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Research Article
Comparison of Cytotoxicity Effects of Propionic Acid Compounds to THLE-2 and HEP-G2 Cells

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Abstract

Background and Objective: The Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are mainly used for analgesia, anti-pyretic and for reducing swelling. The NSAIDs are one of the most widely prescribed medication worldwide, however with many adverse drug reactions such as gastrointestinal disturbances, liver and kidney toxicity. The current study accessed the cyto-effects of 2-(4-(2-methylpropyl)phenyl)propanoic acid (Ibuprofen/2-4-2MPPPA) and 2 other propionic acid derivatives 3-(4-hydroxyphenyl)propionic acid (3-4HPPA) and 3-(4-aminophenyl)propionic acid. **Materials and Methods:** The HEP-G2 cells and THLE-2 cells were incubated with 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 M of compounds. The plates were then further incubated for 72 hrs. Cell viability was determined using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. **Results:** Incubation of liver cells with all 3 derivatives showed a dose-dependent reduction in cell viability for both THLE-2 and HEP-G2 cells. However, HEP-G2 cells were more viable than THLE-2 cells. All 3 derivatives moderately cytotoxic to THLE-2 cells when compared to HEP-G2 cells. Interestingly, Ibuprofen (2MPPPA) was the least cytotoxic. **Conclusion:** This study has proven the safety of ibuprofen as an OTC (Over The Counter) NSAID which is one of the most popular NSAID worldwide. Therefore, these results provided the safety and data for future studies of for propionic acid NSAIDs' efficacy and toxicity studies.

Key words: 3-(4-aminophenyl)propionic acid, 3-(4-hydroxyphenyl)propionic acid, 2-(4-(2-methylpropyl)phenyl)propanoic acid, cytotoxicity, NSAIDs, ibuprofen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In vitro cytotoxic effects are the first assay in any efficacy or toxicity evaluation of potential new drugs. Propionic acids derivatives are a large group of compounds that have many useful pharmacological properties[1]. It is one of the largest subgroups of the Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). The NSAIDs are mainly used for pain relief, fever reduction and for reducing swelling[2,3]. The NSAIDs are one of the most widely prescribed medication worldwide[4].

Ibuprofen (3-(aminophenyl)propanoic acid; is a popular OTC (Over The Counter) NSAID is used primarily for pain, fever, dysmenorrhea and inflammatory conditions like osteoarthritis and rheumatoid arthritis. It is potent and considered one of the safest NSAID[5]. However, unlike other NSAIDs cause many adverse drug reactions, ibuprofen has been reported to also cause adverse drug reactions such as raised liver enzymes, nausea, dyspepsia, gastrointestinal ulceration and/or bleeding and diarrhea[6]. It also may induce more serious but very rare adverse effects that include liver failure, heart failure, hyperkalemia, renal impairment, confusion and bronchospasm[7]. Therefore, in this present study, Ibuprofen was used as the reference drugs to the other closely related propionic acid compounds.

Interestingly, propionic acid derivatives have different biomedical properties such as anti-microbials, 3-hydroxy-2-methylene-3-phenylpropionic acid[8], anti-malarial; 3-hydroxyalkyl-2-methylene-propionic acid[9] and anti-cancer; 2-(2-fluorobiphenyl-4-yl)propanoic acid[10]. In this study, the *in vitro* cytotoxic potential of ibuprofen, 3-(4-aminophenyl) propionic acid and 3-(4-hydroxyphenyl)propionic acid towards human liver cells (normal and cancerous). This is performed as liver damage commonly seen or even rare liver failure is observed clinically in patients undergoing NSAID therapy[4] (Fig. 1).

