About the Cover:

On 21 August 2017, a total or partial solar eclipse was visible across the entire contiguous United States and was broadcast by several news outlets and government agencies. Visibility in Alabama ranged from 80% in Mobile to 98% in northeastern Alabama with the coverage in Tuscaloosa reaching around 90%. This image by NASA can be found at Nasa.gov.

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Combining Everolimus with other Antiangiogenic Drugs Provides Time for Patients to Undergo Non-Drug Based Cancer Treatment

Austin Acks

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Cancer is one of the leading causes of death worldwide, despite extensive research on the treatment of the disease. Cancer cells rely on many natural functions of the human body such as the growth of new blood vessels to bring cancer cells nutrients. The process of new blood vessel development is called angiogenesis. This review addresses the very important angiogenic pathway, the properties of mTOR, and how cancer can be controlled by inhibiting protein translation within this pathway. Unfortunately, the following proposals do not achieve 100% efficacy at inhibiting mTOR, but drugs such as aspirin and everolimus show extensive characteristics of tumor control causing stunted tumor growth and sometimes tumor shrinkage. Both show experimentally significant results in the slowing of metastasis as well, which can give patients valuable time to explore other treatment options to care for their disease more effectively.

Introduction

Due to the unpredictable outcomes of current cancer treatments such as surgery and chemotherapy, alternative ways to inhibit tumor growth are needed. Common cancer treatments include chemotherapy, radiation therapy, stem cell transplant, and other forms of drugless remedies. Despite significant advances in medical research, these treatments are not always successful [3]. Drug repurposing and drug research offer doctors better methods to control tumors from reaching metastasis. By inhibiting the growth of tumors, other treatments such as radiation can prove much more effective thus improving overall treatment. Currently, leading medical researchers are devoting a lot of time to studying the inhibition of angiogenesis to prevent tumors from receiving the essential nutrients they need to grow and reach metastasis. The inhibition of angiogenesis has been studied, but mainly in response to conditions other than cancer. Specific pre-existing drugs have been identified to aid in the reduction in expression of mTOR, a leading protein causing angiogenesis through VEGF-A and HIF-1α production. For example, aspirin has been found to have mTOR limiting capabilities, along with a stronger drug, everolimus, which is beginning to be used in cancer treatment. Everolimus has been found to be more effective in suppressing tumor growth when used in conjunction with gefitinib, an epidermal growth factor receptor (EGFR) antagonist, because angiogenesis can occur at moderate rates even in the presence of an mTOR inhibitor. Therefore, this finding suggests that angiogenesis occurs through multiple pathways because of the increased effect drug coupling has on overall treatment. Although a method for the complete suppression of mTOR and angiogenesis does not yet exist, the combination of aspirin, everolimus, and gefitinib drug treatments shows signs of effective tumor growth inhibition that, coupled with immunotherapy, could provide improved treatment.

Key Players in the Angiogenic Pathway

Angiogenesis plays a vital role in renal cell carcinoma due to cancer cells’ overexpression of vascular endothelial growth factor, hypoxia-inducible factor-1α, and Hippel-Lindau gene alterations. With heightened levels of these growth factors and proteins, patients are at an elevated risk of uncontrolled tumor growth and metastasis. Angiogenesis, a direct result of overexpressed proteins, “is the physiological process of the growth of new blood vessels from preexisting blood vessels” [4]. The propelling factor in angiogenesis turns out to be hypoxia-inducible factor-1α because its transcription is a response to the overall oxygen content in cells. In cases of low concentrations of oxygen in the cell, HIF-1α accumulates in the cytoplasm of the cell until it is transf erred into the nucleus. From there, the HIF-1α spurs the transcription and translation of many growth factors such as VEGF, FGF (fibroblast growth factor), and TGF (transforming growth factor). Each of these proteins allow for different angiogenesis pathways that overcompensate for each other when one is being inhibited as shown by Bianco et al.; when two protein inhibitors were used in conjunction and caused lower concentrations of VEGF than using the singular drug [2]. From this evidence, researchers concluded that angiogenesis indeed occurs through multiple different pathways, therefore targeting them all in conjunction should reduce tumor growth and metastasis capabilities. As angiogenesis is inhibited, necessary nutrients such as oxygen become scarce in the tumor vicinity, leading to slowed cell division and control over tumor growth. Renal cell carcinoma is one of the most vascular reliant of tumors meaning that angiogenesis plays a huge

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role in its development. In renal cells, bFGF mRNA expression is two to three times higher than normal cells, thereby suggesting bFGF mRNA is the most important vascular growth factor for this type of cancer [2]. Because the main angiogenesis promoters differ for different types of cancer, researchers must identify the prominent growth factors in order to treat the disease most effectively. Evidently, HIF-1α-induced translation of FGF is a main target for inhibition in renal cell carcinoma to hinder angiogenesis and decrease tumor nutrition, although treatment should be paired with other growth factor inhibitors to block as many angiogenic pathways as possible.

Bianco et al. Figure 4: A and B show statistically significant results when treating mice with everolimus, gefitinib, and the combination of the drugs. C and D show the number of weeks that each group of mice survived based on their treatment approach.

Treatment of Cancer Using Everolimus

Besides FGF, other proteins involved in the angiogenesis process can be targeted in various types of cancers such as the mTOR protein, also known as rapamycin. As discussed above, FGF is likely the most favorable candidate to target with inhibition, but mTOR inhibition has also been proven to aid in the treatment of renal cell carcinoma. mTOR is activated when cell resources are depleted. In response to this, mTOR “permits translation of proteins that drive cell growth, cell proliferation, and the production of angiogenic growth factors” [1]. In Amato et al., researchers identified a known mTOR inhibitor called everolimus and assessed the benefits of daily dosing in patients with renal cell carcinoma. Thirty-seven patients with metastatic renal cell carcinoma received 10 mg/day of the mTOR inhibitor in a four-week cycle. Improved control of the tumor was recorded according to Response Evaluation Criteria in Solid Tumors using MRI and CT scanning [1]. Of the original thirty-seven patients, five were progression free for six or more months, lending evidence to everolimus’ ability to inhibit angiogenesis and cellular replication. By February 2008, the data cutoff date, twenty-one of the thirty-seven patients were progression free for six or more months. Response to the mTOR inhibitor was much more significant than expected as seventy-three percent of participants experienced stable disease and fourteen percent experienced partial response meaning non-advancement and improvement, respectively. On average, the percent change from baseline tumor size of the thirty-seven patients was negative, meaning that most of the time, tumor reduction or stabilization occurred as shown in the figure above. This is very significant because the success rate of everolimus at preventing metastasis progression is considerable. Some patients still experienced increases in the size of the tumors because everolimus does not completely eliminate all mTOR proteins. Side effects noted during the study were the development of pneumonitis, mucositis, duodenitis, thrombocytopenia, and hyperlipidemia stemming from the high dose of everolimus given to patients. Researchers decreased dosage when these common side effects were observed [1]. Even though HIF-1α, as discussed previously, is the main target for anti-angiogenesis in renal cell carcinoma, mTOR targeting offers a more general treatment to cancer. Seemingly, everolimus coupled with HIF-1α inhibitors would more effectively treat renal cell carcinoma as opposed to using only one of those drugs, unless the combination causes intolerable side effects.

Amato et al. Figure 2: This figure shows that 24/37 patients experienced a decrease in tumor size during the study.

