microbiology research

MDPI

Article

Effects of Common Anti-Inflammatories on Adenovirus Entry and Their Physicochemical Properties: An In-Depth Study Using Cellular and Animal Models

Hector R. Galvan-Salazar [1,2], Marina Delgado-Machuca [3], Gustavo A. Hernandez-Fuentes [3], Nomely S. Aurelien-Cabezas [3], Alejandrina Rodriguez-Hernandez [3], Idalia Garza-Veloz [4], Martha A. Mendoza-Hernandez [2,3], Margarita L. Martinez-Fierro [4], Sergio A. Zaizar-Fregoso [3], Iram P. Rodriguez-Sanchez [5], Fabian Rojas-Larios [3], Mario Del-Toro-Equihua [3], Gabriel Ceja-Espiritu [3] and Ivan Delgado-Enciso [1,3,6,*]

1 Department of Research, Health Services of the Mexican Social Security Institute for Welfare (IMSS-BIENESTAR), Colima 28085, Mexico; hector_rgs@hotmail.com
2 General Hospital Number 1, Mexican Institute of Social Security (IMSS), Villa de Alvarez, Colima 29883, Mexico; mendoza_martha@ucol.mx
3 Department of Molecular Medicine, School of Medicine, University of Colima, Colima 28040, Mexico; karla_machuca@ucol.mx (M.D.-M.); gahfuentes@gmail.com (G.A.H.-F.); nomelyaurelien@gmail.com (N.S.A.-C.); arodrig@ucol.mx (A.R.-H.); alexzaizar09@gmail.com (S.A.Z.-F.); frojas@ucol.mx (F.R.-L.); mequihua@ucol.mx (M.D.-T.-E.); gcejae11@ucol.mx (G.C.-E.)
4 Molecular Medicine Laboratory, Academic Unit of Human Medicine and Health Sciences, Autonomous University of Zacatecas, Zacatecas 98160, Mexico; idaliagv@uaz.edu.mx (I.G.-V.); margaritamf@uaz.edu.mx (M.L.M.-F.)
5 Molecular and Structural Physiology Laboratory, School of Biological Sciences, Autonomous University of Nuevo Leon, San Nicolas de los Garza 66455, Mexico; iramrodriguez@gmail.com
6 Department of Dietetics and Nutrition, Robert Stempel College of Public Health and Social Work, Florida International University, Miami, FL 33199, USA
* Correspondence: ivan_delgado_enciso@ucol.mx; Tel./Fax: +52-312-3161099

Abstract: The severity of adenovirus infection or the success of adenovirus-vectorized gene therapy largely depends on the efficiency of viral entry into cells. Various drugs can alter viral entry. This study evaluated the effects of dexamethasone, paracetamol, diclofenac, ibuprofen, and ketorolac on adenovirus entry into cells in vitro and in vivo. SiHa cell cultures pretreated with dexamethasone, paracetamol, diclofenac, ibuprofen, ketorolac, or no drug were exposed to the Ad-BGal vector. The percentage of cells showing vector entry was quantified microscopically. In vivo, BALB-C mice pretreated for 7 days with the drugs or no drug were exposed to the Ad-BGal vector intravenously (IV) or via oral (VO). Organs showing vector entry were identified by X-Gal staining and eosin counterstaining. Hepatic areas with adenovirus entry were quantified in μm^2. Dexamethasone, paracetamol, and ibuprofen increased adenovirus entry both in vitro and in vivo. Diclofenac increased entry only in vitro. Ketorolac did not affect adenoviral entry. The liver exhibited the most significant changes, with dexamethasone, paracetamol, and ibuprofen increasing adenovirus entry the most. Oral administration of the vector showed that dexamethasone increased its entry into the pharynx. Some physicochemical properties of the drugs (MW (g/mol), LogP, MR [cm^3/mol], tPSA, CMR, LogS, and ClogP) were analyzed, and their possible implications on cell membrane properties that could potentially influence adenovirus entry through mechanisms independent of cellular receptors were discussed. Anti-inflammatory drugs could alter adenoviral infections and adenovirus vector-based gene therapies, necessitating further research.

Keywords: anti-inflammatory drugs; adenovirus; adenoviral vector; infection; gene therapy; vaccines; physicochemical descriptors

check for updates

Citation: Galvan-Salazar, H.R.; Delgado-Machuca, M.; Hernandez-Fuentes, G.A.; Aurelien-Cabezas, N.S.; Rodriguez-Hernandez, A.; Garza-Veloz, I.; Mendoza-Hernandez, M.A.; Martinez-Fierro, M.L.; Zaizar-Fregoso, S.A.; Rodriguez-Sanchez, I.P.; et al. Effects of Common Anti-Inflammatories on Adenovirus Entry and Their Physicochemical Properties: An In-Depth Study Using Cellular and Animal Models. *Microbiol. Res.* **2024**, *15*, 1590–1604. https://doi.org/10.3390/microbiolres15030105

Academic Editor: Takayuki Murata

Received: 3 August 2024
Accepted: 9 August 2024
Published: 19 August 2024

1. Introduction

Adenoviral vectors are a technological platform for vaccine production and gene therapy [1]. They have been used in 20.5% of all gene therapy clinical trials [2]. Adenovirus-vectored vaccines were a response to the COVID-19 pandemic. The ChAdOx1/AZD1222 vaccines from Oxford–AstraZeneca, Gamaleya's Sputnik V, Johnson & Johnson's INJ-7843735/Ad26.COV2.s, and CanSino's Convidicea (Ad5-nCoV) [3,4], all adenovirus-vectored, have been administered worldwide. Additionally, various adenovirus-vectored vaccines against other infectious diseases are under development and research [5,6].

Viral entry into the cell is a crucial step in both wild-type virus infections and virus-based vaccines and gene therapy [7,8]. Adenovirus entry into the cell is mediated by the coxsackie–adenovirus receptor (CAR) and integrins [9,10]. The severity of an infection [11] or the success of an adenovirus-based therapy (vaccines and gene therapy) [12] largely depends on the efficiency of viral entry. However, there are few studies on drugs that enhance or inhibit adenovirus entry into the cell in its wild-type or therapeutic form.

Various drugs can alter the entry of different viruses. One of the most recent and controversial topics was the cell-entry mechanism of the SARS-CoV-2 virus during the COVID-19 pandemic. Researchers investigated whether non-steroidal anti-inflammatory drugs (NSAIDs) and dexamethasone had any effect on SARS-CoV-2 cell entry, potentially increasing COVID-19 severity [13–15]. Dexamethasone increases SARS-CoV-2 viral entry into cells by upregulating its main entry receptors: angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease TMPRSS2 [14]. It was also suggested that diclofenac, naproxen, and nimesulide increase TMPRSS2 expression in liver cells [16], which could modify virus entry. Initially, it was proposed that the use of anti-inflammatory drugs could worsen the disease [17], but this controversy was later resolved by evidence of the clinical benefit of using steroids or NSAIDs in the disease, leading to their widespread use [18–21]. This highlights the importance of generating both experimental and clinical information on how anti-inflammatory drugs influence the viral cell entry of medically important viruses. Owing to the limited information on the effect of dexamethasone, paracetamol, and NSAIDs on adenovirus cell entry, we aimed to analyze this topic using in vitro and in vivo experimental models.

2. Materials and Methods

2.1. Purification and Propagation of the Ad-BGal Vector

The non-replicating adenovirus Ad-CMV/βgal was used as the experimental virus [22]. Viral vectors were obtained following the protocols for recombinant adenoviruses propagated on HEK-293 cells (Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the ViraKit Adeno+>Mini-24 kit (Virapur LLC, San Diego, CA, USA) according to the manufacturer's specifications (Application Manual Qbiogene version 1.4. AdEasy TM Vector System. 2251 Rutherford Rd. Carlsbad, CA 92008 USA. www.qbiogene.com). The viruses were titrated on HEK-293 cells by tissue culture infectivity dose 50 ($TCID_{50}$) as described in the Vector System Application Manual, version 1.4 (Virapur LLC, San Diego, CA, USA).

2.2. Reagents

The anti-inflammatory drugs used were: Dexamethasone (Adrecort®, Allen Laboratorios, Gustavo A. Madero, Mexico City, Mexico), Paracetamol (Salpifar®, PISA Farmacéutica, Iztacalco, Mexico City, Mexico), Diclofenac (Deflox®, Merck, Naucalpan de Juárez, Mexico City. Mexico), Ibuprofen (Gobrosan®, Apotex, Mexico City, Mexico), and Ketorolac (Dolac®, Siegfried Rhein, Álvaro Obregón, Mexico City, Mexico). Diclofenac, ibuprofen, and ketorolac were chosen as representative nonsteroidal anti-inflammatory drugs (NSAIDs) due to their widespread use and different mechanisms of action within the NSAID category, while dexamethasone was selected as the representative steroid due to its potent anti-inflammatory and immunosuppressive properties [23]; all these drugs are included in the Basic Medication Framework of Health in Mexico, considered for groups of analgesia (ibuprofen and ketorolac), endocrinology and metabolism (dexamethasone), and diclofenac (ophthalmology, rheumatology, and traumatology) [24,25].

