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Ibuprofen on Proliferation and Apoptosis of Sarcoma Cells via PI3K/Akt/mTOR Signaling Pathway

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ABSTRACT

Nowadays, there is a serious lack of information about the value-added apoptosis of sarcoma cells in China. Especially in clinical medicine, exploring the effect of ibuprofen on the growth and apoptosis of fibrosarcoma cells under the PI3K/Akt/mTOR signaling pathway can not only effectively prevent us in advance, but also be a great way to break through this field. The main purpose of this study was to investigate the effects of ibuprofen on the proliferation, cell cycle and apoptosis of fibrosarcoma cells through the PI3K/Akt/mTOR signaling pathway. We divided the HTl080 cell line into zero control group, control group and experimental group. The withering group was not inoculated with any cells, while the control group was only added with the same amount of culture medium, while the experimental group was added with 5,10,15,20 concentrations respectively. We found that the apoptosis rate of sarcoma cells in the control group increased from 5.66% to 7.12%, while the apoptosis rate of sarcoma cells in the experimental group increased significantly faster than that in the control group, with an overall increase of 7.16%, from 4.56% to 11.72%. Therefore, we can be surer that ibuprofen has a very good inhibitory effect on the proliferation, cell cycle and apoptosis of fibrosarcoma cells under the PI3K/Akt/mTOR signaling pathway. Therefore, when ibuprofen was injected into the body, it could not only observe the sarcoma cells well but also reflect the good inhibitory effect of ibuprofen on other substances in vivo under the PI3K/Akt/mTOR signaling pathway.

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Introduction

In recent years, more and more tumor signaling pathways have been found (1). Among them, the biggest hot spot is the tumor treatment scheme targeting the PI3K mediated signal transduction pathway (2, 3). In addition, ibuprofen can also promote the development of most substances in sarcoma cells in a favorable direction, which is more convenient for the observation of experimental results and data collection (4).

Ibuprofen (chemical name is isobutyl propionic acid, Ibuprofen) is one of the non-steroidal anti-inflammatory, antipyretic and analgesic drugs (NSAIDS). The side effects of ibuprofen are relatively small (4). At present, there are many different formulations of ibuprofen on the market, such as ordinary tablets, sustained-release tablets, capsules, injections, suspensions, suppositories and creams for injection (5, 6). The role of ibuprofen is not only analgesic, anti-inflammatory and anti-rheumatic, but also cooling. Therefore, in practice, ibuprofen is usually used in combination with other drugs to form a compound preparation with multiple functions of analgesia, anti-inflammation, anti-rheumatism and anti-cold (4).

At present, most of the application of ibuprofen in China is focused on analgesia and anti-rheumatism. In contrast, the research and exploration in antipyretic and anti-cold in clinical medicine is not perfect, which is much less than that of acetylchlorophenol or well-known aspirin (7, 8). In addition, ibuprofen belongs to the class of oral easily absorbed drugs. Although the body's absorption capacity will slow down when mixed with food, the total amount of absorption will not be reduced due to the slower speed. In addition, when ibuprofen is taken with antacids containing aluminum or magnesium, the absorption capacity will not be affected (9). The binding rate of ibuprofen and

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plasma protein is as high as 98%. The plasma concentration will reach a peak within 1.3-2.2 hours after taking ibuprofen. When the dosage of ibuprofen is 200mg, the plasma concentration will be 23-28μg/ml. when the dosage is 400mg, the blood concentration will be 24-46ug/ml; when the dosage is increased to 600mg, and the corresponding blood concentration will rise to 45-59μg/ml. T1/2 after a dose is generally 1.7-2 hours. After 6 hours, the concentration of synovial fluid will reach a balance with that in blood. In the following 12 hours, the concentration of synovial fluid will be higher than that of plasma. In the metabolism of ibuprofen in the liver, about 60% - 80% of ibuprofen will be excreted by urine through the kidney, 90% will be discharged within 24 hours, 1% is the original substance of ibuprofen, and the rest will be discharged out of the body with feces.