With regard to other cancers such as gastric cancer (GC), mTOR presents itself in high quantities, showing activation in 60% of patients. Therefore, the mTOR pathway is one that, if targeted, can apply to many different cases. Everolimus analysis has been very popular recently because of the discovery that mTOR inhibition is quite effective at slowing angiogenesis. In a case studied by Ji Hyun Park et al., a man from Korea was diagnosed with stage IV GC with multiple liver metastases. He was treated with 10 mg/day of everolimus and achieved tumor stability with a partial response over two years [3]. After twenty-nine months, CT scanning revealed a progression in liver metastasis, proving that everolimus treatment alone will not fully inhibit angiogenesis and its role in metastasis. After a pause in treatment to care for the patient’s pneumonia, everolimus treatment resumed and again a
stabilization of liver metastasis was observed for another year. Unfortunately, the patient died in March 2013 [3]. Similar to the renal cell carcinoma study, researchers observed overall improvement while on everolimus treatment. This case, though, “is the first report of a durable control of metastatic GC with everolimus re-treatment in a patient who was previously treated with the drug” [3]. Even though this was the only case of its kind that existed at the time of the study, the results remain significant because of the more recent demonstrations of everolimus treatment in other types of cancer. Studies including this one, the renal cell carcinoma study by Shankhajit et al., and others discussing cases involving breast cancer, thyroid cancer, lung cancer, and cervical cancer have all shown strong mTOR activity. This means that for a wide range of cancer treatment, everolimus or an everolimus-like drug are good candidates for prescription and research. However, everolimus treatment alone is not enough to eliminate the cancer cells from their hosts.

The Combination of Everolimus and Other Treatments

Everolimus is not a perfect inhibitor of angiogenesis because angiogenesis occurs through multiple pathways, but treatment combinations involving everolimus are proving to be promising. In Park et al., the patient was solely treated with everolimus, but this did not yield the most effective results that can be achieved with the combination of drug treatments [3]. The result of the Park et al. study was only partial control of the tumor metastasis, but if everolimus was used in conjunction with another treatment, it’s possible that results could have been more significant at inhibiting tumor growth. In a study conducted by Bianco et al., mTOR inhibition was researched in conjunction with EGFR (epidermal growth factor receptor) inhibition where EGFR is another pathway for angiogenesis [2]. Researchers isolated the growth of cancer cells in soft agar gel and had four samples including a control group, an everolimus group, a gefitinib group (EGFR inhibitor), and a group treated with both everolimus and gefitinib. The colon cancer cells were treated with appropriate doses of the drugs and then analyzed for tumor growth and growth rate in reference to the control group. Both GEO, a typical cancer cell line in the colon, and GEO-GR, a highly resistant cancer cell line in the colon, were tested. In both of the groups being treated with gefitinib and the group being treated with everolimus, tumor growth showed significant decrease in comparison with that of the control. They also found that the combination of the two drugs significantly increased tumor control and decreased VEGF expression compared to the gefitinib-only and everolimus-only groups. This result wasn’t entirely surprising, but researchers didn’t expect to find that “everolimus restores the survival-inhibitory effects of antiEGFR drugs in resistant cancer cells” [2]. Because gefitinib had close to no effect on the control of GEO-GR cell survival and everolimus had about a 10% effect on GEO-GR cell survival, expectations are that the combination of the two would only result in about a 10% cell death. Surprisingly, the gefitinib + everolimus group resulted in a 30% cell death rate, which is much higher than the expected 10%. As a result, it was concluded that the drugs must be acting together in some way that enhances their control over cancer cell division, which might have been helpful in the Park et al. study. Since gefitinib (EGFR inhibitor) had zero effect initially, but the combination with everolimus showed drastic effect, researchers concluded that everolimus, when used with gefitinib, can restore the EGFR inhibitor capabilities of gefitinib even though GEO-GR cells were originally resistant to the drug [2]. This study highlights the importance of research on the combination of angiogenesis-inhibiting drugs because of their potential ability to complement and enhance each other’s effects.

One problem that arises with the treatment using combinations of drugs is that patients may have allergic responses or may not be able to tolerate the required high doses. Therefore, alternative drug choices are imperative. In addition to everolimus, aspirin has been proven to limit angiogenesis and the mTOR pathway as demonstrated in the Zhao et al. study of
mice with the comparison of aspirin doses to everolimus [5]. Although aspirin has been found to help with cancer, “the underlying molecular mechanism remains enigmatic” [5]. In this study, forty mice were randomly divided into four groups including a control, a low-aspirin group, a high-aspirin group, and an everolimus group. Tumor size was measured every other day, revealing tumor growth inhibition rates of 19.3% for low-aspirin, 33.6% for high-aspirin, and 53.7% for everolimus in hepatocarcinoma. Growth inhibition rates for S180 sarcoma were 25.7% for low-aspirin, 40.6% for high-aspirin, and 48.7% for everolimus [5]. As the dosage of aspirin increased, the control of the tumor increased as well, but never fully reached the capability of everolimus. As shown in Zhao et al. Figure 1, higher doses of aspirin resulted in a tumor size of 650 v/mm³ while everolimus treatment resulted in a tumor size of 600 v/mm³ twenty-one days after the start of the experiment [5]. Aspirin expressed very similar results to everolimus in the mice tumor growth, limiting both VEGF-A and HIF-1α expression. The inhibition of these proteins suggests that the protein, mTOR, was inhibited in some way by the aspirin treatment. Not only did aspirin show mTOR inhibition, but it also showed signs of inducing autophagy in cancer cells, which is the intracellular degradation system ultimately leading to cell death. The discovery of aspirin’s efficacy at inhibiting mTOR is very useful when a patient stops responding to everolimus or has a harmful reaction to the potency of the drug.

Zhao et al. Figure 1: This figure shows how aspirin can manage the growth of tumor cells in comparison with everolimus.

Concluding Remarks

Although everolimus has been consistently proven to aid in the prevention of angiogenesis through the inhibition of the mTOR protein, more research must be done to develop ways to inhibit other pathways for angiogenesis. With today’s current advancements in cancer drug treatments and other forms of treatment, it could be very effective to use treatments discussed in Bianco et al. such as everolimus coupled with gefitinib. The mTOR protein seems to be the best focus for targeting in the pharmacological treatment of cancer because of its ability to trigger the translation of proteins essential to angiogenesis pathways [2]. If mTOR could be completely isolated and targeted in a tumor before metastasis, sufficient nutrients would not reach the tumor cells, hence causing apoptosis to occur. Unfortunately, metastasis is not always easy to predict, but targeting mTOR with everolimus and a few other antiangiogenic drugs can cause an overall weakening of tumor growth and the delay of metastasis. With the delay, time is left for doctors to prescribe another type of therapy such as radiation or immunotherapy because these therapies can eliminate the remaining cancer cells. Ideally, everolimus treatment should precede immunotherapy or radiation in order to slow cancer development and allow more time for better treatment.

References


The Mediating Role of Pain Catastrophizing in Health Care Utilization within Low-Socioeconomic Settings

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Introduction

Chronic pain affects approximately 116 million American citizens [25]. Chronic pain is defined as pain lasting for more than three to six months, persisting past normal healing time [14,32]. It can lead to numerous adverse effects, such as poor quality of life, disability, and interference with daily activities [11,51]. In particular, depression and chronic pain are highly comorbid [20,31,41]. It has been found that approximately 60.8% of individuals with chronic pain have probable depression, and 33.8% qualify as having severe depression [45]. Chronic pain also frequently adds additional stress and obstacles in relationships [17,19]. Rates of health care utilization (HCU) tend to be higher among individuals with chronic pain, accounting for 15-20% of physician visits [11,28]. Chronic pain costs approximately $560 to $635 billion dollars annually, costing more than heart disease ($309 billion), cancer ($243 billion), and diabetes ($188 billion) combined [25]. Chronic pain typically necessitates increased health care use, but treatment quality and resources are not equally distributed across the population.