2.3. In Vitro Assay

This assay was performed in triplicate. Cells were maintained in DMEM culture medium, 1% fetal bovine serum (FBS), supplemented with L-glutamine, penicillin, and streptomycin, at 37 °C and 5% CO_2 in a humid environment (95%). In 24-well plates, 1×10^5 SiHa cells per mL were placed. Six hours later, the cells were exposed to various drugs at concentrations similar to the maximum plasma concentrations reached in humans: dexamethasone at 1.25 ng/mL [26], paracetamol at 20 µg/mL [27], diclofenac at 5 µg/mL [28], ibuprofen at 25 µg/mL [29,30], and ketorolac at 2.4 µg/mL [31]. The treatment not exposed to any drug served as a reference. After 48 h of drug incubation, the medium was removed, and 1×10^7 plaque-forming units per milliliter (pfu/mL) of the non-replicative Ad-BGal vector were added. The cells were incubated for 2 h in serum-free DMEM medium. The medium was removed from each well, and the wells were washed three times with 600 µL of DMEM medium. Each well was then filled with 600 µL of DMEM FBS for 24 h. After 24 h of incubation, the medium was discarded, and the wells were washed with 300 µL of PBS 1X, letting it sit for 5 min each time. Immediately, 380 µL of X-gal solution (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (Promega, Madison, WI, USA) was added and incubated overnight at 37 °C. Positive cells were stained blue. Plates were covered with aluminum foil and stored at 4 °C. The percentage of cells showing Ad-BGal vector entry (blue-stained cells) was quantified by microscopic cell counting (×400 magnification) (Zeiss AXIO Observer Z1 Inverted Fluorescence Microscope Pred Observer 7, Dublin, CA, USA). Fifteen microscopic visual fields from three independent experiments were quantified for each drug.

2.4. In Vivo Assay

The in vivo assay was conducted as a prospective, single-blind, 6-arm, parallel-group, randomized, preclinical trial, according to the ARRIVE Essential 10 guidelines for animal research [32]. Male BALB-C mice, 6–8 weeks old and weighing 24–28 g, were included (Envigo®, Coyoacan, Mexico City, Mexico). The experiment consisted of two parts: one with intravenous administration of the adenoviral vector (via tail vein) and the other with oral administration of the vector. For the intravenous section, a total of 173 mice were randomized into six parallel groups using a randomized block design [33]. Five groups were exposed to anti-inflammatory drugs, and one group was not exposed to any treatment. The groups were as follows: non-exposed positive control group (n = 15), non-exposed group (n = 17), dexamethasone group (n = 14), paracetamol group (n = 17), diclofenac group (n = 17), ibuprofen group (n = 16), and ketorolac group (n = 17). For the oral administration section, 10 mice were included per group: non-exposed, dexamethasone, paracetamol, diclofenac, ibuprofen, and ketorolac groups. All animals were housed in acrylic cages with a maximum of 5 mice per cage, at 21 ± 2 °C, with 12 h light–dark cycles and ad libitum access to food and water. The animals were handled according to the Mexican Official Standard for the Use of Laboratory Animals (NOM-062-ZOO-1999) [34] and the Guide for the Care and Use of Laboratory Animals by the US National Academy of Sciences (2011) [35].

Anti-inflammatory drugs were administered at the following doses: dexamethasone at 1 mg/kg/dose [36], paracetamol at 100 mg/kg/dose [37], diclofenac at 1 mg/kg/dose [23], ibuprofen at 15 mg/kg/dose [38], and ketorolac at 5 mg/kg/dose [39]. All treatments were administered orally once per day using an oral feeding tube for 7 days. The drugs were diluted in sterile water immediately before administration, and any leftovers were discarded in accordance with the Mexican Official Standard NOM-087-ECOL-SSA1-2002 [40]. This study was conducted in accordance with the Declaration of Helsinki and the experimental protocols were approved by the Research Ethics Committee of the School of Medicine of the Universidad de Colima, Mexico (Protocol Number: UCOL17-015, 5 June 2017).

On the seventh day of drug exposure, for the intravenous experiment, the mice were injected in the tail vein with 1×10^7 pfu of adenoviral Ad-BGal vector diluted in 100 µL of DMEM medium, except for the not-exposed positive control group, which was injected with 1×10^9 pfu using a 27G hypodermic needle. A dose of 1×10^7 pfu of adenoviral

vector is considered low, as previous studies have shown that intravenous administration of 20 to 20,000 times higher doses per mouse can generate a 9–40% transduction rate in the liver [41–43]. Therefore, it is expected that this dose will result in very low entry of the adenoviral vector into the organs, facilitating the detection of whether any drug is capable of modifying the amount of vector entering different organs.

It is important to note that the group not exposed to drugs serves as a reference for the amount of adenoviral vector that can enter different organs under normal conditions when applying a low dose of the vector (1×10^7), without implying that it is a positive control for vector transduction, as adenovirus entry does not necessarily have to occur at this dose. However, it provides a baseline for comparing whether any of the analyzed drugs can promote vector entry, even at this low dose. The not-exposed positive control group, injected with 100 times more vector (1×10^9 pfu), served as a positive control for the entire methodological process. For the oral administration experiment, 1×10^7 pfu of adenoviral Ad-BGal vector diluted in 100 µL of DMEM medium was administered orally.

Forty-eight hours after the adenoviral vector injection, the mice were euthanized, and the following organs were extracted: liver, heart, intestines, kidneys, lungs, brain, and peritoneum. Euthanasia was performed by trained research personnel via manual cervical dislocation according to the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition [44]. Mice were anesthetized with an intraperitoneal injection of 50 mg/kg of sodium pentobarbital before euthanasia.

2.5. Processing and Histological Analysis

Organs were embedded in Tissue-Tek® O.C.T. Compound (Sakura Finetek USA, Torrance, CA, USA) and frozen in liquid nitrogen. Cryostat sections were collected on gelatin-coated glass slides (10 µm sections). The slides were fixed in 2% paraformaldehyde for 10 min at room temperature, then washed twice with PBS for 20 min each before staining in the X-gal solution. The slides were incubated overnight at 37 °C in the X-gal staining solution (1 mg/mL X-gal solution) [45]. The next morning, slides were washed with PBS and counterstained with eosin.

Histological sections were observed under a Zeiss AXIO Vert.A1 microscope (Carl Zeiss, Coyoacan, Mexico City, Mexico) at ×400 magnification, with the same lighting conditions. Adenoviral vector entry into cells was indicated by blue-stained areas in tissues stained with X-Gal and counterstained with eosin. The organ and number of mice in which adenovirus entry was detected were determined for each treatment group. In hepatic tissues, the area (μm^2) where adenovirus entry was detected was also quantified (per 10,000 μm^2 of hepatic tissue analyzed).

A total of 38,090,000 μm^2 of hepatic area was analyzed in the dexamethasone group (2,720,714.285 μm^2 per mouse), 52,020,000 μm^2 in the paracetamol group (3,060,000 μm^2 per mouse), and 45,055,000 μm^2 in the ibuprofen group (2,815,937.5 μm^2 per mouse).

2.6. Structure–Activity Analysis

The Structure–Activity Relationship (SAR) analysis involved a comprehensive literature review focusing on pharmacophores responsible for the biological activity of the compounds (paracetamol, dexamethasone, diclofenac, ibuprofen, and ketorolac). Molecular illustrations were created using ChemDraw 3D software, version 20.0 (PerkinElmer, Waltham, MA, USA) [46], with each molecule structurally optimized and key properties calculated. The analyzed chemical properties included the molecular weight (MW), representing the compound's mass (g/mol); LogP, the logarithm of the octanol–water partition coefficient indicating lipophilicity, where higher values denote greater lipophilicity; ClogP, the calculated partition coefficient reflecting lipophilicity; MR (molecular refraction expressed in cm^3/mol), measuring the compound's light-bending capability; and tPSA (total polar surface area), quantifying exposed polar surface area. Additional properties examined included the CMR (calculated molecular refraction), offering insights into molecular vol-

ume and polarizability; LogS, the logarithm of water solubility reflecting lower values for less solubility; and pKa, the acid dissociation constant indicating molecular acidity [46,47].