The Akt in ibuprofen can also activate IKK, which makes cross talk exist in the NF-kB signaling pathway. Moreover, phosphatidylinositol 3-kinases (PI3Ks) can improve the proliferation, differentiation, apoptosis and glucose transport of protein family cells (10, 11). When the activity of PI3K increases, it means that there are many different cancers involved. Moreover, PI in PI3K accounts for a small proportion of the components of the cell membrane, and its content is much less than that of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. However, PI is abundant in the cell membrane, up to 12% of the total phospholipids (12). Just like pi-3-phosphate, which is monophosphorylated, can well stimulate cell migration, but cells cannot migrate without phosphorylation.

In the development of human medical treatment, the signal pathway has a very close relationship with the development of tumors (13). In addition, it also plays a very important role in the process of malignant proliferation of tumor cells, as well as the cell metastasis and chemotherapy phase antagonism. Thus, it can be concluded that the P13K-mTOR signaling pathway will become a breakthrough in the treatment of malignant tumors.

The PI3K/Akt/mTOR signal transduction pathway induces tumors in the cell's genesis, mainly during the autophagy process of cells, such as 3-MA (3-methyladenine) which is an enzyme necessary for the formation of autophagosomes. Type PI3K inhibitor can well inhibit the occurrence of autophagy, and there are many different mechanisms to inhibit cell apoptosis (14, 15). Phosphorylation at the post mitochondrial level distinguishes it from apoptosis in other cell structures, such as bad and caspas-es9, and then down-regulates the expression of the tumor suppressor protein 053 in the nucleus. There are many PI3K/Akt signaling pathway targets in the cells, and they are in all stages of tumorigenesis.

Therefore, in the current study, we tried to investigate the effect of ibuprofen on the proliferation and apoptosis of sarcoma cells via PI3K/Akt/mTOR signaling pathway.

Materials and methods

Experimental subjects

The human fibrosarcoma cell line HTl080 was purchased from the Key Laboratory of Immunology of Provincial Hospital of our province, and the cells were placed in an environment of 37°C and 5% $C0_2$. The HTl080 cells were digested and passaged in a high-sugar DMEM medium containing 10^5U/L penicillin and 10% fetal bovine serum and 10^5μg/L streptomycin when they were full of culture flasks. The agent used was 0.25% trypsin.

Detection of proliferative activity

HTl080 cells in the logarithmic growth phase were seeded in 96-well plates and cultured at 1×10^4/well with the addition of 100μL/well. After 24 h culture, the medium was changed, and the non-inoculated cells (zero group), the medium group containing only equal amount (control group) and the ibuprofen group with different concentrations (experimental group, the final concentrations were 5, 10, 15, 20, 50 and 100μmol/L, respectively) were set up, with 6 duplicate holes in each group. The culture was conducted for 12 hours and 24 hours with the addition of activity detection reagents and 10μL of SunBiox™Am-Blue cells for proliferation, respectively, followed by two hours of dark incubation. Finally, the density value (OD) of each hole light was measured by enzyme-linked immunoassay, and the filter wavelength was adjusted to 570 nm, the reference wavelength was 600 nm, and the zero set was adjusted at the same time.

Observational morphology

After digesting HTl080 cells in a logarithmic growth phase, they were made into cell suspension, and 1 x 10^6 cells were seeded in a Petri dish with a diameter of 6 cm. When 80% of the dishes were covered with cells, ibuprofen (100 μmol/L) was added to the experimental group, while no treatment was done to the control group. The morphological changes of the cells in the experimental group after adding ibuprofen under the inverted microscope for 24 h were observed and recorded.HTl080 cells from the ibuprofen group (100 μmol/L, 24 h) and the control group were collected according to AnnexinV-FITC/PI cell apoptosis detection kit treatment specifications. Finally, flow cytometry was used to analyze the proportion of late apoptotic cells and early apoptotic cells in the ibuprofen group (100 μmol/L, 24 h) and control group.

