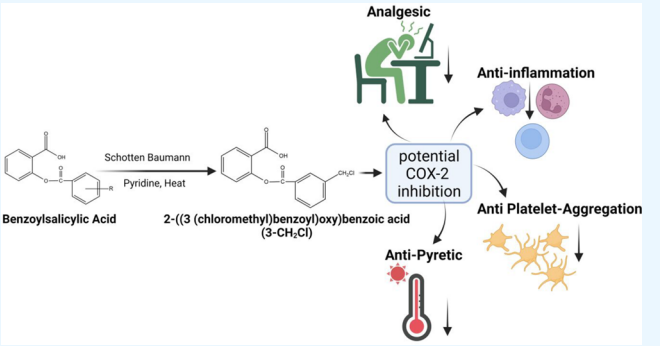
ABSTRACT: Salicylic acid (SA) derivate is well-known for its anti-inflammatory and analgesic activity through cyclooxygenase (COX)-inhibition. Previous studies pointed toward gastric toxicity induced by most salicylic acid derivative compounds, particularly acetylsalicylic acid (ASA). Despite the adverse effect, ASA is still used due to price affordability and additional advantages in preventing platelet aggregation. Recently, a novel salicylic acid derivative called 2-((3 (chloromethyl)benzoyl)oxy)benzoic acid (3-CH_2Cl) was introduced as a potential alternative compound to substitute ASA. Preliminary assessment results of COX-2 specificity, toxicity profile, analgesic, anti-inflammatory, and antiplatelet activity have made 3-CH_2Cl a promising compound for "new" drug development. This review focuses on the discovery, potential activity, and benefits of 3-CH_2Cl and the possible molecular mechanisms of its regulations in health and disease. Thus, this review may prove to be beneficial for the utilization of 3-CH_2Cl as a potential alternative drug to substitute ASA.



INTRODUCTION

About 2500 years ago, Hippocrates, the father of medicine, prescribed willow bark to reduce fever and pain. His finding form the basis of the acetylsalicylic acid (ASA)-discovery.[1] Besides anti-inflammatory and analgesic activity, acetylsalicylic acid (ASA) is famous for preventing platelet aggregation due to its unique capability in inhibiting cyclooxygenases (COX).[2] However, several studies have reported the side effect of ASA, particularly on the gastrointestinal tract.[3−5] In 2019, the American Heart Association (AHA) and American College of Chest Physicians (ACC) recommended avoiding the routine usage of ASA for the treatment of cardiovascular diseases in older patients over 70 years.[6] To maintain the benefit of acetylsalicylic acid and minimize its harmful effects, many compounds bearing salicylic acid residue were invented. Several modifications of salicylic acid such as methyl salicylate, mesalamine, 2-(2-hydroxy benzoyl)oxy benzoic acid (salsalate), and acetyl 3-aminoethyl salicylic acid (Ac3AESA) have been reported.[7] They have been used to treat inflammatory conditions, mainly by inhibiting the COX pathway and the Nuclear Factor Kappa B (NF-κb) pathway.[8] However, the synthesis of these compounds was relatively complex.

The anti-inflammatory activity of NSAIDs originated by salicylic acid derivate is associated with COX inhibition.[9] The two famous COX-isoforms exert different physiological functions. COX-1 mainly catalyzes prostaglandins (PGs) production that regulates vascular homeostasis, platelet function, and gastric and renal cellular integrity. COX-2 catalyzes the production of pro-inflammatory PGs.[10] COX-2 is a constitutive and inducible enzyme.[11] Induction of COX-2 expression is triggered by several pathways, such as activated toll-like receptors (TLR) and NFkβ pathways. The non-selective inhibition of ASA toward COX enzyme is the main reason for its adverse effect due to disruption of cellular homeostasis.[12] Therefore, many studies shifted toward the discovery of drugs with better selectivity toward COX-2 inhibition. Here, in this review, we focus on a novel compound called 2-((3(chloromethyl)benzoyl)oxy)benzoic acid (3-CH_2Cl), its discovery, and potential benefits.