2.7. Statistical Analysis

For descriptive statistics, data were represented as percentages, interquartile mean (IQM), Q1 (25th percentile), and Q3 (75th percentile), and minimum and maximum values. The non-normal distribution of data was confirmed using the Kolmogorov–Smirnov test. Intergroup comparisons were performed using the Mann–Whitney U test. Additionally, for the in vivo assay, intergroup analysis was performed using Fisher's exact test. Statistical analyses were conducted using IBM SPSS software version 20 (IBM SPSS, Chicago, IL, USA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. Results of the In Vitro Assay

In SiHa cells pretreated with anti-inflammatory drugs for 48 h, variations in the percentage of adenoviral entry were evaluated after exposure to 1×10^7 pfu/mL of the non-replicative Ad-BGal vector. The adenovirus entered 30.79% of the untreated reference cells (Q1–Q3 = 30.5–31.2). Pretreatment with diclofenac (54.77%, Q1–Q3 = 50.05–55.15, $p < 0.001$), dexamethasone (45.21%, Q1–Q3 = 45.05–45.50, $p < 0.001$), paracetamol (40.19%, Q1–Q3 = 39.60–40.80, $p < 0.001$), and ibuprofen (40.00%, Q1–Q3 = 39.40–40.50, $p < 0.001$) resulted in increased adenoviral entry compared with the drug-free cells. Only pretreatment with ketorolac showed no significant differences from the untreated (not exposed) with drugs group (33.19%, IQM = 33.19, Q1–Q3 = 33.00–33.45; $p = 0.098$) (Table 1 and Figure 1).

Table 1. Percentage of adenoviral vector entry into SiHa cells (cervical cancer cells).

Group	Not Exposed (*n* = 15) %	Dexamethasone (*n* = 15) %	Paracetamol (*n*= 15) %	Diclofenac (*n*= 15) %	Ibuprofen (*n*= 15) %	Ketorolac (*n*= 15) %
IQM	30.79	45.21	40.19	54.77	40.00	33.19
Q1–Q3	30.50–31.20	45.05–45.50	39.60–40.80	54.05–55.15	39.40–40.50	33.00–33.45
p vs. not exposed	NA	<0.001 *	<0.001 *	<0.001 *	<0.001 *	<0.098

NA: Not applicable. IQM: interquartile mean. Q1: 25th percentile. Q3: 75th percentile. * Statistical significance value: $p < 0.050$. n = number of trials.

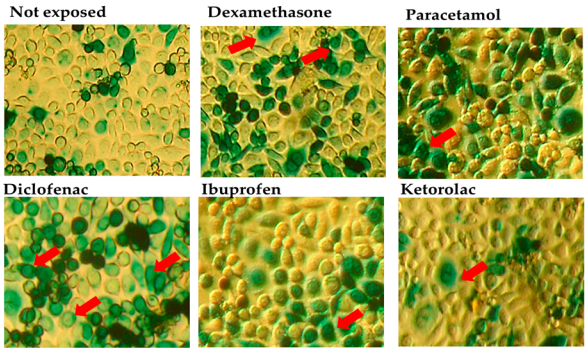

Figure 1. Variations in adenoviral entry into SiHa cells following exposure to anti-inflammatory drugs (X-Gal staining, ×20 magnification). Not exposed (not exposed to drugs group); dexamethasone group; paracetamol group; diclofenac group; ibuprofen group; ketorolac group. Red arrows indicate cells expressing β-Gal (green–blue color), indicating entry of the adenoviral vector with the reporter gene. There is a significant increase in the proportion of cells where adenovirus entered in the diclofenac, dexamethasone, paracetamol, and ibuprofen groups compared with the not exposed to drugs cell group ($p < 0.05$ for all comparisons). The ketorolac group shows no differences compared with the not-exposed group ($p > 0.05$).

3.2. Results of the In Vivo Assay (Intravenous)

In mice pretreated with anti-inflammatory drugs for 7 days, variations in the number of organs showing adenovirus entry (with B-Gal expression) were evaluated forty-eight hours after the intravenous administration of 1×10^7 pfu of the adenoviral vector Ad-BGal.

Table 2 shows in an intergroup analysis that the anti-inflammatory drugs were significantly able to modify adenovirus entry into the liver ($p < 0.001$, Fisher's exact test). In this initial analysis, only the number of mice with any organ positive for adenovirus entry was identified, without considering the quantity of cells or positive area. No vector expression was detected in any organ of the mice in the drug-free group mice. Adenoviral vector entry into liver samples showed intergroup differences ($p < 0.001$, Fisher's exact test). The proportions of positive mice in the liver were 71.42%, 29.41%, and 25% in the groups treated with dexamethasone, paracetamol, and ibuprofen, respectively (Table 2), which was different compared with the not-exposed group ($p < 0.001$, 0.044, and 0.044, respectively). With ketorolac and diclofenac, no significant differences were observed compared with the not exposed to drugs group in livers ($p > 0.05$).

Table 2. Number of mice showing adenoviral entry into cells of different organs after the application of 1×10^7 pfu adenoviral vector via the tail vein.

Group	Not Exposed *n* = 17 (%)	Dexamethasone *n* = 14 (%)	Paracetamol *n* = 17 (%)	Diclofenac *n* = 17 (%)	Ibuprofen *n* = 16 (%)	Ketorolac *n* = 17 (%)	*p* **
Liver	0	10 * (71.42)	5 *(29.41)	0	4 * (25)	0	<0.001
Heart	0	0	0	0	1 * (6.25)	0	0.395
Intestine	0	1 * (7.14)	1 * (5.88)	1 * (5.88)	3 * (18.75)	2 * (11.76)	0.486
Kidney	0	0	0	0	0	0	NA
Lung	0	0	0	0	0	0	NA
Brain	0	0	0	0	0	0	NA
Peritoneum	0	0	0	0	0	0	NA

* Number of mice showing adenoviral vector entry into cells of an organ. NA: Not applicable. Intergroup comparisons were performed using Fisher's exact test. ** Statistical significance value: $p < 0.05$. The not-exposed group serves as a reference for the amount of adenoviral vector that can enter different organs under normal conditions with a low dose of vector (1×10^7), without implying that it is a positive control. $n = 98$.

In intestinal cells, positivity was detected with dexamethasone (7.14%), paracetamol (5.88%), diclofenac (5.88%), ibuprofen (18.75%), ketorolac (11.76%), and not exposed to drugs (0%), without observing a statistically significant difference in the multigroup analysis ($p = 0.486$, Fisher's exact test) (Table 2). Adenovirus entry into cardiac cells occurred only in 6.25% of the mice pretreated with ibuprofen, without a significant difference compared with the not exposed to drugs group ($p = 0.395$). No adenovirus entry was observed in the kidneys, lungs, brain, and peritoneum with or without treatment with any anti-inflammatory drug (Table 2). The not-exposed positive control group, which was injected with 100 times more vector (1×10^9 pfu), showed that, in 100% of the mice, the vector entered hepatic cells.

In light of the liver showing the highest incidence of adenoviral vector entry in mice, we conducted additional analysis to quantify the positive hepatic area for adenovirus entry per 10,000 μm^2 of liver tissue analyzed. As previously stated, adenovirus entry was absent in untreated mice (mean area = 0.0 μm^2/10,000 μm^2). Treatment with dexamethasone resulted in adenovirus entry with a mean area of 10.2082246 μm^2/10,000 μm^2 of analyzed liver tissue, significantly differing from the not exposed to drugs group ($p < 0.001$) (see Table 3). Mice treated with paracetamol and ibuprofen also showed positive adenovirus entry areas, with mean areas of 0.149938272 μm^2 and 0.007111308 μm^2/10,000 μm^2 of analyzed liver tissue, respectively, demonstrating significant differences compared with the not exposed to drugs group ($p = 0.018$ and 0.031, respectively) (Table 3 and Figure 2).

Table 3. Hepatic area with adenovirus entry.

Group	Not Exposed (n = 17) (μm^2)	Dexamethasone (n = 14) (μm^2)	Paracetamol (n = 17) (μm^2)	Ibuprofen (n = 16) (μm^2)
IQM (μm^2/10,000 μm^2)	0.0000	10.2082	0.1499	0.0071
Q1–Q3 (μm^2)	0.0000	0.0000–37.1358	0.0000–5.2957	0.0000–0.1484
Min–Max (μm^2)	0.0000–0.0000	0.0000–1065.3062	0.0000–47.0628	0.0000–0.7234
p vs. not exposed	NA	<0.001 *	0.018 *	0.031 *

IQM (Interquartile mean (mean bounded by values between Q1 and Q3). Q1: 25th percentile. Q3: 75th percentile. Min–Max: Minimum and maximum values. Results are expressed in μm^2/10,000 μm^2 of analyzed hepatic tissue. Comparisons were performed with the Mann–Whitney U test. * Statistical significance value: $p < 0.05$. NA, Not Applicable.