There are substantial chronic pain disparities based on sex, race, and socioeconomic status (SES) [25,53]. Low-SES communities present complex disparities due to the intricate interactions between lower levels of income, literacy, and education, and higher rates of racial/ethnic minorities [16]. Within low-SES settings, individuals encounter various obstacles in obtaining health care services such as limited access, difficulties with transportation and child-care services, financial difficulties, and insurance coverage. Studies of various factors of SES such as income, education, and literacy, have consistently demonstrated inverse relationships with pain [16,26,30,40,44].

The relationships between factors of SES and HCU are currently unclear due to limited research within low-SES settings. Studies have shown decreased access to primary care within low-income settings, and increased utilization of emergency health care among individuals with low-income and racial/ethnic minorities [10,18,36]. Many studies have shown limited opioid prescriptions in communities with a high proportion of minority inhabitants [33]. Individuals with higher SES have been found to use more specialized care, prescription medication, and medical imaging [18]. Within primary care settings, there is variability in results regarding HCU based on levels of income and racial/ethnic minority status. While some studies have reported decreased primary care utilization among low-SES and racial/ethnic minorities, other studies have found increased HCU [5,18,34]. There is a particular paucity of research within community health centers, in which patients experience large fee reductions for physician visits.

Literacy presents an interesting obstacle in healthcare management. Primary literacy is an individual’s ability to read, write, and speak English, and compute and solve problems at levels of proficiency.
necessary to function on the job and in society, to achieve one’s goals, and develop one’s knowledge and potential [24]. Literacy relates to the ability to obtain, process, and understand health information necessary for health management [24]. Navigating the medical system with low literacy may contribute to poor health outcomes, human rights abuse, and exploitation [13]. Patients with low-literacy levels have demonstrated higher rates of hospitalizations and emergency room visits, and decreased likelihood of regular physician visits or designated places of care [4,48].

There are few studies on HCU within low-SES settings. In particular, the potential mechanisms explaining the relationships between SES with HCU are not currently understood. Previous research has demonstrated strong relationships between pain-related variables (e.g., disability, pain severity, pain interference) and psychological variables (e.g., depression and anxiety) with HCU [2,3,11]. Many studies have demonstrated that increased disability, pain severity, and pain interference are associated with higher rates of HCU [2,27]. Depression has been shown to be a strong predictor of increased HCU [2,8]. Pain catastrophizing is a well-established concept in chronic pain, described as strongly held negative thoughts about pain and its influence on one’s life [49]. Less research has been conducted on pain catastrophizing and HCU. However, rates of HCU have been shown to be higher in individuals with depression and pain-related fears than depression alone [12].

Therefore, this study proposed exploratory analyses investigating potential pain-related and psychological mediators between poverty and literacy with HCU. The future of medical care and efficient HCU is centered on better understanding of how to effectively utilize healthcare. This is especially important in areas with high disparities in quality and access to health care.

Methods

Design/Setting

Medical records and pre-treatment data were compiled from patients with chronic pain receiving care at low-income clinics located in rural and suburban Alabama. Data was collected as part of the Learning About My Pain (LAMP) trial, a randomized comparative effectiveness study of group-based psychosocial interventions (PCORI Contract #941, Beverly Thorn, PI; clinicaltrials.gov identifier NCT01967342). Patients were recruited through a network of Community Health Centers administered by Whatley Health Services (WHS), resulting in a total of 290 participants. Whatley Health Services (WHS) is a privately owned, non-profit corporation serving low-income patients in Central and West Alabama. Participant coordinators, flyers, and word of mouth served as recruitment tools and contributed to the total participant population. Participants were compensated $45 at the conclusion of the pre-randomization assessment.

Inclusion/Exclusion Criteria

The inclusion criteria included the following: at least 19 years of age, at least one chronic pain diagnosis, experienced pain for at least half of the days for three months (not including malignant pain such as cancer or HIV), were able to speak and understand English, and had access to a method of communication (e.g., telephone).

Participants were excluded if any of the following criteria were met: significant cognitive impairment (measured by the Short Portable Mental Status Questionnaire [37], current serious uncontrollable psychological issues (e.g., schizophrenia), active substance abuse, below first-grade literacy levels, major changes in the four weeks prior to pre-treatment assessment regarding current pain or psychotropic medication, and current external psychosocial treatment for pain (did not include psychotherapy for non-pain issues).

Measures

Data Collection

Participant coordinators were trained by the PI to critically analyze medical records for HCU specific to chronic pain (more information is provided below in “medical records”). Records were examined retrospectively 12-months prior to study onset. Pre-treatment measures included: demographics, economic/educational, pain, and psychological variables. The demographic data were recorded using the Brief Demographic Questionnaire (BDQ), which included gender, age, income, highest level of educational attainment, and race. Due to overall low levels of income, this study examined income through calculating a poverty status variable that is based on the dichotomization of self-reported income into above and below household-adjusted poverty thresholds (https://aspe.hhs.gov/poverty-guidelines). The level of education was based on self-reported grade level. Trained assessors collected data on portable electronic tablets or paper backups. For clarification, these measures were all read out loud to the participants except the primary and health literacy measures.

Literacy

Using the Wide Range Achievement Test-4 Word-Reading subtest (WRAT) [55], each participant’s primary literacy was accessed. Based on a 0 or 1 scoring for incorrect and correct pronunciation of 55 words, increasing in complexity, the participant’s lit-
eracy was scored. Scores were converted based on a normative scale into grade level equivalency (WRAT GLE). The WRAT-4 has excellent internal consistency reliability ($\alpha = .92$) and alternate-form test-retest (.85) reliabilities [55].

\section*{Disability}

Disability was assessed through the Patient-Reported Outcomes Measurement Information System Physical Function v1.0 Short Form 201 (PROMIS PF-20). The National Institutes of Health (NIH) developed this measure based on self-reported capability of performing routine activities. Some examples of this 20-items scale include walking, climbing stairs, carrying groceries, and putting on shoes. The participant was asked to score the first 14 items based on their difficulty to perform certain activities with a 1 (unable to do) to a 5 (without any difficulty). The final 6 items ask how much their health limits certain activities with a 1 indicating unable to do and a 5 meaning not at all. For the current study, the PROMIS PF-20 was reverse scored so that greater impairment and disability has higher scores. The total score range was a 20 for the highest functioning and lowest disability, and a 100 for the highest disability and lowest functioning. The PROMIS PF-20 demonstrates excellent internal consistency [6].

\section*{Depressive Symptoms}

The Patient Health Questionaire-9 (PHQ-9) is a 9-item self-report measure created from the full PHQ to measure depressive symptoms [29]. Each item on the scale is measured from 0 (not at all) to 3 (nearly every day). The total scores range from 0 to 27, with higher scores indicating higher severity of depressive symptoms. The internal reliability of the PHQ-9 is high, with Cronbach’s $\alpha$ of .89. Test-retest reliability is also reported as excellent [29].

\section*{Pain Catastrophizing}

Pain Catastrophizing Scale (PCS) measures levels of catastrophic thinking about pain [49]. Pain catastrophizing is defined as highly negative thoughts about pain and its impact on one’s life. Using a 13-item, 5-point Likert scale ranging from 0 (not at all) to 4 (all the time), indicating the degree to which the participant has these thoughts and feelings attributed to their pain. Higher scores indicate higher levels of catastrophic thinking. The PCS internal reliability is excellent with $\alpha = .87$ [49].

\section*{Medical Records}

Health care utilization were collected from the medical records obtained from WHS. Health care utilization was operationalized as the sum of health care visits specifically for chronic pain to WHS over a 12-month period prior to study onset. Participant coordinators were trained by the PI to examine medical records. Visits that were not related to chronic pain, such as sinus infections or viruses, were not recorded as health care visits for chronic pain.