SYNTHESIS, CHARACTERIZATION AND TABLET FORMULATION OF 2-((3-(CHLOROMETHYL) BENZOYL)OXY)BENZOIC ACID

Through modification of the Schotten-Baumann acylation-reaction, numerous structure modifications could be elicited in

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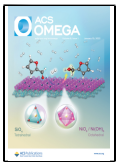

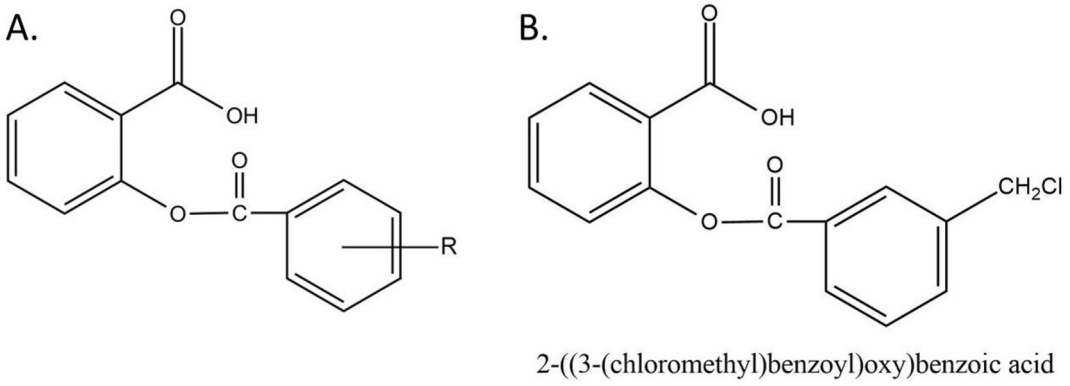

Figure 1. Salicylic acid main structure and derivate: (A) benzoylsalicylic acid; (B) 2-((3(chloromethyl)benzoyl)oxy)benzoic acid (3-CH_2Cl).

the phenolic group of SA. The main structure was called benzoyl salicylate (Figure 1A). Based on the above reaction, Natalia et al. (2013) reported the *in-silico* invention of 64 new compounds, in which 14 of them have been successfully synthesized (see Table 1). The *in-silico* docking experiment of

Table 1. Different Structural Modification of Benzoyl Salycilate (-R), Their Analgesic-Activity Effective Dose (ED_{50}), and Potential Interaction (G-Score) with COX-2

no.	group (R)	analgesic activity (ED_{50}) in murine models	G-score COX-2
1	3-CH_2Cl	15.73	−9.48
2	4-CH_2Cl	19	−8.79
3	4-OCF_3	22.62	−8.00
4	2-Cl	40.31	−8.77
5	4-$C(CH_3)_3$	26.65	−9.21
6	3,5-Cl_2	25.32	−9.03
7	4-C_4H_9	58	−8.92
8	H	32	−7.83
9	4-NO_2	43	−8.28
10	4-OCH_3	23	−6.83
11	4-CH_3	22	−8.36
12	3-Cl	20.09	−7.39
13	4-F	21.09	−7.97
14	4-CF_3	21	−8.00

14 compounds with human COX-2 receptor protein (PDB: 5F1A) was performed using the Grid-Based Ligand Docking with Energetic (Glide) scoring system.[13] Thus, 3-CH_2Cl was chosen for further research because this compound (Glide Score 3-CH_2Cl = −9.48 kcal/mol) had better affinity than ASA (Glide Score, −5.88 kcal/mol). The 2D binding interaction has been presented by Caroline and colleagues.[14] Furthermore, the lipophilic parameter (CLogP) of 3-CH_2Cl is higher than that of ASA (CLogP 3-CH_2Cl = 3.495; CLogP ASA = 0.804). This indicates that 3-CH_2Cl has more nonpolar properties and theoretically could enter the cytoplasm through the cell membranes better than ASA. Even though COX-2 closely resembles COX-1, COX-2 active site could bind to larger chemical structures through the difference of amino acid (isoleucine in COX-1 and valine in COX-2) at position 523.[9] This substitution is sufficient to broaden the volume of COX-2-active sites to recognize bigger substrates than COX-1.[9] It is well-known that ASA could acetylate serine 530 (Ser530) in COX-2, resulting in the inhibition of COX-2 activity.[15] There is still no supporting data regarding the potential interaction site of 3-CH_2Cl with COX-2 (see question mark in Figure 3 Number 8).