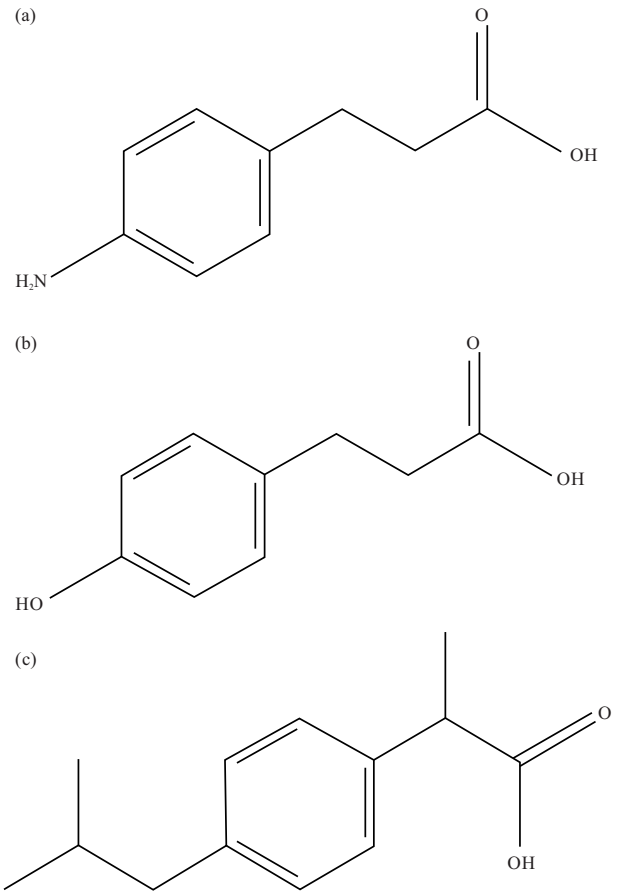

Fig. 1(a-c): Chemical structures of propionic acid derivatives, (a) 3-(4-aminophenyl)propionic acid, (b) 3-(4-hydroxyphenyl) propionic acid and (c) 2-(4-(2-methylpropyl)phenyl)propanoic acid (Ibuprofen)
(a) MW = 165.19, (b) MW = 166.17 and (c) MW = 206.29

MATERIALS AND METHODS

Study area: This study was conducted in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia from 2016 (screening) till 2019 (mechanistic study).

Chemicals and plating of cells: Ibuprofen (3-(4-aminophenyl) propanoic acid), 3-(4-aminophenyl)propionic acid and 3-(4-hydroxyphenyl)propionic (Sigma Chemicals, US) were dissolved in 0.1% Dimethyl Sulfoxide (DMSO; Sigma Chemicals, US) at 100 mM stock solution. A serial dilution with either Dulbecco's modified Eagle's medium (DMEM; Life Technologies, US) or LHC-8 medium were obtained from Life Technologies (US). Cell line from human hepatocellular carcinoma (HEP-G2) cells and cell line of normal human liver (THLE-2 cells) were obtained from American Type Culture Collection (Rockville, US)[11]. The THLE2 cells have shown to express phenotypic characteristics and retain phase I and II metabolizing enzymes. Cells were grown in DMEM for HEP-G2 and LHC-8 medium for THLE-2 cells. As recommended by the manufacturer, all incubations were supplemented with 10% fetal calf serum (Sigma Chemicals, US). Cells were harvested when reached 90 to 100% confluency. Cell concentrations were determined by Trypan blue (Sigma Chemicals, US) exclusion[11]. The 1×10^5 cells was pipetted into each well of the 96-well microtiter plate, incubated overnight at 37°C with 95% O_2/5% CO_2 prior to treatment.

Dosing and 3-(4,5-Dimethylthiazole-2-yl)-2,5-Diphenyltetrazolium Bromide assay: Approximately 24 hrs of incubation, serial dilution with final concentration of 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μM of compounds were added to the wells. Detailed procedures have been published by Imamura *et al.*[12] and Thiruchenthooran *et al.*[13]. The plates were then further incubated for 72 hrs. Cell viability was determined using 3-(4,5-Dimethylthiazole-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay[11,13].

Statistical analysis: Data are expressed as Mean+SD of 4 separate experiments using GraphPad Prism 5 software. Statistical significance was defined at $p<0.05$ using analysis of variance. Significant treatment means were further subjected to Tukey's post test.