Experimental Methods

In this paper, the variance was used to compare and analyze the different concentrations and times of the ibuprofen group, and t-test flow was used to compare the sample mean of protein expression and analysis results between the two groups. Measurement data refers to the data obtained in the process of observing and observing the quantity of a certain quantitative index in a unit in statistics. Measurement data usually have measurement units, and the expression of units is to reflect the size of values, such as blood pressure, glycated hemoglobin, high-density lipoprotein, etc. Although an important part of statistical analysis includes hypothesis testing, t-test has high test efficacy and is convenient to calculate. The hypothesis test is a statistical inference method to infer whether there may be differences in the whole population from samples. However, the theoretical basis of the t-test is t-distribution, which is most frequently used in hypothesis test methods of all measurement data. Let X and Y be independent, and X-N (0,1), Y-X^2_n, then we will denote the distribution of this random variable as T-T_n, which is called the t distribution with n degrees of freedom (df), as shown in Equation 1.

$$t = \frac{X}{\sqrt{Y/n}} \quad [1]$$

The probability density function of the t distribution is as follows (2):

$$t_{(x,n)} = \frac{\Gamma\left(\frac{n+1}{2}\right)}{\sqrt{n\pi}\Gamma\left(\frac{n}{2}\right)}\left(1+\frac{x^2}{n}\right)^{-\frac{n+1}{2}} \quad (2)$$

Results and discussion

Effects of Ibuprofen on the Proliferation of HTl080 Cells

In this experiment, human fibrosarcoma HTl080 cells were intervened with ibuprofen in vitro, and it was found that different concentrations of ibuprofen could inhibit the proliferation of HTl080 cells at different times. The inhibition rate of the cells as shown in Figure 1 when the dose of ibuprofen was 5 μmol/L and 10μmol/L.

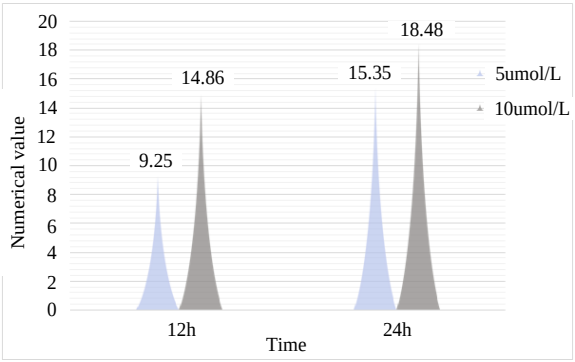

Figure 1. Inhibition rate of ibuprofen on cells at doses of 5μmol/L and 10μmol/L

As can be seen from Figure 1, when the dose of ibuprofen was 5μmol/L, the inhibition rate was 9.25 after 12 hours and 15.35 after 24 hours. When the dose of ibuprofen was 10μmol/L, the inhibition rate was 14.86 after 12 hours and 18.48 after 24 hours.

The inhibition rates of Ibuprofen on cells at doses of 15μmol/L and 20μmol/L were shown in Figure 2.

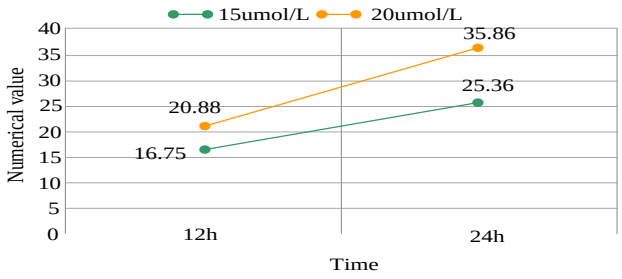

Figure 2. Inhibition rate of ibuprofen on cells at doses of 15μmol/L and 20μmol/L

In Figure 2, when the dose of ibuprofen was 15μmol/L, the inhibition rate was 16.75 after 12 hours and 25.36 after 24 hours. When the dose of ibuprofen was 20μmol/L, the inhibition rate was 20.88 after 12 hours and 35.86 after 24 hours.