The synthesis of 3-CH_2Cl was relatively simple. Using biphasic aqueous basic conditions (using acetone as a solvent of the reaction), 3-CH_2Cl was formed spontaneously when the precursor compound 3-chloromethylbenzoyl chloride reacted with SA under 5 min exposure of 600-W microwave irradiation[14] or heat induced reflux method[16] (see Figure 2). To increase the yield of the reaction, a tertiary amine pyridine was added directly to SA before being mixed with the precursor.

2-(3-Chloromethyl)benzoyloxy)benzoic acid was produced by reacting salicylic acid and 3-(chloromethyl)benzoyl chloride in acetone as solvent with pyridine as catalyst. The reaction was carried out under 600 W microwave irradiation exposure for 5 min.

The chemical reactions for the synthesis of 3-CH_2Cl could be seen on Scheme 1.[13] In brief, pyridine attacks the chloride

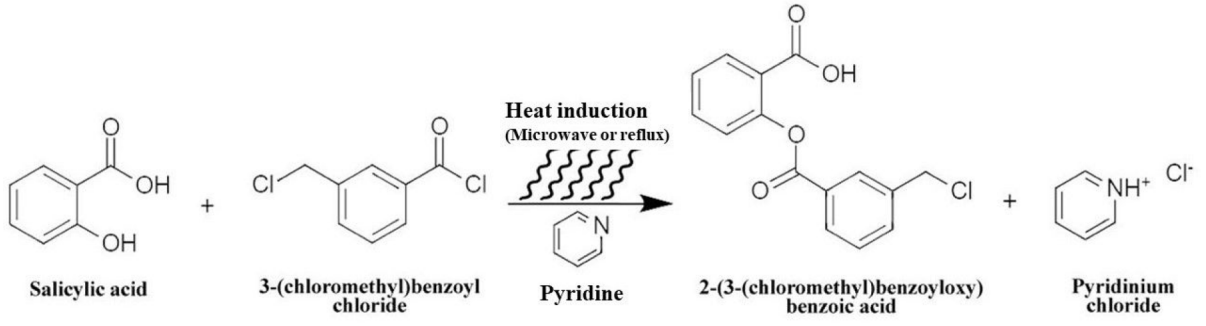

Figure 2. Synthesis of 2-(3-chloromethyl)benzoyloxy)benzoic acid.

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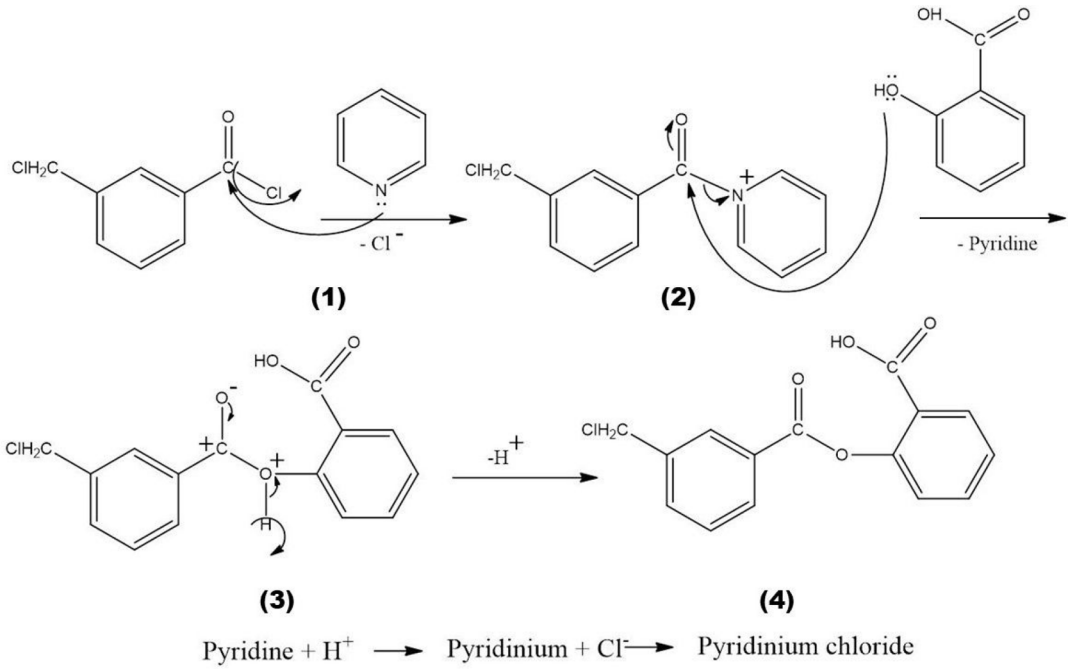
Scheme 1. Chemical Reaction for the Synthesis of 2-((3(Chloromethyl)benzoyl)oxy)benzoic Acid (3-CH_2Cl)