\section*{Statistical Analyses}

SPSS version 24.0 was used in the analysis of data [23]. Pearson product-moment correlations were examined among all continuous independent and dependent variables and point-biserial correlations were examined when evaluating dichotomous with continuous variables (Table 1). Mediation models examined the relationship between poverty status and literacy with HCU using a bootstrapping technique (with $n = 5000$ bootstrap re-samples) [38]. Bootstrapping is a nonparametric resampling procedure that does not include an assumption of multivariate normality. Bootstrap samples are empirically generated and are used to calculate the indirect effects in the resamples. Precision of the test statistic is estimated by means of 95% bias-corrected bootstrap confidence intervals (CIs). Estimates of indirect effects are significant when the bootstrapped confidence intervals do not contain zero. Mediation models were conducted with Hayes’ SPSS macro, PROCESS with 5000 bootstrap resampling [23].

Mediation models identified potential mediators based on the relations between the independent and dependent variables with the proposed mediators. Potential mediators examined include pain-related variables (pain severity, pain interference, disability) and psychological variables (depression and pain catastrophizing). In order to be examined as mediation models, it was determined that the independent variable (poverty status or literacy) was significantly related to the mediator variable (path a) and the dependent variable (HCU for chronic pain) was significantly related to the mediator variable (path b) (Figure 1). Based on previous research [22], the independent variable did not need to be significantly related to the dependent variable for mediation to occur (direct path c).
Results

Participant Results

There were 290 participants included in this study. The study sample included a majority of study participants who were below poverty status (n = 210, 72.4%), female (n = 205, 70.7%), and Black/African American (n = 194, 66.9%). Study participants identified an average of five types of pain (SD = 3) and an average of six sites of pain (SD = 3). Average pain duration was 16.6 years (SD = 12.2). Average number of treatment visits for chronic pain to Whatley Medical Center over a one-year period was five to six visits (about one visit every other month).

Mediation Models

Based on bivariate correlations, only PCS and BPI-Severity were considered potential mediators among the relationship between literacy with HCU. PCS and BPI-Severity were first examined in separate mediation models and then were examined within the same mediation model. Only PCS was considered a potential mediator among the relationship between poverty status with HCU. Depression, pain interference, and disability were not viable mediators due to the lack of significant relationships with either the independent or dependent variables.

The indirect effects demonstrated that PCS fully mediated the relationship between literacy and HCU. Results suggested that reduced primary literacy was associated with greater pain catastrophizing, which in turn, was associated with greater HCU. Similarly, the indirect effects also showed that BPI-Severity fully mediated the relationship between literacy and HCU. Results suggested that reduced primary literacy was associated with greater pain severity, which in turn, was associated with greater HCU. When entering pain catastrophizing and pain severity into the same mediation model, only pain catastrophizing was a significant mediator.

The indirect effects demonstrated that PCS fully mediated the relationship between poverty status and HCU. Below poverty status was associated with greater pain catastrophizing, which in turn, was associated with greater HCU.

Discussion

Chronic pain is a highly prevalent disease with especially harmful impact in low-income settings. Income-related disparities in access to health care are especially large in the United States even after controlling for insurance coverage and race/ethnicity [9,46]. Low-SES settings present with various obstacles in pain management such as difficulties in transportation and access to treatment, financial limitations, and inadequacy of treatments offered. Many patients feel frustrated and burdened with the continual seeking of health care services to ease the suffering associated with chronic pain, frequently with little to no improvement in pain management. Due to heavy burdens associated with HCU in low-SES populations, understanding the factors contributing to the relationships between factors of SES with HCU are essential for chronic pain management. This study found an important mediating role of pain catastrophizing in the relationship between factors of SES disparity (income and literacy) and HCU for chronic pain in low-income community health centers. Being below poverty status and having lower levels of literacy were associated with increased pain catastrophizing, which in turn was associated with increased HCU.

Primary and health literacy are essential skills in navigating healthcare. Disparities in literacy are common around the world. Within the United States, there are 40-44 million functionally illiterate individuals, the majority being white, native-born, and elderly [24]. In modern society, illiteracy is synonymous with hopelessness and poverty [13]. Research has demonstrated a pattern of lower levels of literacy among individuals with low-SES suggesting the important role of literacy in low-income communities [1].

Chronic pain demands substantial cognitive resources [21]. Low-SES settings are associated with greater levels of stress, and stress demands cognitive resources [7,21]. When combining the stress of chronic pain, the stress associated with low-SES settings, and low levels of literacy, one’s ability to process, learn, and remember new information may be hindered [40,47,52,54]. The association between low literacy and poor health outcomes might be in part explained by the excess burdens of cognitive demands, and barriers to obtaining health information and navigating the complex medical system.

This study suggests that pain catastrophizing plays an important role in the relationship between income and literacy with HCU. It was interesting that pain severity was a significant mediator when included individually in the mediation models, and then was non-significant when pain catastrophizing was included in the model. The results suggest that pain catastrophizing, rather than pain itself, accounted for more of the variance in the relationship between factors of SES disparity and HCU. As a result of higher cognitive load, individuals with low-SES and low-literacy may be at a greater risk for negative cognitive patterns, such as pain catastrophizing [45]. As an example, individuals might be more inclined to interpret pain as dangerous, serious, and unmanageable. In addition, due to high levels of stress, stigma, and discrimination, individuals might be predisposed to negative cognitions.

Limitations

Although a unique examination of the relationship between factors of disparity and HCU, this study is limited by its cross-sectional nature and therefore cannot establish causality. For example, it might
be possible that individuals with pain catastrophizing are vulnerable to a downward spiral of SES and negative health outcomes, potentially increasing health care visitation. However, it is unlikely that individuals with higher tendencies for pain catastrophizing later developed low-literacy levels. Another possible limitation is that the number of visits recorded in the study does not indicate efficiency of medical care. Within medical settings with limited resources, health care services are typically inadequate for addressing the needs of patients with chronic pain. Therefore, patients with chronic pain in low-SES settings may need to repeatedly visit health care services due to the inadequacies in treatment. The study is also limited by examining primary care alone, and does not have information on number of visits to other care settings. Given the low-SES setting, it is likely that there was little to no use of specialized and multidisciplinary care, however, the study was not able to control for these extraneous health care visits. Generalizability may be limited to highly underserved populations with multiple disparities. Future studies would benefit from examining the relationship between other forms of disparities (e.g., language barriers, sex, age) and HCU.

Conclusion

Negative cognitive patterns, such as pain catastrophizing, play an important role in the mechanisms of disparities and HCU. This study found that the relationship between literacy and poverty status with HCU is fully mediated by pain catastrophizing. Within low-SES settings, inadequate education, increased rates of dropout, discrimination, stigma, and higher levels of stress interact in complex manners to influence academic achievement and literacy [43]. In order to help fix this massive problem, especially with individuals who have a daily battle with chronic pain, it is important to offer treatments targeted to reduce catastrophizing. Treatments adapted for the particular literacy needs of low-SES populations might be especially important. Adapted literacy psychosocial treatments that target negative cognitive patterns have been shown to be efficacious with pain management [50]. Previous research has found that psychosocial treatments are not only effective in pain management, but also decrease health care costs [42]. In conclusion, pain catastrophizing helps to explain the relationship between factors of disparity and HCU within low-income communities. Future efforts to implement and sustain biopsychosocial treatments with literacy adaptations might help important for pain management.

References


**Prescription Drug Price-Gouging in America**

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While health care models vary greatly around the world, they all rely on the biological vulnerabilities that make human illness possible. Some people are more susceptible than others due to factors such as genes and the environment, and American pharmaceutical companies capitalize on these vulnerable patients by increasing the prices of medications, thus, maximizing profits. The media has coined the term “price-gouging” to define this injustice. It is illegal when collusion occurs among businesses to set costs high and control the market, known as price-fixing, and American citizens have been bearing the burden of its consequences. While Congress has begun to take action against conspiring pharmaceutical companies, major players such as “Pharma Bro” Martin Shkreli, former CEO of Turing Pharmaceuticals, have been able to get away with absurd pricing decisions without proof of involving other companies. By analyzing the historical context of the pharmaceutical industry, compiling lived experiences found in abundance throughout the internet, and identifying potential for improvement in the present day, those with a deeper understanding of this issue can create public awareness and eventual change.