The inhibition rate of Ibuprofen on cells at doses of 50μmol/L and 100μmol/L was shown in Figure 3.

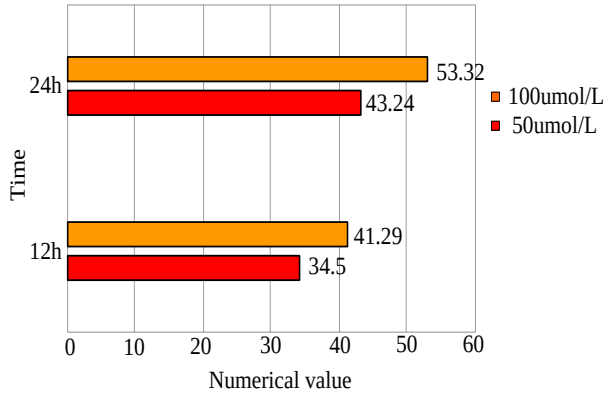

Figure 3. Inhibition rate of ibuprofen on cells at doses of 50μmol/L and 100μmol/L

As can be seen from the data in Figure 3, when the dose of ibuprofen was 50μmol/L, the inhibition rate was 34.50 after 12 hours and 43.24 after 24 hours. When the dose of ibuprofen was 20μmol/L, the inhibition rate was 41.29 after 12 hours and 53.32 after 24 hours.

Effects of Ibuprofen on Apoptosis and Protein Expression of HTl080 Cells

The early apoptotic rate of HTl080 cells was increased after ibuprofen (100μmol/L) acted on HTl080 cells for 24 h. This experiment showed that the P13K-mTOR signaling pathway was involved in the growth regulation of fibrosarcoma cells. The expression of p-Akt and p-mTOR protein (PMTORP) in HTl080 cells decreased after ibuprofen treatment. Ibuprofen could inhibit cell growth by inhibiting P13K, reducing the activation of Akt and inhibiting the activity of its downstream signaling molecule mTOR.That is, ibuprofen can inhibit proliferation and promote apoptosis of HTl080 cells by inhibiting the P13K-mTOR signaling pathway, in which the effect of ibuprofen on apoptosis of HTl080 cells was detected by flow cytometry as shown in Figure 4.

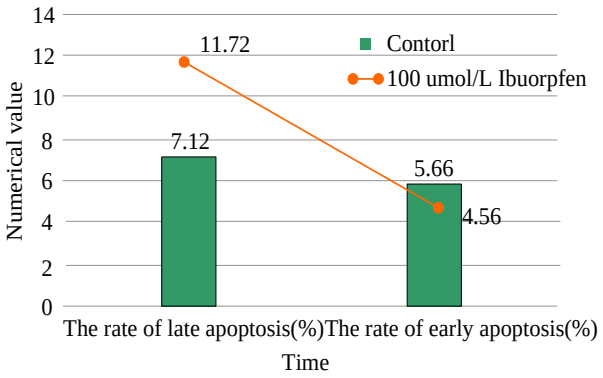

Figure 4. Flow cytometry assay of the apoptosis rate of HTl080 cells in different groups

As shown in Figure 4, the apoptotic rate of sarcoma cells in the control group increased from 5.66% to 7.12% before and after the experiment, while the apoptotic rate of sarcoma cells in the experimental group increased significantly faster than that in the control group, with an overall increase of 7.16%, from 4.56% to 11.72%.

The expression of the p-Akt protein in both groups is shown in Table 1.

Table1. Effects of ibuprofen on P-akt protein expression in HTl080 cells

Group	N	P-Akt Protein
Control	3	0.62
100umol/L Ibuprofen	3	0.22

From the data in Table 1, we can see that the control group has a value of 0.62 after the experiment and the experimental group has a value of 0.22.P-Akt is the activated form of Akt, and only activated Akt has biological characteristics, which can activate the anti-apoptotic mechanism. Therefore, the balance between cell proliferation and apoptosis can be regulated by regulating the expression of Akt, and the cell cycle can be regulated to achieve the purpose of inhibiting tumor growth. The expression of PMTORP in both groups is shown in Table 2.