ion in the benzoyl chloride group (**1**), because chloride ion act as a good leaving group. This reaction resulted in the formation of another compound (**2**) which is more electrophilic than the first compound (**1**). After that, the carbonyl-carbon atom in the compound (**2**) is attacked by phenolic hydroxyl groups from SA, resulting in another compound (**3**). At the end, the pyridium ion is released, and binds to the free chloride ion, resulting in pyridium chloride (see text below the compound **3**). The detachment of pyridium-ion was accompanied by the electron movement, resulting in the formation of 3-CH_2Cl (4).

The absolute yield of white crystalline solid pure 3-CH_2Cl obtained from the above reaction was 73.27 ± 4.66%. Several methods such as thin-layer chromatography (TLC), infra-red (IR) spectra, nuclear magnetic resonance spectroscopy (^{13}C-NMR and ^{1}H-NMR), and high-pressure liquid chromatography (HPLC) could be used to detect the purity and stability of 3-CH_2Cl.[17] Accelerated stability testing has been performed with crystalline 3-CH_2Cl. 3-CH_2Cl has 3 years tentative shelf life at 25 °C with the storage humidity of 75 ± 5%. To deliver an accurate dosage orally, Hadinugroho and colleagues suggested the tablet-form of 3-CH_2Cl using sodium lauryl sulfate (SLS) as surfactants, and croscarmellose sodium (CS) as a disintegrating agent. Based on the linear and quadratic models, the optimum tablet formula was 3-CH_2Cl (300 mg), SLS (0.92%), CS (2.33%), Neusilin (9.38%), microcrystalline cellulose (5%), and spray-dried lactose (ad 800 mg) respectively.[16]

PHARMACOKINETICS PROPERTIES OF 2-((3-(CHLOROMETHYL) BENZOYL)OXY)BENZOIC ACID

To investigate the 3-CH_2Cl absorption pattern, the pharmacokinetic studies have been performed in a rat model using high-pressure liquid chromatography diode array detector (HPLC-DAD) method[18] with a single dose of 45 mg/kg body weight (BW). The maximum plasma concentration of 3-CH_2Cl (C_{max}) was 0.57 ± 0.02 μg/mL. The time of maximum plasma concentration was (T_{max}) of 28.9 ± 1.1 min. The total systemic exposure of 3-CH_2Cl (AUC_{total}) was 66.3 ± 1.0 μg min/mL, and the elimination half-life ($T_{el}^{1/2}$) was 39.4 ± 3.9 min. The elimination life constant (K_{el}) was 0.018 ± 0.002 min^{-1}. Based on these findings, 3-CH_2Cl exerts a slower onset of action and longer elimination time than ASA.[18]

Interestingly, the high lipophilic properties (log P = 3.73) and longer elimination time of 3-CH_2Cl indicated that this compound was extensively distributed in the deep and very deep tissues during absorption.[19] Compared with the pharmacokinetic profile of ASA, several studies[20,21] reported that ASA increased dramatically in the first 5 min and then declined rapidly, whereas the concentration of its degradation product, salicylic acid, constantly increased. In contrast, no degradation product such as salicylic acid was observed with 3-CH_2Cl until 3 years at 25 °C with a relative humidity of 75 ± 5%.