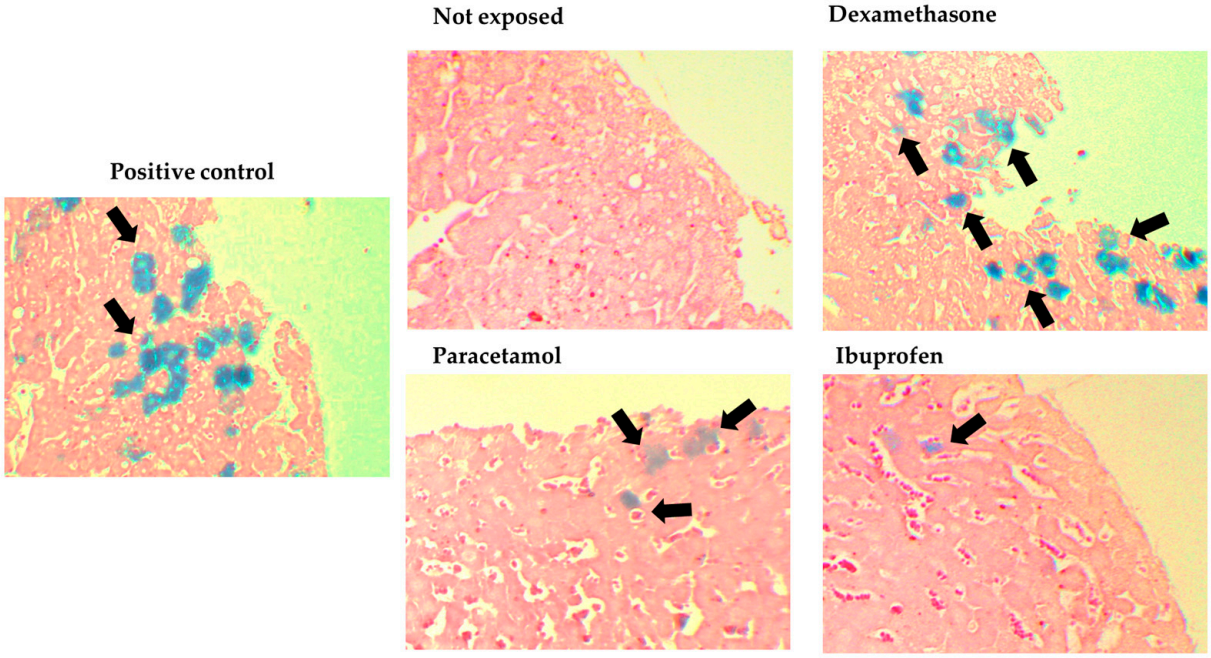

Figure 2. Microphotograph at 10× magnification of liver tissue from a mouse pretreated with dexamethasone, paracetamol, and ibuprofen vs. not exposed to drugs; administered with the adenoviral vector Ad-BGal at 1×10^7 pfu. After 48 h of intravenous inoculation with the adenoviral vector, the mice were euthanized, and their tissues were processed. The tissue samples were stained with X-Gal and counterstained with eosin. The experimental setup included a positive control (1×10^9 pfu), which was not exposed to drugs. The black arrow indicates an area with adenoviral entry (X-Gal stained, shown in blue color).

3.3. Results of the In Vivo Assay (Oral Administration)

The proportion of mice in which adenoviral vector entry was detected in the tongue, pharynx, and esophagus was analyzed. Table 4 shows a significant difference in adenoviral entry in the pharynx (p = 0.001, intergroup test), with dexamethasone causing positivity in 70% of the cases, followed by 10% in the ibuprofen group, and no entry in the other groups. No differences were found between groups for the tongue and esophagus, although a notable 40% positivity in the esophagus was observed with dexamethasone (Table 4).

Table 4. Number of mice showing adenoviral entry into cells of different organs after oral administration of 1×10^7 pfu of adenoviral vector.

Group	Not Exposed $n = 10$ (%)	Dexamethasone $n = 10$ (%)	Paracetamol $n = 10$ (%)	Diclofenac $n = 10$ (%)	Ibuprofen $n = 10$ (%)	Ketorolac $n = 10$ (%)	p **
Tongue	0	0	0	0	0	0	NA
Pharynx	0	7 * (70%)	0	0	1 * (10.0)	0	0.001
Esophagus	0	4 * (40.0)	0	0	1 * (10.0)	1 * (10.0)	0.201

* Number of mice showing adenoviral vector entry into cells of an organ. NA: Not applicable. Intergroup comparisons were performed using Fisher's exact test. ** Statistical significance value: $p < 0.05$. The not-exposed group serves as a reference for the amount of adenoviral vector that can enter different organs under normal conditions with a low dose of vector (1×10^7), without implying that it is a positive control. $n = 60$.

3.4. Structure Relationships Analysis

Considering the results of adenovirus penetration percentages following exposure to the different drug-treated models, it is consistent with previous work to propose a possible theory to explain the observations. The chemical structure and physicochemical properties of the treatment compounds reveal some structural characteristics. First, the five compounds are rich in heteroatoms (O and N) and have conjugated double-bond systems, which restrict their movement in space. This might not only influence their target site, but could also affect other targets indirectly. To establish a broader chemical perspective of these compounds (paracetamol, dexamethasone, diclofenac, ibuprofen, and ketorolac), they were modeled to obtain their approximate physicochemical properties [47,48]. Table 4 presents various chemical characteristics of the five evaluated drugs. The obtained physicochemical parameters reflect properties of polarity and solubility in polar and nonpolar media, which could be primarily responsible for the observed effects.

In Table 5, significant differences can be observed in the values of LogP and tPSA, which varied among the structures. However, there was no direct correlation between the increase or decrease in these physicochemical factors and the obtained results. Nonetheless, some key points can be noted among the compounds used for pretreatment and the adenovirus penetration capacity. Comparing Table 5 with Tables 1 and 2, we identified some patterns that could help us to understand the adenovirus penetration response in the evaluated systems. Analyzing these chemical properties is crucial to understand the different adenovirus penetration patterns observed in the in vitro and in vivo models used.

Table 5. Comparison of the chemical properties of five drugs (paracetamol, dexamethasone, diclofenac, ibuprofen, and ketorolac).

Chemical Properties	Dexamethasone	Paracetamol	Diclofenac	Ibuprofen	Ketorolac
MW (g/mol)	392.20	151.06	295.02	206.13	255.27
LogP	0.72	0.55	4.12	3.75	1.64
MR [cm^3/mol]	103.95	40.25	73.53	61.2	68.78
tPSA	94.83	49.33	49.33	37.3	57.61
CMR	10.3188	4.1737	7.6677	6.124	6.9954
LogS	−2.682	−1.058	−4.712	−3.119	−3.048
ClogP	1.7852	0.494	4.57624	3.679	1.622

MW (Molecular Weight). LogP (logarithm of the octanol–water partition coefficient). MR (molecular refraction). tPSA (total polar surface area). CMR (calculated molecular refraction). LogS (logarithm of the solubility in water). ClogP (calculated partition coefficient reflecting lipophilicity). pKa (acid dissociation constant).

Firstly, we found that LogP and ClogP indicate the lipophilicity of a substance [49]. Greater lipophilicity could facilitate penetration through cell membranes, increasing adenovirus entry [49]. Diclofenac, with the highest LogP (4.12) and ClogP (4.57624) values, shows a high percentage of entry into SiHa cells and liver tissues. However, this pattern was

not consistent in all cases, as ibuprofen, despite having a high LogP (3.75), showed lower penetration in liver tissues compared with dexamethasone. Secondly, the polar surface area (tPSA) is related to a molecule's ability to interact with water [50]. A high tPSA may result in lower permeability through lipid membranes. Dexamethasone, with a high tPSA (94.83), still shows high penetration in liver tissues, suggesting that other factors are also important. Additionally, LogS indicates the water solubility of a compound [51]. Very low solubility could limit bioavailability, affecting adenovirus penetration. Dexamethasone and diclofenac have lower negative LogS values (−2.682 and −4.712, respectively), which could indicate lower water solubility, but this is not clearly reflected in adenovirus penetration. Finally, pKa influences drug ionization at physiological pH levels, affecting solubility and permeability [51]. Dexamethasone's multiple pKa values (12.406, 14.124, and 14.861) could influence its behavior at different pH levels in the body, but the correlation with adenovirus entry is not direct.