Table 2. Effects of ibuprofen on PMTORP expression in HTl080 cells

Group	N	P-MTOR
Control	3	0.70
100μmol/L Ibuprofen	3	0.31

Table 2 shows that after the experiment, the expression of PMTORP in the control group is still 0.70, while the expression of PMTORP in the 100umol/L ibuprofen experimental group has decreased to 0.31. Therefore, ibuprofen has a great influence on the expression of PMTORP.

At present, the domestic research on ibuprofen in the medical field is mainly limited to nerve tissue, breast cancer, lung cancer, leukemia, and so on (2). Therefore, the research on the role of sarcoma in the P13K/Akt signal transduction pathway is not enough. How to carry out experiments on the inhibitory effect of ibuprofen on sarcoma cells and the collection of experimental data is our next focus. In this paper, fibrosarcoma cell line HTl080 was cultured at 37°C and 5% CO_2, and then digested and passaged to observe the efficacy of ibuprofen. The data obtained from the experiment were compared and analyzed to prove the accuracy of the experiment. Then, taking P13K/Akt signal transduction pathway as the starting point, the proliferation and apoptosis of fibrosarcoma were discussed and analyzed. It is not only conducive to observing the role of ibuprofen, but also to open up a new treatment path in the medical field.

In order to explore the mechanism of ibuprofen regulating the migration and invasion of QGY-7703 cells via PI3K/Akt/mTOR signaling pathway, Sutherland *et al*. (16) randomly divided the QGY-7703 cells into control group (Group C) and experimental group (group B), and then divided the experimental group into three subgroups (group B1-3), group B1 with 250μmol/L ibuprofen, B2 with 500μmol/L ibuprofen, group B3 with 1000μmol/L ibuprofen and control group with fulminant Exposed in RPMI l640 nutrient solution. They used the Transwell method to detect the migration and invasion of cells after 24, 48 and 72 hours of incubation. Real-time PCR was used to detect the expression levels of PI3K, PTEN and MMP-9, and Western blot was used to detect the expression levels of PTEN, Akt, p-Akt, mTOR and p-mTOR. Although ibuprofen can inhibit the migration and invasion of liver cancer cell line QGY-7703 in his experiment, it is not clear that this mechanism is related to PI3K/Akt/mTOR signaling pathway (16).

Soft tissue sarcoma (STS) is a group of heterogeneous malignant tumors, accounting for 1% of adult malignant tumors. The treatment of STS should be individualized and multimodal. However, complete surgical resection and clear margin are still the mainstream of treatment. Commonly used chemotherapy drugs such as doxorubicin and ifosfamide have been proved to be effective in less than 30% of these cases. Therefore, Sheehan *et al*. (17) detected the apoptosis and antiproliferative effects of trail and TRD on rhabdomyosarcoma (A-204), leiomyosarcoma (SK-LMS-1) and epithelioid cell sarcoma (VA-ES-BJ). Flow cytometry (FACS) analysis (propidium iodide/annexin V staining) was used to quantify cell survival, apoptosis and necrosis. Gene expression was analyzed by DNA microarray and verified by RT-PCR. Protein levels were recorded by western blot analysis. Cell proliferation was detected by BrdU ELISA. Their experimental results provide experimental support for in vivo trials to evaluate the role of trial and TRD in STS, but in terms of individualization, it does not guarantee the accuracy of the data (17).