TOXICITY STUDY OF 2-((3-(CHLOROMETHYL) BENZOYL)OXY)BENZOIC ACID

The well-known side effect of ASA is associated with gastric mucosal damage. To investigate whether 3-CH_2Cl has a similar disadvantage with ASA, the toxicity studies of both compounds according to the OECD guidelines in the animal model were performed.[22] Observation in the rat analgesic model[14,23] indicated significantly less harmful toxicity parameters following the administration of 3-CH_2Cl, better than ASA. Furthermore, the lethal dose (LD_{50}) of 3-CH_2Cl is below 2.000 mg/60 kg body weight (BW), which is similar to ASA. The histopathological observation of animal groups treated with 3-CH_2Cl (50 mg/kg BW) showed a significant reduction of gastric mucosal erosion in comparison to ASA in equal concentration.[22] In addition, the treatment of rats with 3-CH_2Cl did not have any harmful impact on liver, heart, lung, and kidney morphological and histological functions.[24]

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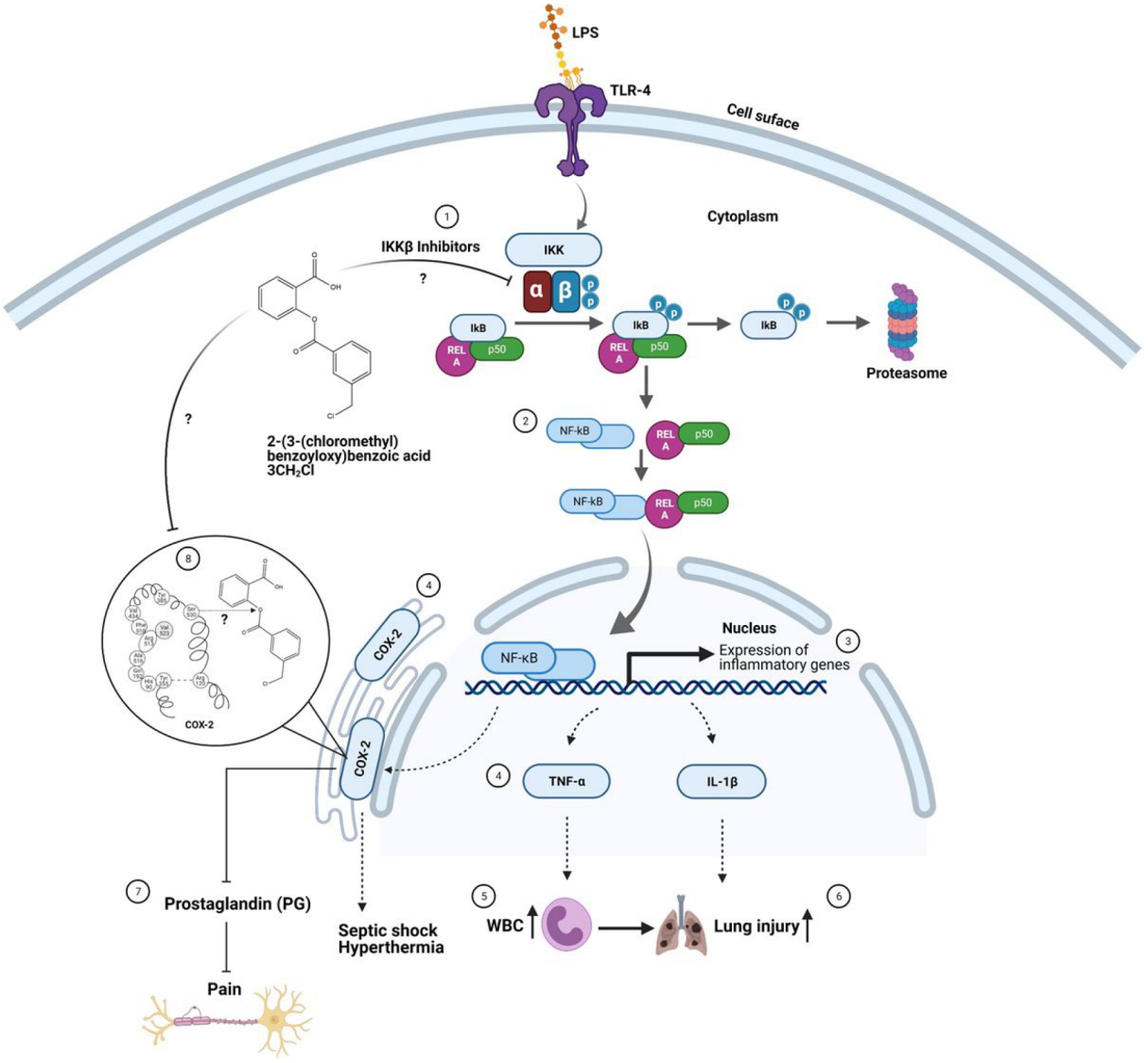