Considering the above, we propose that adenovirus penetration seems to be influenced by a combination of chemical properties, with lipophilicity and polar surface area playing important roles. However, there is no simple correlation, suggesting that other specific factors of the drug and biological system, like receptors, are also important.

In the in vivo model, multiple tissues were evaluated, finding that adenovirus penetrated more in the liver and intestine compared with other organs like the heart, peritoneum, kidneys, and brain. This could be due to several biological and physiological reasons. In the case of the liver, this might be due to its high vascularization, filtering function, and high expression of specific receptors [52]. In the intestine, some of its primary characteristics are its large mucosal surface [53], significant amount of mucosa-associated lymphoid tissue (MALT), which can interact with pathogens and facilitate adenovirus entry, and the gut microbiota that can influence intestinal permeability and the immune response, affecting adenovirus penetration [53,54]. These factors could explain the obtained results.

It is clear that the adenovirus entry process is controlled by various factors, many of which are linked to the passage control exerted by the cell membrane, which could be compromised due to the surrounding microenvironment [55]. Up to this point, we know that these drugs will distribute differently within the system they are dissolved in due to their previously discussed physicochemical properties. This also depends on the type of tissue they are in, considering its vascularization, irrigation, and composition. Lastly, it is necessary to understand the mechanisms of action of these drugs on cell membranes. For instance, one hypothesized mechanism of adenovirus entry is the exploitation of plasma membrane sphingomyelin conversion into ceramides by ASMase, enhancing virus endocytosis and protein VI-mediated membrane rupture [56]. Consequently, we hypothesize that, if the membrane is altered compared with a normal cell, this could either benefit or hinder adenovirus entry.

4. Discussion

Some anti-inflammatory drugs, such as dexamethasone, ibuprofen, and paracetamol, have been shown to increase adenoviral entry both in vitro and in vivo. However, diclofenac exhibited this capability only in vitro, while ketorolac did not modify adenoviral entry either in vitro or in vivo. Notably, dexamethasone was the drug that most significantly increased adenoviral entry, both in vitro and in vivo via intravenous and oral administration, particularly facilitating virus entry in the liver and pharynx, respectively.

In line with our findings, another study demonstrated that dexamethasone enhances modified adenoviral entry in SiHa cells [57], although this effect had not been previously shown in vivo. Nonetheless, there are also reports that present contradictory evidence. It has been documented that pretreatment with dexamethasone decreases CAR and α5β1 integrin expression in various cancer cell lines, potentially leading to decreased adenoviral gene transfer in SiHa cells [58], although this expression has not been demonstrated in vivo.

In our study, we cannot confirm that increased adenoviral vector entry into dexamethasone-treated SiHa cells is due to enhanced receptor expression. In fact, it has been reported that

dexamethasone does not affect CAR levels in several cancer cell lines [59]. Research has shown that pretreatment with antioxidant drugs enhances adenovirus entry into cells by increasing CAR expression on the cell surface [60], which could explain our findings. However, a limitation of our study is that we did not analyze CAR and integrin expression.

There are no previous in vitro or in vivo studies demonstrating that NSAIDs modify adenovirus entry into cells or affect CAR and integrin expression. However, it has been reported that diclofenac, naproxen, and nimesulide can increase surface cell receptors for the entry of other viruses, like SARS-CoV-2 [16], suggesting a possible relationship between NSAID exposure and virus entry.

Our study demonstrated that pretreatment with paracetamol, diclofenac, ibuprofen, and dexamethasone facilitated adenoviral vector entry into SiHa cells, whereas ketorolac did not. However, since the in vitro tropism of adenoviral vectors does not necessarily correlate with in vivo tropism [61], we conducted animal model experiments to study in vivo behavior. We found that the pretreatment of mice with dexamethasone, paracetamol, and ibuprofen increased adenoviral entry into the liver, which had not been previously reported. Nevertheless, while these drugs may increase adenoviral entry into the liver, it does not necessarily imply adverse clinical effects from their administration during viral infection. Previous reports have indicated that dexamethasone pretreatment reduces hepatic leukocyte infiltration [62] and hemoconcentration induced by adenovirus [63]. Thus, despite increased adenoviral entry into the liver due to dexamethasone pretreatment, inflammatory effects from enhanced adenoviral entry may be mitigated by dexamethasone itself.

However, the interaction between anti-inflammatories and paracetamol in the clinical course of adenoviral infection remains uncertain, warranting further investigation. Complications secondary to adenoviral infection in children treated with ibuprofen and paracetamol have been documented [64,65]. Moreover, it has been reported that treatment with diclofenac and ketoprofen increases TNF-α production in cell cultures [66]. Both TNF-α [58] and interleukin 6 [67,68] could increase CAR levels and enhance adenovirus entry into cells. While studies on the role of TNF-α in CAR expression are contradictory [69], more research is needed in this area. Future studies are essential to evaluate the complex relationship between the administration of these drugs and the clinical course of adenoviral infections, particularly in the context of adenoviral vector gene therapies. Increased cellular entry of adenovirus due to anti-inflammatories may even be beneficial in certain gene therapy and vaccine settings. Importantly, the effects of the drugs analyzed cannot be generalized to all adenoviruses, as some adenoviruses do not depend on CAR for cell entry [70]. But it is also important to note that drugs could affect adenovirus entry by receptor-independent mechanisms.

Recent studies have demonstrated the effects of these evaluated compounds on membranes, altering their lipid composition or position. We know that the membrane is a dynamic system constantly undergoing turnover due to cellular microenvironment needs. For example, previous studies have shown that drugs can alter cell membrane properties: Ibuprofen can disturb the molecular order of liquid crystalline phospholipid membranes, increasing the mobility of the head groups and acyl chains of phospholipids [71], which could facilitate adenovirus entry. Diclofenac induces changes in the structural properties of the external phospholipid layers of the gastric mucosa, which could affect membrane permeability [72] and viral penetration. Similarly, for Dexamethasone, studies show that, after four days of treatment, Golgi membranes and liposomes from treated rats were found to have greater fluidity than controls, and it can alter the expression of adhesion molecules and other cellular receptors [73]. Finally, although information on Ketorolac's effects on membranes is limited, it has been found to exhibit high BBB permeability and antibacterial potency, suggesting an alteration in cell membranes [74]. This could also influence viral penetration in prokaryotic systems, as opposed to eukaryotes as observed in this study. This opens the door to new investigations into the potential use of this drug in this model. While the application of Ketorolac for transfecting prokaryotic models, such as certain bacteria

remains speculative, its ability to alter cell membranes may warrant further exploration in this context.

The main limitation of the in vivo study was the low experimental dose of 1×10^7 pfu of the vector per mouse, which resulted in no detectable vector entry in the organs of the not exposed to drugs group, at least with the detection technique used. While this allowed for the easy detection of drugs that clearly increase adenoviral entry into different organs, it did not allow for the determination of whether any drug reduces adenoviral entry into cells, as our reference had a value of zero. Future experiments with higher doses of adenovirus and additional drugs could provide more precise information on the increases and decreases in adenoviral entry into various organs. The use of other detection techniques, such as qRT-PCR or immunohistochemistry for adenoviral vectors, could also offer greater sensitivity in future investigations. It is important to note that, while the not-exposed group with the 1×10^7 pfu dose did not show virus entry, this does not invalidate the experiment, as it was not a positive control, but did represent a limitation, as mentioned. Another limitation was the lack of analysis of receptor expression and other tissues, such as adipose tissue. However, it opens the door to new research avenues that aim to analyze the expression of viral receptors in the cells and tissues treated with the drugs to identify possible treatment-induced changes and also conducting cell permeability assays to determine if the drugs alter the integrity of cell membranes. Additionally, it allows the examination of the changes in the local and systemic immune response in treated animals to better understand the impact of the drugs on viral susceptibility.

5. Conclusions

In conclusion, our study demonstrates that anti-inflammatory drugs, such as dexamethasone, paracetamol, and ibuprofen, can significantly increase adenoviral vector entry into cells both in vitro and in vivo. Specifically, dexamethasone was found to be the most effective, notably enhancing adenoviral entry into the liver, pharynx, and esophagus. The liver exhibited the highest increase in adenoviral penetration, likely due to its rich blood supply and specific receptor interactions influenced by these drugs.