Bray et al. (18) transfected mir-181b-5p mimics and inhibitors into Kaposi sarcoma cell line SLK by Lipofectamine transfection. She studied the effect of mir-181b-5p on the proliferation and apoptosis of SLK cells and provided a theoretical basis for further study of the role of mir-181b-5p in the pathogenesis of Kaposi sarcoma. they also transfected human Kaposi's sarcoma cell line SLK with liposome. MTT assay was used to detect the proliferation of SLK cells. Flow cytometry was used to detect the apoptosis rate of each group 48 hours after transfection. However, their experiment is too theoretical, and there is no good experimental data to support it, the results obtained are different (18). Human fibrosarcoma cell line htl080 was cultured in high glucose DMEM medium containing 10% fetal bovine serum, 10^5U/L penicillin and 105μg/L streptomycin at 5% $C0_2$ and 37°C. Then t-test was used to analyze the data, so as to get more accurate experimental results, and more clearly see the therapeutic effect of ibuprofen on sarcoma cells (18).

In the domain of regulatory subunits, not only SH2 and SH3 are contained, but also they can interact with corresponding binding sites in target proteins (19-21). These factors can activate the receptor of tyrosine kinase (RTK) and induce phosphorylation. Moreover, the phosphorylated residues provide a new docking site for pi3kp85 on the heterodimerization receptor.

However, under certain circumstances, the receptor phosphorylation in PI3K will recruit an adaptor protein. For example, after insulin activates the receptor, there must be an insulin protein (IRS) to promote the binding between the two. Similarly, when integrin (non-RTK) was reactivated, PI3K was anchored from p85 by FAK.

PIP3 in PI3K signaling pathway is a domain, many proteins in it will have a pleckstrin homology as an anchor, so it can be combined with pi-3,4-p2 or pi-3,4,5-p3. The interaction between these three substances can control the binding of protein and membrane. In this way, the activity of the protein can be well regulated. The interaction between protein and lipid will also cause changes in protein conformation, thus improving the function of the protein (22). The production of the second messenger PIP3 on the plasma membrane is the result of PI3K Signal activation. Moreover, PIP3 binds to Akt and PDK1 (phosphoinositide-dependent kinase-1), which contain PH domain in cells, promotes the phosphorylation of PDK1, and its interaction with ser308 of Akt protein also leads to the activation of Akt.

Skin metastatic sarcomas account for 2% and 3% of skin metastatic carcinomas in women and men, respectively. Undifferentiated sarcomas, fibrosarcomas, leiomyosarcomas, chondrocytes sarcomas, rhabdomyosarcomas and Ewing sarcomas together constitute skin metastatic sarcomas (23). In addition, metastatic sarcomas of the distal skin can also be formed in subcutaneous tissue, soft tissue beneath it (such as malignant fibrous histiocytoma and epithelioid cell sarcoma), and dermis.

Fibrosarcoma, as one of the most common malignant bone tumors, is now mainly treated by surgery. However, one of the drawbacks of fibrosarcoma is that it is easy to recur after surgical resection. Therefore, drug therapy also plays an important role in the treatment of fibrosarcoma (24, 25). Gallic acid (GA) is a polyphenolic compound widely existing in plants and has a wide range of pharmacological effects. The reason that attracts people's attention is its role in the field of anti-tumor, but the role of GA in anti-fibrosarcoma has not been reported (26). In the exploration of the possible mechanism of the anti-tumor effect of GA, the main material is HT1080 human fibrosarcoma cells. Flow cytometry and MTT were used to observe the effect of apoptosis and proliferation of HT1080 cells under the action of GA. In addition, Bax protein and Bcl-2 expression can be observed by immune-histochemical techniques (27).

Apoptosis is a process of cell suicide and an active behavior regulated by genes. Bax and Bcl-2 play an abnormal key role in the choice of apoptosis or not. Among them, the most deeply studied apoptotic regulatory gene is the Bcl-2 family (28). As an apoptosis suppressor gene, Bcl-2 is a major family member of the Bax gene. The expression of Bcl-2 protein and Bax protein is in the form of the heterodimer. To promote the occurrence of apoptosis, it can biologically produce the effect of antagonizing Bcl-2. Members of the Bcl-2 protein family, while inhibiting apoptosis; also allow cell survival and apoptosis to reach a relatively balanced state. The low expression of Bcl-2 and high expression of Bax acting on mitochondria can open the transport pore of mitochondrial membrane permeability, and the destruction of the outer membrane can also increase the permeability of the mitochondrial membrane. Meanwhile, a large number of apoptosis-related molecules such as cytochrome Smac, C and so on can play the role of activating C aspase-9 in the cytoplasm, and activate downstream C aspase-3, leading to apoptosis (29).