**Introduction**

The socioeconomic status of consumers is irrelevant when evaluating supply-and-demand for a product that may be necessary for survival, such as medication. Many people in America are left with no choice but to work another job (if their condition permits), go into debt, or stop treatment when their insurance fails to cover it or tries to mitigate costs by requiring patients to pay a larger share, setting quantity limits, and asking patients to try other less expensive drugs first, in a process known as step therapy” [17]. For Medicare Part D beneficiaries, a coverage gap known as the “doughnut hole” can prevent their plan from paying for expensive drugs once a certain amount has already been spent, putting the responsibility on the patient to pay more out-of-pocket. When price-fixing takes place, these problems are exacerbated. Big pharma takes advantage of the industry’s lack of competition to create artificial demand for their medications and drive prices up, thus, forcing a greater number of people into step therapy, a coverage gap, or worse in a much faster timeline. In turn, families sink into a deeper financial hole and do not receive adequate care. A 2015 Kaiser poll found that “about three-quarters of those who say costs are unreasonable say that it is more because pharmaceutical companies set the drug prices too high, while just 10 percent say it is more because the health insurance companies require people to pay too much of the cost for drugs” [14]. The anecdotes recounted below will confirm the feeling shared by a vast majority of Americans: they are being cheated and are paying for it with their lives. Undoubtedly, impoverished communities are hit the hardest by price-gouging, but no one is immune to its effects and few have been held accountable.

**History of the Industry**

Although history has shown the United States to be a world leader in pharmaceutical development, the country has failed those who should benefit most due to price-gouging in recent years. The industry can be traced back to the late 1800s when pharmaceutical firms arose from apothecaries and chemical companies and established relationships with academic labs to innovate products with medical applications. Specifically, in the U.S., casualties during the Mexican-American War resulted in a need to treat soldiers with malaria, cholera, dysentery, and yellow fever, so medications were imported in accordance to the Import Drugs Act of 1848. This law called for drugs to be inspected upon entry into the country by the United States Pharmacopoeia. However, there was little regulation of drug production itself. Firms worked to keep drug recipes secret since patents did not yet exist to protect them. This became a liability when contaminated diphtheria antitoxins and smallpox vaccines were fatally administered to 23 Americans in 1901, so the government had to intervene and improve transparency. The Biologics Control Act of 1902 and Food and Drugs Act of 1906 required labs to obtain manufacturing and distribution licensing as well as list ingredients, expiration date, identification of product, and other information on bottles to ensure safety. When former federal bureaus were consolidated in 1930 to form the current Food and Drug Administration, “pre-marketing approval of all new drugs was made mandatory and proof for scientific safety study was asked” among other drastic regulations that continue into the present [12]. Despite well-intended legislation, the government that initially advocated for the health of its citizens through pharmaceutical control now indirectly permits the contrary by means of excessive regulations that shift power to big pharma.
and their capitalistic ventures. For example, the golden age of the pharmaceutical industry brought breakthrough medications to the public such as Valium, Tagamet, and Prozac in the 1960s-1980s, but “the enormous expense and risks involved in research and development caused many to merely ape their competitors, trying to get a cut of market-share using ‘me too’ formulations rather than innovating novel medications” since it was financially safer to buy the rights to a drug or wait for patents to expire [21]. AstraZeneca employed this technique when they produced Nexium in 2001, which was just an isometric version of an old drug that was no longer patent protected. Market exclusivity rights allow companies to abuse their monopoly of a drug even further. Ratified by the Hatch-Waxman Act of 1984, these rights are granted by the FDA to a newly approved drug that meets requirements and are considered separate from those granted by the patent and trademark office, supposedly for the purpose of balancing competition with generic drugs and the formulation of new ones. However, pharmaceutical companies began to exploit exclusivity. In a tactic known as “evergreening,” companies will make small, inconsequential changes to a medication, apply for another patent, and advertise it as improved. “Hard-switching” is another strategy in which “manufacturers stop selling an older drug about to go generic and replace it with a new high-price market-exclusive product” that patients are prescribed without knowledge of the generic when it is finally ready [16]. Regardless, since patents may expire before the drug is approved or can be issued afterward, exclusivity rights essentially guarantee the drug’s parent company sole profits for at least a few years after hitting the market depending on the type of medication.

Real Problems for Real People

The business strategies employed by pharmaceutical companies can induce great financial strain on those who must make tough decisions regarding their course of treatment. Jacqueline Racener was diagnosed with leukemia in 2015 and did not want to undergo chemotherapy, but “her annual income at the time disqualified her for copay-assistance” with the relatively new Imbruvica drug that was the alternative [20]. To accommodate the nearly $8000 cost per year, she had to step down from her full-time job and work part-time, cutting her income by about 40 percent. In the future, she expects she will also have to refinance her home and ask her adult children for assistance. Situations like Racener’s contribute to the evidence that price-gouging affects the middle to upper class too. Although “the length of US market exclusivity is one of the reasons why so many companies want to introduce their treatments in the US first,” it simultaneously blocks the entrance of a significantly lower priced generic drug, “which account for about 80 percent of all prescriptions [and] have been one of the few bargains in U.S. health care” [2, 5]. For many Americans, generics are the only affordable option, but the difference between patent protection in health care versus other industries comes down to choice. As Dr. Robert Pearl puts it, “if you don’t want to purchase Venetian glass, you can decide it’s too expensive. In contrast, if your child is born with a genetic defect, you have no choice but to obtain the medication available for treatment regardless of price” [11]. This rings too true for parents with children suffering from rare diseases (fewer than 200,000 cases in the U.S.) that can only be treated with a highly specific medication called an orphan drug. Fewer target consumers do not warrant massive sale margins after costly research and development, but the 1983 Orphan Drug Act gave companies tax-credit incentives to offset their investment. Lawmakers have since argued for the Act’s repeal, claiming it has been misused over the years to dramatically increase revenue as orphan drugs have come to account for the majority of those approved by the FDA. The tax breaks awarded to big pharma were no longer enough when the companies realized they could upcharge an orphan drug and remain unchecked by competitors who are likely not producing it. For example, the parents of Luke Whitbeck, a 2-year-old with a rare genetic disorder called Gaucher disease, pay an annual $300,000 for the Cerezyme orphan drug that keeps him alive. His mother, Meg Whitbeck, told NPR they’re “not going to not treat Luke, but we’re also never going to be able to pay these bills,” and that the totals are “almost laughable” when she opens them [18]. Their insurance provider, UnitedHealthCare, requires them to reauthorize their coverage at random intervals, sometimes a week, and the Whitbecks are terrified it will be denied one day. Others argued strongly against the repeal of the Orphan Drug Act because without it, their loved ones may not have any treatment option for their rare disease at all, despite current prices. Some orphan drugs are available at discounted prices with company-sponsored copay programs due to the incentives of the bill, but not all affected families are that fortunate. Americans shouldn’t have to make a decision between their life savings or their lives. Even prices of generic medication have been on the rise since the market became saturated in 2009. The Senate Subcommittee on Primary Health and Aging held a hearing in 2014 to address the issue, but costs still skyrocketed for some, like 52-year-old Carol Ann Riha. She was in “sticker shock” when her local pharmacy charged $19 and $101 for generic Pravastatin and hormone-replacement medication, respectively, that were once $4 and $40 per month in out-of-pocket expenses before 2015 [5].