RESULTS AND DISCUSSION

Incubation of liver cells with 3-(4-aminophenyl)propionic acid (3-4APPA) showed a dose-dependent reduction in cell viability for both THLE-2 and HEP-G2 cells (Table 1). However, HEP-G2 cells were more viable than THLE-2 cells as the HEP-G2 cells were statistically more viable than THLE-2 cells at the two highest concentrations, 50 and 100 μM of 3-4APPA (Table 1). Table 2 demonstrated the viability of cells with 3-(4-hydroxyphenyl)propionic acid (3-4HPPA). Similar trends were observed as HEP-G2 cells were more resistant to 3-4HPPA and both THLE-2 and HEP-G2 cells were dose-dependently affected by 3-4HPPA (Table 2). Incubations of liver cells with 2-(4-(2-methylpropyl)phenyl)propanoic acid (2-4-2MPPPA) or Ibuprofen also revealed similar trends to 3-4APPA and 3-4HPPA (Table 3). Interestingly, when compared between the three compounds, 3-4APPA is the most cytotoxic to THLE-2 cells. Figure 2 illustrated 3-4APPA caused cytotoxicity to THLE-2 cells with the lowest viability of 79.50% when compared to the other 2 compounds at the same concentration of 100 μM. The 2-4-2MPPPA or Ibuprofen was the least toxic to both THLE-2 and HEP-G2 cells. This is the cause of it being one of the safest NSAID and most popular OTC NSAID[7]. Indeed, previous studies of NSAIDs revealed certain NSAIDs have more potential to induce adverse drug reactions to the other types[3,4,6]. More potent NSAIDs such as piroxicam and mefenamic acid able to induce more severe adverse drug reactions (ADRs) *in vitro* and *in vivo*[12].

The main reason of 3-4APPA being more cytotoxic to THLE-2 and partly HEP-G2 cells when compared to 3-4HPPA and 2-4-2MPPPA is the aminophenyl-moiety that is

Table 1: Viability of hepatocytes treated with various concentration of 3-(4-aminophenyl)propionic acid

	Viability of cells (%)	
3-(4-aminophenyl)propionic acid (μM)	THLE-2	HEP-G2
0	105.12 ± 5.27^{ax}	102.47 ± 3.59^{ax}
1.5625	106.75 ± 4.21^{ax}	106.17 ± 4.92^{ax}
3.125	104.02 ± 3.89^{ax}	102.78 ± 3.81^{ax}
6.25	100.01 ± 1.40^{ax}	101.18 ± 2.70^{ax}
12.5	99.70 ± 5.47^{ax}	100.93 ± 3.73^{ax}
25	95.23 ± 2.59^{ax}	99.02 ± 4.30^{ay}
50	87.57 ± 4.25^{bx}	97.54 ± 3.38^{aby}
100	79.50 ± 4.12^{cx}	95.75 ± 3.79^{bcy}

n = 4/group from four separate experiments, $^{a-c}$Means with different superscripts differ significantly ($p<0.05$) in the same column and $^{x-z}$Means with different superscripts differ significantly ($p<0.05$) in the same row

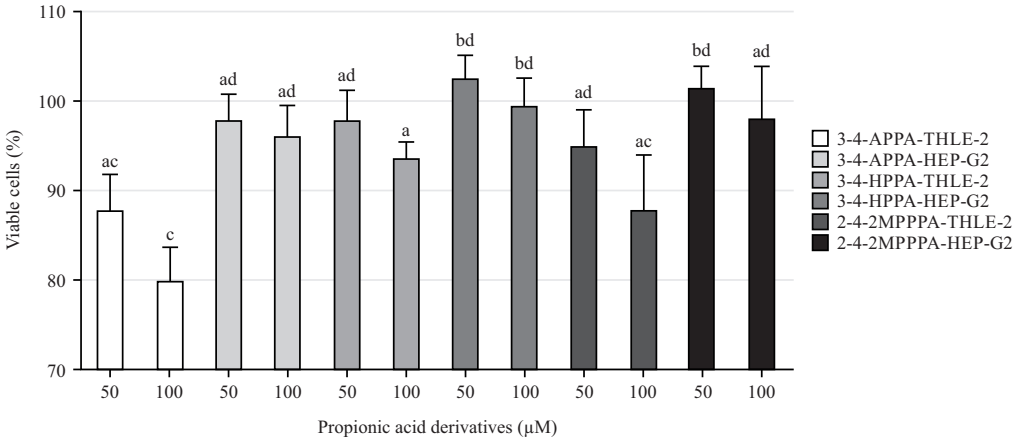

Fig. 2: Percentage cell viability of liver cells incubated with 50 and 100 μM propionic acid derivatives

n = 4/group from four separate experiments, [a-d]Means with different superscripts differ significantly ($p<0.05$), APPA: (Aminophenyl)propionic acid, HPPA: (Hydroxyphenyl)propionic acid and MPPPA: (Methylpropyl)phenyl)propanoic acid