In this paper, we investigated the effect of different doses of ibuprofen on the proliferation and apoptosis of human fibrosarcoma cell line htl080 under the P13K/Akt signal transduction pathway. We found that different doses of ibuprofen have different effects at different time points. The longer the time is, the better the effect will be. The higher the dosage is, the higher the inhibition effect will be.

Ibuprofen can also significantly inhibit the expression of phosphorylated Akt. In combination with the PI3K/Akt/mTOR signaling pathway, ibuprofen can also inhibit the proliferation and differentiation of sarcoma cells, and promote the development of most substances in sarcoma cells. However, at this stage, the academic research or experimental development in this field is still the weak link in the national medical field, and the above-mentioned experiment is a way to solve the problem of sarcoma to some extent. After we deeply explore this problem, the results obtained will certainly let

patients have new hope and let clinical medicine have a new direction.

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None.

Interest conflict

The authors declare no conflict of interest.

References

1. Bilal I, Xie S, Elburki MS, Aziziaram Z, Ahmed SM, Jalal ST. Cytotoxic effect of diferuloylmethane, a derivative of turmeric on different human glioblastoma cell lines.
2. Ortega MA, Fraile-Martínez O, Asúnsolo Á, Buján J, García-Honduvilla N, Coca S. Signal transduction pathways in breast cancer: the important role of PI3K/Akt/mTOR. J Oncol 2020; 2020.
3. Ercisli MF, Lechun G, Azeez SH, Hamasalih RM, Song S, Aziziaram Z. Relevance of genetic polymorphisms of the human cytochrome P450 3A4 in rivaroxaban-treated patients. Cell Mol Biomed Rep 2021; 1(1): 33-41.
4. Mazaleuskaya LL, Theken KN, Gong L et al. PharmGKB summary: ibuprofen pathways. Pharmacogenet Genomics 2015; 25(2): 96.
5. Jolliffe HG, Gerogiorgis DI. Plantwide design and economic evaluation of two Continuous Pharmaceutical Manufacturing (CPM) cases: Ibuprofen and artemisinin. Comput Chem Eng 2016; 91: 269-288.
6. Derry S, Wiffen PJ, Moore RA, Bendtsen L. Ibuprofen for acute treatment of episodic tension-type headache in adults. Cochrane Database Syst Rev 2015; (7).
7. Numpilai T, Muenmee S, Witoon T. Impact of pore characteristics of silica materials on loading capacity and release behavior of ibuprofen. Mater Sci Eng C 2016; 59: 43-52.
8. Dziadkowiec J, Mansa R, Quintela A, Rocha F, Detellier C. Preparation, characterization and application in controlled release of Ibuprofen-loaded Guar Gum/Montmorillonite Bionanocomposites. Appl Clay Sci 2017; 135: 52-63.
9. Du J, Mei C-F, Ying G-G, Xu M-Y. Toxicity thresholds for diclofenac, acetaminophen and ibuprofen in the water flea Daphnia magna. Bull Environ Contam Toxicol 2016; 97(1): 84-90.
10. Walsh P, Behrens N, Carvallo Chaigneau FR et al. A randomized placebo controlled trial of ibuprofen for respiratory syncytial virus infection in a bovine model. PLoS One 2016; 11(4): e0152913.
11. Gazal G, Al-Samadani KH. Comparison of paracetamol, ibuprofen, and diclofenac potassium for pain relief following dental extractions and deep cavity preparations. Saudi Med J 2017; 38(3): 284.
12. Bardanzellu F, Neroni P, Dessì A, Fanos V. Paracetamol in patent ductus arteriosus treatment: efficacious and safe? Biomed Res Int 2017; 2017.
13. Marchlewicz A, Domaradzka D, Guzik U, Wojcieszyńska D. Bacillus thuringiensis B1 (2015b) is a gram-positive bacteria able to degrade naproxen and ibuprofen. Water Air Soil Pollut 2016; 227(6): 197.
14. Ding T, Yang M, Zhang J et al. Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom Navicula sp. J Hazard Mater 2017; 330: 127-134.
15. Bhadra BN, Ahmed I, Kim S, Jhung SH. Adsorptive removal of ibuprofen and diclofenac from water using metal-organic framework-derived porous carbon. Chem Eng J 2017; 314: 50-58.
16. Sutherland MR, Yoder BA, McCurnin D et al. Effects of ibuprofen treatment on the developing preterm baboon kidney. Am J Physiol Renal Physiol 2012; 302(10): F1286-F1292.
17. Sheehan WJ, Mauger DT, Paul IM et al. Acetaminophen versus ibuprofen in young children with mild persistent asthma. N Engl J Med 2016; 375(7): 619-630.
18. Bray M, Stucchi I, Fumagalli M et al. Blood withdrawal and infusion via umbilical catheters: effect on cerebral perfusion and influence of ibuprofen. Neonatology 2003; 84(3): 187-193.
19. Yang Z, Su R, Luo S et al. Comparison of the reactivity of ibuprofen with sulfate and hydroxyl radicals: an experimental and theoretical study. Sci Total Environ 2017; 590: 751-760.
20. Amir M, Akhter MW, Alam O. Synthesis, characterization, and biological evaluation of furoxan coupled ibuprofen derivatives as anti-inflammatory agents. Monatsh Chem 2016; 147(3): 493-508.
21. Iovino P, Chianese S, Canzano S, Prisciandaro M, Musmarra D. Degradation of ibuprofen in aqueous