Modern-Day Crisis

It is not uncommon for an average American to have active prescriptions for five or more medications at one time, which would add up fast for people like Riha. Low-income seniors were hit especially hard in 2003, when President George W. Bush proposed Medicare Part D. Not only was the “doughnut hole” coverage gap created, but government programs were no longer allowed to negotiate drug prices with pharmaceutical companies. The task was instead given to insurance companies when the plan took effect in 2006,
so Medicaid and Medicare have since been forced to pay full price for drugs even if a cheaper alternative exists. Insurers are not the best for the job since most “work with pharmacy benefit managers, who negotiate rebates and discounts on the company’s behalf — often in exchange for preferential placement on their list of covered medicines” [6]. The 2010 introduction of the Affordable Care Act had many benefits to healthcare, including the requirement for pharmaceutical companies to make payments toward closing the “doughnut hole”, but it also played a role in price-gouging. It did not allow the government to negotiate drug prices despite President Obama’s campaign promise to include it and, most significantly, “drugmakers won an unprecedented, guaranteed 12-year monopoly for biologic drugs,” which are based on cells rather than chemicals [9]. Therefore, the length of time in which the industry can avoid generic, or in this case, “biosimilar” competition for these modern drugs goes far beyond that of patents and market exclusivity rights. For patients with rheumatoid and psoriatic arthritis, the biologic Cosentyx has been a fantastic option, but not if they can’t pay for it. Rose Maloney, a Medicaid recipient, “battled first with psoriasis and then with the onset of psoriatic arthritis, autoimmune diseases that give her severe joint pain and skin rashes” for 12 years, and yet prior treatments have been unable to ease her constant pain until Cosentyx [3]. However, Maloney’s insurance stopped approving the drug, so she had weigh the level of her pain with the $40,000-per-year price tag. If the pharmaceutical industry worked with these patients instead of working for their own interests, America would be healthier, both physically and in wealth.

The Downfall of Big Pharma

In the past two years, coverage on news networks and social media has sparked an outrage over high-profile price-fixing scandals among large pharmaceutical companies. Jeffrey Glazer, former CEO of Heritage Pharmaceuticals, has been the first and only person charged by government for conspiring with other manufacturers, a violation of U.S. antitrust laws, despite its current prevalence across the industry. A class action lawsuit linking Heritage with companies such as Mylan and Teva is underway, so Glazer may not even serve jail time if he cooperates in the investigation. Allegedly, he “masterminded a scheme to raise the price of glyburide by 200 percent” by pushing employees to call other companies and discuss a collusive increase in the price of their generics, including the type 2 diabetes medication [19]. Price-fixing can happen outside of the office too. The Connecticut attorney general expanded a lawsuit brought forth by 45 states in 2016 when it was discovered that Emcure, the parent company of Heritage Pharmaceuticals, conspired with other companies by means of “a dinner attended by at least 13 presidents, CEOs and senior drug executives at a steakhouse in New Jersey in 2014” and with “female sales representatives regularly swapping price information at ‘GNOs,’ the industry shorthand for girls’ night out” [10]. Mylan denied collusion with Heritage, but they came under fire for spiking the price of EpiPens from around $100 to over $600 in 2016 after acquiring its rights in 2007. Although the epinephrine medication inside is nothing new, the auto-injector is patented, therefore subject to exploitation. Some have resorted to filling syringes with epinephrine in order to save money, but this isn’t nearly as safe, especially for children.

Drastic Measures

Uproar in the mainstream caught the attention of patients and healthcare providers, who worked tirelessly to research alternatives and successfully create demand for cheaper auto-injectors to compete. Public awareness and pressure has been the most effective method of combating price-gouging, but Mylan’s recent generic version of EpiPens is still around $300, which is triple the original cost. Children tend to need more on hand, which is a bigger expense at the pharmacy, so some desperate parents search for Canadian retailers who will accept their prescriptions. However, it is illegal to import prescription drugs into the U.S. because the FDA cannot oversee them. The drug importation advocacy group, RxRights, has gathered testimonies from across the nation of people who are suffering because of this law, such as Jerry, who insists “without a Canadian pharmacy I would be dead from diabetes” because he is self-employed and cannot afford his medication otherwise [13]. A 2004 bill with bipartisan support passed in Kansas and a few other states to implement a prescription drug importation program called I-SaveRx, which is a testament to the consensus among government officials and their constituents. Unfortunately, FDA interference and lack of advertising have since shut down the program, leaving legal drug prices at the will of an unjust pharmaceutical industry. Even the largest patient advocacy groups working to influence Capitol Hill have met resistance to any attempt to lower prices. Their silence of this issue can be explained by the millions of dollars donated by companies to ensure that “when they prod drug companies, it is generally for better — not less expensive — treatments” [15]. In fact, these companies spend more money lobbying Congress than conducting research and development, and it has only increased since President Trump promised to take on price-gouging, claiming big pharma is “getting away with murder.” He also intended on allowing Medicare to negotiate discounts, but abandoned the concept following meetings with industry lobbyists. Teva Pharmaceuticals, who has been accused of doubling the cost of diaper rash medication Nystatin, “spent $2.67 million, up 115 percent from a year ago as several companies embroiled in controversies raised their outlays significantly” in lobbying efforts [7]. Another money-making tactic utilized by big pharma to their advantage is marketing. Although the FDA requires pre-marketing approval as mentioned, there are no current restrictions on how much money that can be spent on it. Drugmakers also save money by cutting out research and development all together. When Marshall Allen was diag-
nosed with frozen shoulder, his doctor prescribed Vimo-
vo. After reading up on the controversial drug, he dis-
covered that Horizon Pharma made a net profit of $455
million from it since 2014 even though it is merely a
“convenient” concoction of two cheap over-the-counter
drugs, Aleve and Nexium, for respective pain and stom-
ach issues [1]. The same company has made $465 mil-
lion off another drug mixture containing Advil and Pep-
cid, called Duexis. In addition to acquiring old patents
and combining drugs, companies have been merging
recently to keep margins up. This limits the number of
competitors in the industry. In 2015, rivals Pfizer and
Allergan agreed to merge into the world’s largest phar-
maceutical company by sales in order to dodge U.S. tax-
es, which “could easily mean annual tax savings for
Pfizer of more than $100 million” now that “the compa-
ny's operational headquarters would remain in New
York City, but its principal executive offices would be in
Ireland” [22].

Concluding Remarks

For the countless individuals who have shared
their stories online or read them in comradery, the ploys
of big pharma do not come as a surprise, but it far from
lessens the anger associated with price-gouging in
American healthcare. On March 9th, 2018, Martin
Shkreli was convicted of securities fraud and sentenced
to seven years in prison [8]. Although unrelated to his
unjust inflation of drug prices, millions will remember
him for this legacy whether or not it is validated by the
court system. From the evolution of pharmaceutical
firms in the late 1800s to big pharma of the present day,
much can be learned from the grievances of the past.
Through education and awareness, advancement is not
only possible, but necessary. Government regulations
need to be reconsidered, policies must be changed, and
punishments should be imposed on the corrupt industry
that prospers at the hands of the suffering.

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Electrochemical Detection of Doxorubicin and Salinomycin

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Introduction

Research in the area of controlled drug delivery systems has been ongoing since the 1950’s. The field has advanced from extended release formulations to long circulating drug carrier systems [1]. However, a major barrier that still exists is the elimination of the burst effect or premature release of drug [2,3]. In order to design more efficient carrier systems, it will be necessary to evaluate the burst release kinetics [4]. This will require analytical methods which can deliver real time drug release data. Current methods involve collection of released drug followed by analysis using spectrophotometric techniques such as UV-VIS or HPLC (High-Performance Liquid Chromatography). HPLC is used to separate and analyze components of a mixture. These procedures are difficult and do not provide adequate sampling of data points at early times in the release profile when the burst release is in effect. Moreover, the instrumentation can be expensive and is not amenable for monitoring drug release either triggered magnetically or by a laser. Recently, a method involving time-resolved fluorescence was published [5]. However, this method also relies on taking aliquots of released drug for analysis. A need exists for the development of a real time method to monitor drug release which requires a minimal investment in equipment and is easy to use.