Figure 3. Hypothetical mechanism of action of 3-CH_2Cl. During induction of LPS, 3-CH_2Cl may exert anti-inflammatory activity by preventing the dissociation of IKKβ (1), which causes NFκB-inactivation (2). Suppression of NFκB activity could inhibit the transcription of pro-inflammatory genes (3) and their expressions such as COX-2, IL-1β, and TNF-α (4). Physiologically, the inhibition of those proteins leads to reduction of WBC concentration (5), minimizes the severity of ALI (6), and inhibits pain or exert analgesic activity (7). Meanwhile, the inhibition of COX-2 by 3-CH2Cl remains structurally unclear (8).

Though this was toxicity observation, it is worth investigating 3-CH_2Cl as a potential ASA-Substitution.

ANALGESIC AND ANTIPLATELET ACTION OF 2-((3-(CHLOROMETHYL) BENZOYL)OXY)BENZOIC ACID

The inhibition of pain (or analgesic activity) by NSAIDs could inhibit the production of prostaglandins in the central nervous system,[25] mainly through COX inhibition. Several studies have been performed in rats to investigate the potential analgesic action of 3-CH_2Cl ranging from 12.5 mg/kg BW to 200 mg/kg BW,[21] representing the human dose from 12.5 mg/60 kg to 2.000 mg/60 kg BW. The dose-dependent increases of nociceptive response time were observed during oral administration to rats of 3-CH_2Cl on heat-induced Plantar Anasthesiometer. Additionally, the dose-dependent decrement pattern was observed in nociceptive response count after the induction of rats with 0.6% acetic acid in the "writhing" test. Compared with 3-CH_2Cl, ASA has a significantly higher nociceptive response count and lower nociceptive response time. It gave us primary evidence that 3-CH_2Cl could act as a better analgesic agent than ASA through the peripheral and central nervous systems. However, the direct effect of 3-CH_2Cl as a potential analgesic drug in blood prostaglandin concentration is still under investigation.

Another advantage of salicylic acid derivates is the antithrombotic activity to prevent the cardiovascular blockade caused by platelet aggregation.[25] 3-CH_2Cl seems to be another candidate for the antithrombotic drug. After oral treatment with 500 mg/60 kg BW of 3-CH_2Cl, flow cytometry-based aggregation assay with Agonis-treated murine platelet demonstrated a significant decrease of platelet aggregation events.[14] Moreover, the antiaggregation result was confirmed with the

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classical tail-bleeding time analysis. It is well-known that platelet aggregation is induced by the activation of different receptors such as thrombin receptors, prostaglandin thromboxane A2 ($TBXA_2$) receptor, and ADP receptors (P2RY1 and P2RY12).[26−28] This preliminary observation hints that the inhibition mechanism of platelet function by 3-CH_2Cl might be caused by the interaction of this salicylic acid derivates with the platelet COX. This interaction may prevent the production of $TBXA_2$, and thus diminish the $TBXA_2$ activation. To confirm the specific platelet aggregation pathway of 3-CH_2Cl, further study should be performed with the presence of platelet receptor blockade, particularly focused on the $TBXA_2$ receptor.