Table 2: Viability of hepatocytes treated with various concentration of 3-(4-hydroxyphenyl)propionic acid

	Viability of cells (%)	
3-(4-hydroxyphenyl)propionic acid (μM)	THLE-2	HEP-G2
0	104.87±3.75^{ax}	102.62±3.84^{ax}
1.5625	106.03±1.37^{ax}	103.15±5.30^{ax}
3.125	104.70±3.15^{ax}	101.02±2.76^{ax}
6.25	100.03±4.44^{ax}	102.15±3.02^{ax}
12.5	101.75±5.21^{ax}	104.13±2.23^{ax}
25	99.27±2.45^{ax}	101.98±4.37^{ax}
50	97.51±3.75^{bx}	102.21±2.92^{ax}
100	93.20±2.18^{cx}	99.15±3.44^{ay}

n = 4/group from four separate experiments, [a-c]Means with different superscripts differ significantly ($p<0.05$) in the same column and [x-z]Means with different superscripts differ significantly ($p<0.05$) in the same row

Table 3: Viability of hepatocytes treated with various concentration of 2-(4-(2-methylpropyl)phenyl)propanoic acid (ibuprofen)

	Viability of cells (%)	
2-(4-(2-methylpropyl)phenyl)propanoic acid (μM)	THLE-2	HEP-G2
0	102.30±4.32^{ax}	104.23±4.72^{ax}
1.5625	103.19±5.47^{ax}	106.25±3.42^{ax}
3.125	103.68±2.14^{ax}	101.89±3.98^{ax}
6.25	104.25±4.02^{ax}	104.85±3.68^{ax}
12.5	101.25±1.36^{ax}	102.43±2.40^{ax}
25	102.49±3.75^{ax}	103.45±4.02^{ax}
50	94.73±4.37^{bx}	101.25±2.68^{ay}
100	87.54±6.53^{bx}	97.73±6.12^{ay}

n = 4/group from four separate experiments, [a-c]Means with different superscripts differ significantly ($p<0.05$) in the same column and [x-z]Means with different superscripts differ significantly ($p<0.05$) in the same row

capable of inhibiting certain enzymes critical of cellular functions[14,15]. The compounds 3-4APPA and 3-4HPPA are closely related to a naturally occurring propionic acid derivative 3-nitropropionic acid which has been reported to be a potent in disrupting mitochondrial membrane electron transport through inhibiting the enzyme succinate dehydrogenase[16]. This maybe another factor for the increased cytotoxic potential of 3-4APPA and 3-4HPPA. However, these cytotoxic potentials of these three propionic acid derivatives are not potent against the cancer cells when compared to other NSAIDs such as piroxicam[12].

Adverse drug reactions are commonly reported for all classes of NSAIDs and prescribed medications. Although some new improved drugs maybe lack of ADRs[17], this is a major concern clinically as deaths. Reports of mild transient elevation of serum transaminases are quite common to rare severe

hepatotoxicity[4]. New strategies have been developed to reduce these ADRs such as improved drug delivery using nano-encapsulations[18]. With nano-delivery, better efficacy of NSAIDs is produced at lower doses. This will reduce the NSAIDs ADRs significantly as demonstrated with piroxicam[19].

CONCLUSION

This present study revealed 2-4-2MPPPA or Ibuprofen was the least toxic to hepatocytes when compared to another closely related propionic acid derivatives, 3-4APPA and 3-4HPPA. This study has proven the safety of ibuprofen as an OTC NSAID which is one of the most popular NSAID worldwide and will be the basis for future studies in the development of newer propionic acid based NSAIDs.

SIGNIFICANCE STATEMENT

The NSAIDs are a large group of drugs with many adverse drug reactions mainly in the liver and stomach. The present study was to evaluate 3 compounds from the propionic acid group in their cytotoxic potential in normal and liver cancer cell lines with ibuprofen (2-(4-(2-methylpropyl)phenyl) propanoic acid) as the reference. Ibuprofen is considered to be the safest NSAID available OTC. This is an early evaluation of the cytotoxic potential of these compounds and results will be helpful in future *in vitro* and *in vivo* studies of efficacy or toxicity studies.

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