solution with UV light: the effect of reactor volume and pH. Water Air Soil Pollut 2016; 227(6): 1-9.
22. Krasniqi V, Dimovski A, Klarica Domjanović I, Bilić I, Božina N. How polymorphisms of the cytochrome P450 genes affect ibuprofen and diclofenac metabolism and toxicity. Arh Hig Rada Toksikol 2016; 67(1): 1-7.
23. He Y, Langenhoff AA, Sutton NB et al. Metabolism of ibuprofen by Phragmites australis: uptake and phytodegradation. Environ Sci Technol 2017; 51(8): 4576-4584.
24. Luo S, Wei Z, Spinney R et al. UV direct photolysis of sulfamethoxazole and ibuprofen: an experimental and modelling study. J Hazard Mater 2018; 343: 132-139.
25. Ahiskalioglu EO, Ahiskalioglu A, Aydin P, Yayik AM, Temiz A. Effects of single-dose preemptive intravenous ibuprofen on postoperative opioid consumption and acute pain after laparoscopic cholecystectomy. Medicine 2017; 96(8).
26. Schill EM, Lake JI, Tusheva OA et al. Ibuprofen slows migration and inhibits bowel colonization by enteric nervous system precursors in zebrafish, chick and mouse. Dev Biol 2016; 409(2): 473-488.
27. Yuan C, Hung C-H, Li H-W, Chang W-H. Photodegradation of ibuprofen by TiO2 co-doping with urea and functionalized CNT irradiated with visible light–Effect of doping content and pH. Chemosphere 2016; 155: 471-478.
28. Li Y, Wu B, Zhu G et al. High-throughput pyrosequencing analysis of bacteria relevant to cometabolic and metabolic degradation of ibuprofen in horizontal subsurface flow constructed wetlands. Sci Total Environ 2016; 562: 604-613.
29. DePeter KC, Blumberg SM, Becker SD, Meltzer JA. Does the use of ibuprofen in children with extremity fractures increase their risk for bone healing complications? J Emerg Med 2017; 52(4): 426-432.