In contrast, ketorolac had minimal impact on adenoviral entry, and diclofenac showed increased entry only in vitro. These findings suggest that the chemical properties and physiological effects of these drugs can modify adenoviral vector behavior, with potential implications for their use and consideration in future therapies. Further research is needed to explore how these drugs affect viral entry mechanisms and to assess their clinical relevance, particularly in the context of adenoviral vector-based therapies and infections.

Author Contributions: Conceptualization, I.D.-E.; Data curation, M.L.M.-F. and G.C.-E.; Formal analysis, H.R.G.-S., M.D.-M., G.A.H.-F., A.R.-H. and M.A.M.-H.; Funding acquisition, I.D.-E.; Investigation, H.R.G.-S., M.D.-M., N.S.A.-C., A.R.-H., M.L.M.-F. and S.A.Z.-F.; Methodology, H.R.G.-S., F.R.-L. and M.D.-T.-E.; Project administration, I.D.-E.; Resources, S.A.Z.-F. and G.C.-E.; Software, H.R.G.-S., I.G.-V., I.P.R.-S. and F.R.-L.; Supervision, I.D.-E.; Validation, I.G.-V., M.A.M.-H., I.P.R.-S. and M.D.-T.-E.; Visualization, I.D.-E.; Writing—original draft, G.A.H.-F. and N.S.A.-C.; Writing—review and editing, G.A.H.-F. and I.D.-E. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the University of Colima Faculty of Medicine and the Fundación para La Ética, Educación e Investigación del Cáncer del Instituto Estatal de Cancerología de Colima, A.C., Mexico.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and the experimental protocols were approved by the Research Ethics Committee of the School of Medicine of the Universidad de Colima, Mexico (Protocol Number: UCOL17-015, 5 June 2017).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in this article; further inquiries can be directed to the corresponding author/s.

Acknowledgments: The authors would like to express their gratitude to Julio V. Barrios Nuñez from the University of Colima (Colima, Mexico) for their assistance with English language editing). G.A. Hernandez-Fuentes would like to express their gratitude the financial support from CONAHCYT, Mexico for his postdoctoral studies (633738).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Forni, G.; Mantovani, A. COVID-19 Vaccines: Where We Stand and Challenges Ahead. *Cell Death Differ.* **2021**, *28*, 626–639. [CrossRef] [PubMed]
2. Ginn, S.L.; Amaya, A.K.; Alexander, I.E.; Edelstein, M.; Abedi, M.R. Gene Therapy Clinical Trials Worldwide to 2017: An Update. *J Gene Med.* **2018**, *20*, e3015. [CrossRef] [PubMed]
3. Pormohammad, A.; Zarei, M.; Ghorbani, S.; Mohammadi, M.; Razizadeh, M.H.; Turner, D.L.; Turner, R.J. Efficacy and Safety of COVID-19 Vaccines: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Vaccines* **2021**, *9*, 467. [CrossRef] [PubMed]
4. Khan, W.H.; Hashmi, Z.; Goel, A.; Ahmad, R.; Gupta, K.; Khan, N.; Alam, I.; Ahmed, F.; Ansari, M.A. COVID-19 Pandemic and Vaccines Update on Challenges and Resolutions. *Front. Cell Infect. Microbiol.* **2021**, *11*, 690621. [CrossRef] [PubMed]
5. Shoushtari, M.; Roohvand, F.; Salehi-Vaziri, M.; Arashkia, A.; Bakhshi, H.; Azadmanesh, K. Adenovirus Vector-Based Vaccines as Forefront Approaches in Fighting the Battle against Flaviviruses. *Hum. Vaccin. Immunother.* **2022**, *18*, 2079323. [CrossRef] [PubMed]
6. Sakurai, F.; Tachibana, M.; Mizuguchi, H. Adenovirus Vector-Based Vaccine for Infectious Diseases. *Drug Metab. Pharmacokinet.* **2022**, *42*, 100432. [CrossRef] [PubMed]
7. Wang, I.-H.; Burckhardt, C.J.; Yakimovich, A.; Greber, U.F. Imaging, Tracking and Computational Analyses of Virus Entry and Egress with the Cytoskeleton. *Viruses* **2018**, *10*, 166. [CrossRef]
8. Einfeld, D.A.; Roelvink, P.W. Advances towards Targetable Adenovirus Vectors for Gene Therapy. *Curr. Opin. Mol. Ther.* **2002**, *4*, 444–451. [PubMed]
9. O'Neill, A.M.; Smith, A.N.; Spangler, E.A.; Whitley, E.M.; Schleis, S.E.; Bird, R.C.; Curiel, D.T.; Thacker, E.E.; Smith, B.F. Resistance of Canine Lymphoma Cells to Adenoviral Infection Due to Reduced Cell Surface RGD Binding Integrins. *Cancer Biol. Ther.* **2011**, *11*, 651–658. [CrossRef]
10. Smith, T.A.G.; Idamakanti, N.; Marshall-Neff, J.; Rollence, M.L.; Wright, P.; Kaloss, M.; King, L.; Mech, C.; Dinges, L.; Iverson, W.O.; et al. Receptor Interactions Involved in Adenoviral-Mediated Gene Delivery After Systemic Administration in Non-Human Primates. *Hum. Gene Ther.* **2003**, *14*, 1595–1604. [CrossRef]
11. Greber, U.F.; Flatt, J.W. Adenovirus Entry: From Infection to Immunity. *Annu. Rev. Virol.* **2019**, *6*, 177–197. [CrossRef]
12. Wang, W.-C.; Sayedahmed, E.E.; Mittal, S.K. Significance of Preexisting Vector Immunity and Activation of Innate Responses for Adenoviral Vector-Based Therapy. *Viruses* **2022**, *14*, 2727. [CrossRef]
13. de Bruin, N.; Schneider, A.-K.; Reus, P.; Talmon, S.; Ciesek, S.; Bojkova, D.; Cinatl, J.; Lodhi, I.; Charlesworth, B.; Sinclair, S.; et al. Ibuprofen, Flurbiprofen, Etoricoxib or Paracetamol Do Not Influence ACE2 Expression and Activity In Vitro or in Mice and Do Not Exacerbate In-Vitro SARS-CoV-2 Infection. *Int. J. Mol. Sci.* **2022**, *23*, 1049. [CrossRef]
14. Shahbaz, S.; Oyegbami, O.; Saito, S.; Osman, M.; Sligl, W.; Elahi, S. Differential Effects of Age, Sex and Dexamethasone Therapy on ACE2/TMPRSS2 Expression and Susceptibility to SARS-CoV-2 Infection. *Front. Immunol.* **2022**, *13*, 1021928. [CrossRef] [PubMed]
15. Fang, L.; Karakiulakis, G.; Roth, M. Are Patients with Hypertension and Diabetes Mellitus at Increased Risk for COVID-19 Infection? *Lancet Respir. Med.* **2020**, *8*, e21. [CrossRef] [PubMed]
16. Saheb Sharif-Askari, N.; Saheb Sharif-Askari, F.; Mdkhana, B.; Al Heialy, S.; Ratemi, E.; Alghamdi, M.; Abusnana, S.; Kashour, T.; Hamid, Q.; Halwani, R. Effect of Common Medications on the Expression of SARS-CoV-2 Entry Receptors in Liver Tissue. *Arch. Toxicol.* **2020**, *94*, 4037–4041. [CrossRef] [PubMed]
17. Cabbab, I.L.N.; Manalo, R.V.M. Anti-Inflammatory Drugs and the Renin-Angiotensin-Aldosterone System: Current Knowledge and Potential Effects on Early SARS-CoV-2 Infection. *Virus Res.* **2021**, *291*, 198190. [CrossRef]
18. Mendoza-Hernandez, M.A.; Guzman-Esquivel, J.; Ramos-Rojas, M.A.; Santillan-Luna, V.V.; Sanchez-Ramirez, C.A.; Hernandez-Fuentes, G.A.; Diaz-Martinez, J.; Melnikov, V.; Rojas-Larios, F.; Martinez-Fierro, M.L.; et al. Differences in the Evolution of Clinical, Biochemical, and Hematological Indicators in Hospitalized Patients with COVID-19 According to Their Vaccination Scheme: A Cohort Study in One of the World's Highest Hospital Mortality Populations. *Vaccines* **2024**, *12*, 72. [CrossRef] [PubMed]
19. Bahl, A.; Johnson, S.; Chen, N.-W. Timing of Corticosteroids Impacts Mortality in Hospitalized COVID-19 Patients. *Intern. Emerg. Med.* **2021**, *16*, 1593–1603. [CrossRef]
20. Guzman-Esquivel, J.; Galvan-Salazar, H.; Guzman-Solorzano, H.; Cuevas-Velazquez, A.; Guzman-Solorzano, J.; Mokay-Ramirez, K.; Paz-Michel, B.; Murillo-Zamora, E.; Delgado-Enciso, J.; Melnikov, V.; et al. Efficacy of the Use of Mefenamic Acid Combined with Standard Medical Care vs. Standard Medical Care Alone for the Treatment of COVID-19: A Randomized Double-blind Placebo-controlled Trial. *Int. J. Mol. Med.* **2022**, *49*, 29. [CrossRef]
21. Kelleni, M.T. Early Use of Non-Steroidal Anti-Inflammatory Drugs in COVID-19 Might Reverse Pathogenesis, Prevent Complications and Improve Clinical Outcomes. *Biomed. Pharmacother.* **2021**, *133*, 110982. [CrossRef] [PubMed]