THE ANTI-INFLAMMATORY ACTION OF 2-((3-(CHLOROMETHYL) BENZOYL)OXY)BENZOIC ACID: A POTENTIAL DRUG FOR THE TREATMENT OF ACUTE LUNG INJURY (ALI)

Further studies to examine the advantage of 3-CH_2Cl as an anti-inflammatory agent have been performed.[17] Treatment of intravenous (i.v.) LPS in Wistar rats with 500 mg/60 kg BW of 3-CH_2Cl (rat dosage converted to human) significantly reduced pro-inflammatory cytokines TNF-α and IL-1β concentrations in cardiac blood plasma. In line with this observation, 3-CH_2Cl could stabilize the rat body temperature, and prevent the rats from undergoing hypothermic and hyperthermic LPS-induced septic shock. Besides, the reduction of cardiac white blood cell concentration, less pulmonary edema, and the reduction of hepatocyte injury have been observed in the 3-CH_2Cl treated LPS-rat group. A significant reduction of lung fibroblasts in histological examination gives us an insight into the potential usage of 3-CH_2Cl as a therapeutic drug with a broad spectrum of acute lung injury (ALI) related diseases such as sepsis, pneumonia, aspiration, trauma, pancreatitis, blood transfusions, smoke or toxic gas inhalation, or even COVID-19 infection. Even though the molecular mechanism of 3-CH_2Cl in alleviating inflammation is still poorly investigated, the above-mentioned preliminary studies could lead us to the potential mechanism of 3-CH_2Cl, particularly through interaction and inhibition of nuclear factor-kappa beta inhibitor protein (IKKβ),[29] moving toward the inhibition of downstream NF-$\kappa\beta$ signaling pathways, and reduction of pro-inflammatory genes such as COX-2, TNF-α, and IL-1β, a typical mechanism of LPS-induced inflammation associated with TLR-4 receptor.[30]

CONCLUSION

The hypothetical pathway of 3-CH_2Cl as an analgesic drug through COX-2 inhibition and as an anti-inflammatory drug through inhibition of NF-$\kappa\beta$ signaling pathways in LPS-induced TLR-4 activation was shown in Figure 3 (adapted from Tjahjono et al., 2021). The molecular interaction of 3-CH_2Cl in the COX-2 active site is still unclear. Based on the literature review regarding the interaction of ASA with IKKβ and current 3-CH_2Cl data observation, we proposed that during LPS induced inflammation, 3-CH_2Cl could inhibit the activation of IKKβ by LPS-specific TLR-4, preventing the dissociation of inhibitor kappa beta protein (I$\kappa\beta$) and the downstream association of transcription factor complexes between NF-$\kappa\beta$, RelA, and p50 proteins to induce the expression of various pro-inflammatory proteins such as COX-2, TNF-α, and IL-1β. The inhibition of TNF-α and IL-1β expression could prevent the typical LPS induced physiological changes such as ALI and accumulation of WBC. The inhibition of COX-2 could presumably reduce prostaglandin production, induce analgesia and antipyretic action, and prevent septic shock hyperthermia. Pharmacokinetic studies showed that 3-CH_2Cl exhibited a slower onset of action and longer elimination time better than ASA. Interestingly, 3-CH_2Cl could reduce LPS-induced ALI. Even though very limited data about the molecular mechanism, the less harmful impact of 3-CH_2Cl in toxicity study, and the analgesic-, anti-inflammatory-, and antiplatelet activity of this compound encourage us to further examine the potential function of this compound as an alternative drug to substitute ASA, additional work is needed to elucidate the signaling mechanism of 3-CH_2Cl related their physiological activity. Moreover, the development of nanotechnology-based models of 3-CH_2Cl is very promising as a future perspective to further broaden the immunomodulatory potentials of this compound.

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Notes

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