Doxorubicin, Fig. 1, is used to treat breast cancer, ovarian cancer, multiple myeloma, and Kaposi’s sarcoma. It is commonly administered as a pegylated liposomal formulation known as DOXIL© or Caelyx© [6]. This formulation improves the solubility of doxorubicin and extends the time of circulation through the bloodstream. Release of doxorubicin from liposomal carriers can be monitored by UV VIS spectroscopy at 485 nm [7]. Generally, released drug is dialyzed away from the carrier; the dialysate is sampled periodically and analyzed. In this case, doxorubicin can bind to dialysis tubing, which can make measurements inaccurate. However, UV Vis could not be used because it is not a real time measurement of drug release.

Doxorubicin has well-characterized electrochemistry. It has both quinone and hydroquinone groups which can be reduced (-0.6 V) and oxidized (+0.25 V) electrochemically [8]. There have been efforts made to monitor doxorubicin release from the DOXIL© carrier electrochemically and it was found that concentrations in the micromolar range could be detected [9,10,11].

Salinomycin, Fig. 2, is a member of the monocarboxylic polyether antibiotic family. It was originally used to prevent coccidiosis, a parasitic disease found in poultry. Recently, it has been shown to be highly effective at killing human breast cancer stem cells [12,13] and various other cancer stem cells including colorectal cancer, pancreatic cancer, and prostate cancer. Cancer stem cells represent a subpopulation of tumor cells that have the capacity to self-renew and can give rise to the types of malignant cancer cells that comprise a tumor. It has also been effective against drug-resistant cancers.

Salinomycin absorbs at 285 nm but has a very small extinction coefficient. The extinction coefficient determines how much the compound dampens light, so since salinomycin has a small coefficient, its effects on light are less easily seen and measured. In order to effectively detect release of the drug by HPLC, it must be chemically derivatized before the
analysis to enhance the UV absorption. This requires dialyzing released drug, reacting the drug with a chemical reagent, then performing the HPLC analysis [14]. Again, this is labor intensive, and does not allow for real-time evaluation of drug release.

**Materials and Methods**

Doxorubicin HCl was purchased from Aldrich Chemical Co. and used as received. Salinomycin sodium salt, silver wire, and Hg (I) chloride were purchased from Acros Chemical Co. All other chemicals were purchased from VWR. The Ag/AgCl reference electrode was purchased from CH Instruments Inc.

The electrochemical cell was a three-electrode setup with a Ag/AgCl reference electrode, a Pt wire counter electrode, and a Hg-Ag working electrode. The potentiostat was a Pine Research Instruments WaveNow. The electrolyte utilized was a phosphate-buffered saline (PBS) containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.8 mM KH$_2$PO$_4$. Solutions of Salinomycin and Doxorubicin were created at various concentrations ranging from 2.53 to 175.9 µM in PBS electrolyte solution.

Silver wires with a diameter of one millimeter were cut to the required length (~ 4 inches) and insulated using heat shrink tubing obtained from Walmart, leaving bare wire at each end. The silver wire was immersed in 6.659 mM HgCl$_2$ in PBS and the potential was set to -500 mV vs Ag/AgCl to affect the electrochemical reduction of Hg onto the Ag surface. The time for reduction was varied from ten seconds to ten minutes to vary the amount of Hg on the Ag surface.

All electrochemical experiences were run under dry nitrogen, including CV (cyclic voltammetry), DPV (differential pulse voltammetry), and BE (bulk electrolysis). The nitrogen bubbled through the solution while stirring with a magnetic spin bar removed any contaminants that were on any of the three electrodes.

**Results**

**Preparation of the Mercury Amalgam Coated Silver Electrodes**

Hg amalgam coated silver electrodes were prepared by the electrochemical reduction of Hg$_2^+$ on the surface of Ag wire. All electrochemical experiments were conducted under dry nitrogen. The optimal length of bare Ag wire in the solution was found to be 3 mm. Bulk electrolysis to deposit the Hg was allowed to run for 10 minutes. This afforded a thick, smooth coating of Hg. Optical micrographs of the silver wire before and after deposition are shown in Figure 3.

In aqueous solution, the mercury electrode had a high overpotential for electrochemical reduction of protons. This opened the electrochemical window for cathodic scans to a more negative potential. The cyclic voltammogram in Fig. 3 shows the reduction potential for protons had been moved to beyond -1.6 V vs Ag/AgCl. The high overpotential for proton reduction at the Hg amalgam coated silver electrode allowed electrochemical characterization at potentials beyond -1.0 V.

**Electrochemical Sensing of Doxorubicin**

Doxorubicin (Fig. 1) is a cancer drug that is used in Doxil©. It is electrochemically active and the quinone functional group can undergo a two-electron reduction to give the corresponding hydroquinone. This provided a means of using electrochemistry to sense the concentration of doxorubicin in aqueous solution. Both Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) were used to characterize the electrochemistry, but DPV was found to be more sensitive at nanomolar concentrations (Figure 5). The peak was fit to a Gaussian function to give the peak potential, the peak height (µA) and the peak area. The electrochemical characterization of DOX yielded reproducible correlations between the concentration of drug and current peak. At low concentrations, the curves are linear. However, at higher concentration the data fall below the linear curve. It is suspected that doxorubicin adsorbs the electrode surface, which makes it unavailable for electron transfer and causes the measured current to be lower than expected. However, the plot is linear in the concentration range expected for magnetically triggered release from the polymer micelle delivery system.

**Electrochemical Sensing of Salinomycin**

Salinomycin (Fig. 2) is very useful for killing breast cancer stem cells. The DPV in Fig. 7 shows a sharp reduction peak at -1.3 V vs Ag/AgCl. The peak was fit to a Gaussian function to give values of peak position, peak height (µA) and peak area. Figure 8 shows the plot of peak height as a function of salinomycin concentration. At low concentrations (< 25 µM) the curves were linear. At higher concentrations, the data fell below the line. Again as with doxorubicin, salinomycin adsorbs to the electrode at high concentration, blocking the electrode surface, leading to poor electron transfer kinetics, which decreases the current. Fortunately, for the purpose of detecting release of salinomycin from the polymer micelles the concentration is in the linear range.
Conclusions

The results of this experiment demonstrated that a real-time detection of both doxorubicin and salinomycin at nanomolar concentrations is possible, and is applicable to other electrochemically active cancer drugs such as Paclitaxel. The electrochemical technique is both affordable and relatively easy to use. The outlined method will allow for the concentration of free drug during early release from polymer micelle carrier systems. This also allows for the characterization of the burst effect—rapid release of drug at very short times. This discovery will help create an understanding for the burst effect and lead the way to being able to target cancer cells using the magnetic triggered release drugs.

References


According to the Centers for Disease Control, Alabama had the United States’ highest infant mortality rate (IMR) in 2014. In 2015, the Alabama Department of Public Health reported the IMR for White residents was 5.2%, but among Black and Other residents it was 14.4%. The goal of this project is to provide geographical data and case studies to better understand the implications of Alabama’s IMR and to address factors in the overall gap in treatment between demographic groups. This project includes geographical analyses using rates of infant mortality by county and demographic group, location of hospitals with obstetric centers, and rural and low income communities across Alabama. Data suggest the IMR tends to be highest for Whites in rural counties, and especially those with fewer hospitals in the region, but higher for African Americans in counties surrounding large cities. Case studies of birth experiences from low-income minority women in rural areas were compared to case studies of higher income women from urban and suburban areas. Obstetric care experience for rural-dwelling minority women was characterized by different barriers than for urban and suburban-dwelling women, such as access to care and transportation. These narratives, when combined with geographical analyses, elucidate treatment gaps in underserved populations that inform where improvement can be made in Alabama’s obstetric care.