22. Delgado-Enciso, I.; Cervantes-García, D.; Martínez-Dávila, I.A.; Ortiz-López, R.; Alemany-Bonastre, R.; Silva-Platas, C.I.; Lugo-Trampe, Á.; Barrera-Saldaña, H.A.; Galván-Salazar, H.R.; Coronel-Tene, C.G.; et al. A Potent Replicative Delta-24 Adenoviral Vector Driven by the Promoter of Human Papillomavirus 16 That Is Highly Selective for Associated Neoplasms. *J. Gene Med.* **2007**, *9*, 852–861. [CrossRef] [PubMed]
23. Malhotra, S.; Rana, D.; Patel, V. Comparison of Analgesic, Anti-Inflammatory and Anti-Pyretic Efficacy of Diclofenac, Paracetamol and Their Combination in Experimental Animals. *Int. J. Basic. Clin. Pharmacol.* **2013**, *2*, 458. [CrossRef]
24. Estados Unidos Mexicanos; Consejo de Salubridad General Edición 2018 Del Cuadro Básico y Catálogo de Medicamentos. Available online: https://www.dof.gob.mx/nota_detalle.php?codigo=5544613&fecha=23/11/2018#gsc.tab=0 (accessed on 31 July 2020).
25. Jasso, L.; Lifshitz, A.; Arrieta, O.; Burgos, R.; Campillo Serrano, C.; Celis, M.Á.; De la Llata, M.; Domínguez, J.; Halabe, J.; Islas-Andrade, S.; et al. Importancia Del Cuadro Básico de Medicamentos En La Prescripción Médica. *Gac. Med. Mex.* **2020**, *156*, 610–611. [CrossRef]
26. Blizzard, C.; McLaurin, E.B.; Driscoll, A.; Silva, F.Q.; Vantipalli, S.; Metzinger, J.L.; Goldstein, M.H. Plasma Pharmacokinetic Parameters of Dexamethasone Following Administration of a Dexamethasone Intracanalicular Insert in Healthy Adults. *Clin. Ophthalmol.* **2021**, *15*, 2055–2061. [CrossRef] [PubMed]
27. Graham, G.G.; Davies, M.J.; Day, R.O.; Mohamudally, A.; Scott, K.F. The Modern Pharmacology of Paracetamol: Therapeutic Actions, Mechanism of Action, Metabolism, Toxicity and Recent Pharmacological Findings. *Inflammopharmacology* **2013**, *21*, 201–232. [CrossRef]
28. Mermelstein, F.; Hamilton, D.A.; Wright, C.; Lacouture, P.G.; Ramaiya, A.; Carr, D.B. Single-Dose and Multiple-Dose Pharmacokinetics and Dose Proportionality of Intravenous and Intramuscular HPβCD-Diclofenac (Dyloject) Compared with Other Diclofenac Formulations. *Pharmacother. J. Human. Pharmacol. Drug Ther.* **2013**, *33*, 1012–1021. [CrossRef] [PubMed]
29. Davies, N.M. Clinical Pharmacokinetics of Ibuprofen. *Clin. Pharmacokinet.* **1998**, *34*, 101–154. [CrossRef]
30. Kulesza, A.; Zielniok, K.; Hawryluk, J.; Paczek, L.; Burdzinska, A. Ibuprofen in Therapeutic Concentrations Affects the Secretion of Human Bone Marrow Mesenchymal Stromal Cells, but not Their Proliferative and Migratory Capacity. *Biomolecules* **2022**, *12*, 287. [CrossRef]
31. Resman-Thrgoff, B.H. Ketorolac: A Parenteral Nonsteroidal Antiinflammatory Drug. *DICP* **1990**, *24*, 1098–1104. [CrossRef]
32. Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE Guidelines 2.0: Updated Guidelines for Reporting Animal Research. *PLoS Biol.* **2020**, *18*, e3000410. [CrossRef]
33. Festing, M.F.W. The "Completely Randomised" and the "Randomised Block" Are the Only Experimental Designs Suitable for Widespread Use in Pre-Clinical Research. *Sci. Rep.* **2020**, *10*, 17577. [CrossRef]
34. de Aluja, A.S. Laboratory Animals and Official Mexican Norms (NOM-062-ZOO-1999). *Gac. Med. Mex.* **2002**, *138*, 295–298.
35. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals*, 8th ed.; National Academies Press: Washington, DC, USA, 2011; ISBN 978-0-309-15400-0.
36. Owoeye, O.; Malomo, A.O.; Elumelu, T.N.; Salami, A.A.; Osuagwu, F.C.; Akinlolu, A.A.; Adenipekun, A.; Shokunbi, M.T. Radiation Nephritis: Anti-Inflammatory Effect of Dexamethasone in Adult Wistar Rats (Rattus Norvegicus). *Int. J. Morphol.* **2008**, *26*, 69–74. [CrossRef]
37. Lahoti, A.; Kalra, B.; Tekur, U. Evaluation of the Analgesic and Anti-Inflammatory Activity of Fixed Dose Combination: Non-Steroidal Anti-Inflammatory Drugs in Experimental Animals. *Indian J. Dent. Res.* **2014**, *25*, 551. [CrossRef]
38. Kalra, B.; Shalini; Chaturvedi, S.; Tayal, V.; Gupta, U. Evaluation of Gastric Tolerability, Antinociceptive and Antiinflammatory Activity of Combination NSAIDs in Rats. *Indian J. Dent. Res.* **2009**, *20*, 418. [CrossRef] [PubMed]
39. Guo, C.X.X.; Irwin, M.G.; Cheung, K.M.C.; Chan, D. An Effective Dose of Valdecoxib in Experimental Mouse Models of Pain. *Methods Find. Exp. Clin. Pharmacol.* **2007**, *29*, 383. [CrossRef]
40. Estados Unidos Mexicanos; Secretaría de Medio Ambiente y Recursos Naturales Norma Oficial Mexicana. *Protección Ambiental-Salud Ambiental-Residuos Peligrosos Biológico-Infecciosos-Clasificación y Especificaciones de Manejo*; Secretaría de Gobernación: Mexico city, Mexico, 2003.
41. Nakatani, T. Assessment of Efficiency and Safety of Adenovirus Mediated Gene Transfer into Normal and Damaged Murine Livers. *Gut* **2000**, *47*, 563–570. [CrossRef] [PubMed]
42. Smith, T.; Idamakanti, N.; Kylefjord, H.; Rollence, M.; King, L.; Kaloss, M.; Kaleko, M.; Stevenson, S.C. In Vivo Hepatic Adenoviral Gene Delivery Occurs Independently of the Coxsackievirus–Adenovirus Receptor. *Mol. Ther.* **2002**, *5*, 770–779. [CrossRef]
43. Chia, S.H.; Geller, D.A.; Kibbe, M.R.; Watkins, S.C.; Fung, J.J.; Starzl, T.E.; Murase, N. Adenovirus-Mediated Gene Transfer to Liver Grafts: An Improved Method to Maximize Infectivity. *Transplantation* **1998**, *66*, 1545–1551. [CrossRef]
44. Leary, S.R.A.; Underwood, W.; Raymond, A.; Samuel, C.; Greenacre, C.; Gwaltney-Brant, S.; Grandin, T.; McCrackin, M.A.; Meyer, R.; Miller, D.; et al. *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*; American Veterinary Medical Association: Schaumburg, IL, USA, 2020; Volume 1.
45. Chen, X.-S.; Troiano, N.; Kacena, M. β-Galactosidase Detection as an Indicator of Endogenous PTHrP in Cartilage. *Biotech. Histochem.* **2008**, *83*, 89–96. [CrossRef] [PubMed]
46. PerkinElmer Informatics. *ChemDraw 3D*; Version 20.0; PerkinElmer Informatics: Waltham, MA, USA, 2020.
47. Saxena, A.K.; Gupta, A.K.; Bhatia, K.S. Physicochemical Significance of ChemDraw and Dragon Computed Parameters: Correlation Studies in the Sets with Aliphatic and Aromatic Substituents. *J. Math. Chem.* **2024**, *1*, 1–26. [CrossRef]

48. Dearden, J.C. Prediction of Physicochemical Properties. In *Computational Toxicology. Methods in Molecular Biology*; Humana Press: Totowa, NJ, USA, 2012; Volume 929, pp. 93–138.
49. Chandrasekaran, B.; Abed, S.N.; Al-Attraqchi, O.; Kuche, K.; Tekade, R.K. Computer-Aided Prediction of Pharmacokinetic (ADMET) Properties. In *Dosage Form Design Parameters*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 731–755.
50. Prasanna, S.; Doerksen, R. Topological Polar Surface Area: A Useful Descriptor in 2D-QSAR. *Curr. Med. Chem.* **2009**, *16*, 21–41. [CrossRef] [PubMed]
51. Bergazin, T.D.; Tielker, N.; Zhang, Y.; Mao, J.; Gunner, M.R.; Francisco, K.; Ballatore, C.; Kast, S.M.; Mobley, D.L. Evaluation of Log P, PKa, and Log D Predictions from the SAMPL7 Blind Challenge. *J. Comput. Aided Mol. Des.* **2021**, *35*, 771–802. [CrossRef] [PubMed]
52. Ogoke, O.; Oluwole, J.; Parashurama, N. Bioengineering Considerations in Liver Regenerative Medicine. *J. Biol. Eng.* **2017**, *11*, 46. [CrossRef] [PubMed]
53. Johansson, K.R.; Sarles, W.B. Some Considerations of the Biological Importance of Intestinal Microorganisms. *Bacteriol. Rev.* **1949**, *13*, 25–45. [CrossRef] [PubMed]
54. McLoud, T.C.; Boiselle, P.M.; Trotman-Dickenson, B. Diseases of Altered Immunologic Activity. In *Thoracic Radiology*; Elsevier: Amsterdam, The Netherlands, 2010; pp. 216–241.
55. Flatt, J.W.; Butcher, S.J. Adenovirus Flow in Host Cell Networks. *Open Biol.* **2019**, *9*, 190012. [CrossRef] [PubMed]
56. Carlin, C.R. New Insights to Adenovirus-Directed Innate Immunity in Respiratory Epithelial Cells. *Microorganisms* **2019**, *7*, 216. [CrossRef]
57. Kanerva, A.; Lavilla-Alonso, S.; Raki, M.; Kangasniemi, L.; Bauerschmitz, G.J.; Takayama, K.; Ristimäki, A.; Desmond, R.A.; Hemminki, A. Systemic Therapy for Cervical Cancer with Potentially Regulatable Oncolytic Adenoviruses. *PLoS ONE* **2008**, *3*, e2917. [CrossRef]
58. Brüning, A.; Runnebaum, I.B. CAR Is a Cell–Cell Adhesion Protein in Human Cancer Cells and Is Expressionally Modulated by Dexamethasone, TNFα, and TGFβ. *Gene Ther.* **2003**, *10*, 198–205. [CrossRef]
59. Hemminki, A.; Kanerva, A.; Liu, B.; Wang, M.; Alvarez, R.D.; Siegal, G.P.; Curiel, D.T. Modulation of Coxsackie-Adenovirus Receptor Expression for Increased Adenoviral Transgene Expression. *Cancer Res.* **2003**, *63*, 847–853. [PubMed]
60. Jornot, L.; Morris, M.A.; Petersen, H.; Moix, I.; Rochat, T. N-acetylcysteine Augments Adenovirus-mediated Gene Expression in Human Endothelial Cells by Enhancing Transgene Transcription and Virus Entry. *J. Gene Med.* **2002**, *4*, 54–65. [CrossRef] [PubMed]
61. Sharma, A.; Li, X.; Bangari, D.S.; Mittal, S.K. Adenovirus Receptors and Their Implications in Gene Delivery. *Virus Res.* **2009**, *143*, 184–194. [CrossRef] [PubMed]
62. Seregin, S.S.; Appledorn, D.M.; McBride, A.J.; Schuldt, N.J.; Aldhamen, Y.A.; Voss, T.; Wei, J.; Bujold, M.; Nance, W.; Godbehere, S.; et al. Transient Pretreatment With Glucocorticoid Ablates Innate Toxicity of Systemically Delivered Adenoviral Vectors Without Reducing Efficacy. *Mol. Ther.* **2009**, *17*, 685–696. [CrossRef] [PubMed]
63. Xu, Z.; Smith, J.S.; Tian, J.; Byrnes, A.P. Induction of Shock After Intravenous Injection of Adenovirus Vectors: A Critical Role for Platelet-Activating Factor. *Mol. Ther.* **2010**, *18*, 609–616. [CrossRef] [PubMed]
64. Zhao, H.; Liu, Y.; Feng, Z.; Feng, Q.; Li, K.; Gao, H.; Qian, S.; Xu, L.; Xie, Z. A Fatal Case of Viral Sepsis and Encephalitis in a Child Caused by Human Adenovirus Type 7 Infection. *Virol. J.* **2022**, *19*, 154. [CrossRef] [PubMed]
65. Straussberg, R.; Harel, L.; Levy, Y.; Amir, J. A Syndrome of Transient Encephalopathy Associated With Adenovirus Infection. *Pediatrics* **2001**, *107*, e69. [CrossRef] [PubMed]
66. Tsuboi, I.; Tsuboi, I.; Tanaka, H.; Nakao, M.; Shichijo, S.; Itoh, K. Nonsteroidal Anti-Inflammatory Drugs Differentially Regulate Cytokine Production in Human Lymphocytes: Up-Regulation of TNF, IFN-γ and IL-2, in Contrast to down-Regulation of IL-6 Production. *Cytokine* **1995**, *7*, 372–379. [CrossRef] [PubMed]
67. Atasheva, S.; Shayakhmetov, D.M. Cytokine Responses to Adenovirus and Adenovirus Vectors. *Viruses* **2022**, *14*, 888. [CrossRef]
68. Gupalo, E.M.; Buryachkovskaya, L.I.; Chumachenko, P.V.; Mironova, N.A.; Narusov, O.Y.; Tereschenko, S.N.; Golitsyn, S.P.; Othman, M. Implication of Inflammation on Coxsackie Virus and Adenovirus Receptor Expression on Cardiomyocytes and the Role of Platelets in Patients with Dilated Cardiomyopathy. *Cardiovasc. Pathol.* **2022**, *60*, 107452. [CrossRef]
69. Chen, X.; Liu, R.; Liu, X.; Xu, C.; Wang, X. Protective Role of Coxsackie-Adenovirus Receptor in the Pathogenesis of Inflammatory Bowel Diseases. *Biomed. Res. Int.* **2018**, *2018*, 7207268. [CrossRef]
70. Lamprinou, M.; Sachinidis, A.; Stamoula, E.; Vavilis, T.; Papazisis, G. COVID-19 Vaccines Adverse Events: Potential Molecular Mechanisms. *Immunol. Res.* **2023**, *71*, 356–372. [CrossRef] [PubMed]
71. Kremkow, J.; Luck, M.; Huster, D.; Müller, P.; Scheidt, H.A. Membrane Interaction of Ibuprofen with Cholesterol-Containing Lipid Membranes. *Biomolecules* **2020**, *10*, 1384. [CrossRef] [PubMed]
72. Pereira-Leite, C.; Jamal, S.K.; Almeida, J.P.; Coutinho, A.; Prieto, M.; Cuccovia, I.M.; Nunes, C.; Reis, S. Neutral Diclofenac Causes Remarkable Changes in Phosphatidylcholine Bilayers: Relevance for Gastric Toxicity Mechanisms. *Mol. Pharmacol.* **2020**, *97*, 295–303. [CrossRef] [PubMed]

73. Dudeja, P.K.; Dahiya, R.; Brown, M.D.; Brasitus, T.A. Dexamethasone Influences the Lipid Fluidity, Lipid Composition and Glycosphingolipid Glycosyltransferase Activities of Rat Proximal-Small-Intestinal Golgi Membranes. *Biochem. J.* **1988**, *253*, 401–408. [CrossRef]
74. Basu, S.; Varghese, R.; Debroy, R.; Ramaiah, S.; Veeraraghavan, B.; Anbarasu, A. Non-Steroidal Anti-Inflammatory Drugs Ketorolac and Etodolac Can Augment the Treatment against Pneumococcal Meningitis by Targeting Penicillin-Binding Proteins. *Microb. Pathog.* **2022**, *170*, 105694. [CrossRef]