Introduction

Infant mortality rate remains one of the most important health statistics because whether an infant survives its initial year of life is one of the most widely accepted indicators of population health [9]. Infant mortality is tied to community health status, poverty and socioeconomic status levels in a community, and availability and quality of health services and medical technology. Infant mortality rate is defined as the number of infant deaths per 1,000 live births. Global efforts have been taking place to reduce infant mortality rates in both high and low-income countries.

Trends in reducing infant mortality in the United States began in the early 20th century, with advancements in sanitation and use of antibiotics. As the 20th century progressed, infant mortality improved even more due to greater developments and research in medical technology and in the field of obstetrics. In the 1970s in particular, there was a focus on improving access to obstetric care in resource-deficient areas. However, in more recent years progress has slowed, and a racial and ethnic gap continues to exist in public health rankings and the Centers for Disease Control and Prevention rank Alabama #48 out of 50 states in infant mortality with large racial disparities, making Alabama a state in dire need of improvement to maternal and neonatal care[1].

Women in rural communities often find themselves travelling long distances to receive prenatal and perinatal care due to limited obstetric care availability within their communities. Women from rural areas who travel outside of their community have been associated with poorer prenatal care compliance [6]. If they lack adequate transportation, not only are they likely to have difficulty making it to their prenatal care visits but they are also at risk for delaying or foregoing prenatal care completely. Increased stress related to travel and birthing in an unfamiliar setting outside of the community can interfere with the progress of labor [8]. Thus, many women in rural areas who travel outside of their community are more likely to experience more birth-associated complications.

However, individual-level risk factors may not be able to account for such disparities in obstetric care experience and infant mortality rates because larger social forces have great bearing on disease risk and care outcomes in a population [2]. Research has continuously supported the idea that racism and social inequality are major contributors to racial disparities in health [4]. This means that due to high levels of stress and internalized racism, many minority women are susceptible to adverse birth outcomes before they even become pregnant [3]. Due to the variation that exists in individualized experiences of pregnancy, birth, and neonatal care, causal relationships to birth outcomes have a large number of influential factors.

Currently there exists a gap in the literature regarding the ethnographic study of childbirth. With 99% of women delivering in hospitals, it is important to study the health care culture that creates variation in obstetric care experience and produces disparities in maternal and neonatal outcomes [7]. It has been shown that African American mothers receive differential treatment when receiving care and are less likely to get medical advice and information about medical complications and risks [5]. The purpose of this exploratory study is to identify where gaps in infant mortality may exist and where public health efforts can be concentrated to improve infant mortality and obstetric care experience across demographic groups in the state of Alabama.
Methods

Data was collected for this study via geographic analyses and case studies. Geographical analyses were conducted using the program ArcGIS and data obtained from the US Bureau of the Census, the Centers for Disease Control and Prevention, the Alabama Department of Public Health, and the Alabama Hospitals Association. Maps in this study depict trends in infant mortality from 2013 among two demographic groups, “White” and “Black and Other,” median income, and the distribution of hospitals in the state of Alabama. Case studies were collected in the form of oral histories. Oral histories are very unstructured interviews where no questions are asked; the informant has the ability to tell their story to the researcher freely. This eliminates the problem of questions leading an informant toward a particular answer the researcher is seeking. Two case studies were collected from “White and Urban” mothers and two case studies were collected from “Minority and Rural” mothers. Given that identity and experience are inherently intersectional, measuring only one variable would not give a full picture, thus race and urban-rural classification were chosen to represent the larger differences between intersectional demographic groups.

Results and Discussion

Geographic Analyses

Figure 1: Median Annual Household Income by County in Alabama, 2013

Figure 2: Distribution of Hospitals with Obstetric Wards by Bed Count in Alabama, 2015

Figure 3: Infant Mortality Rate by County in Alabama, 2013

Figure 1 and 2 demonstrate that in counties with higher median annual household income there tends to be hospitals with obstetric wards that have a larger numbers of beds. In counties with low median annual household income tend to have much smaller hospitals or a lack hospitals with obstetric wards completely. When compared to Figure 3, there are observable trends in low income counties reporting “no data” for infant mortality rate because there are no hospitals with obstetric wards in these counties. These counties tend to be those with low median household incomes. It is important to note that many of the counties
with high infant mortality rate and lack of hospitals with obstetric wards tend to be rural.

Figure 4: White Infant Mortality Rate by County in Alabama, 2013

Figure 5: Black and Other Infant Mortality Rate by County in Alabama, 2013

Figure 4 and 5 show that there is little overlap in counties where the White infant mortality rate is highest and where the Black and Other infant mortality rate is highest. White infant mortality rate tends to be highest in rural areas that are low income and lack hospitals with obstetric wards. It can be hypothesized that this is because of a general lack of resources and long distances to travel in rural areas where infant mortality rate tends to be highest. This contrasts the Black and Other infant mortality rate which is highest in and around major cities in Alabama, often not far from large hospitals and in some cases in counties with relatively high median income. From this it can be hypothesized that because high infant mortality rate for minority women tends to be in these counties, it may not be access to care that plagues them. Instead, this may support previous research that suggests places with higher income inequality, such as around cities, and the long-term social effects of internalized racism may play a large role in explaining the gap between races in infant mortality rate [2].

Case Studies

Trends were noticed among rural minority women in the case studies. Both rural women delayed receiving prenatal care until they were five months pregnant. In one case, this was because the mother found out she was pregnant very late into her pregnancy, and in the other case the mother was too nervous to tell her family so she delayed getting medical care until she became noticeably pregnant. Transportation was another factor that made it more difficult for these women to access medical care. In one case study, a hospital was only twenty minutes away but the mother relied on family and friends to drive her to her prenatal appointments and to the hospital for her birth because she didn’t have access to a car. In the second case study, the subject lived fifty miles from the nearest hospital and had to take almost a whole day off from work to get care. Both women from these case studies also had medical interventions during the birth of their children in the form of an epidural and a Caesarean section. To this day, they maintain good relationships with their doctors and still see the same doctor for annual gynecological check-ups. One woman cited that her doctor still keeps up with her child’s academic accomplishments and personal life and said, “I wouldn’t change a thing, I could not have asked for better doctors.” Both women also noted that there had been recent hospital closures in their county in the last decade and that although smaller hospitals were located near them, they lacked obstetric wards.

Trends among urban White women were markedly different from that which characterized the experiences of minority women. Whereas rural-dwelling minority women had little choice in hospitals, two interviewed urban White women utilized care at hospitals that were farther than their nearest hospital.
They found doctors that offered the services that suited their wants and needs. This is likely because both women sought alternative care in addition to obstetric care, given that they hired doulas, or birth partners. They both told of how much support their doulas were able to offer both before and after birth. Overall, urban White subjects in both case studies were unhappy with their obstetric care, citing it as a “checklist experience.” Despite having such positive experiences with their respective doulas, they faced stigma from friends and family regarding their desires for natural births.

Limitations

One of the limitations of this study comes from how the Alabama Department of Public Health chooses to organize their data. Because only two groups, “White” and “Black and Other,” are accounted for, it is difficult to get an idea of how infant mortality among different minority groups breaks down further. Additionally, while collecting case studies in the form of oral histories has its benefits, it prevents the researcher from asking targeted questions pertaining to the research question. However, given that this is an exploratory study, oral histories reveal directions for future research.

Conclusion

There are a few improvements that can be made academically to understand how variation in obstetric care outcome gets produced, and also structurally to prevent large disparities in infant mortality from continuing to exist. First, there is a need for more ethnographic study of obstetric care in the United States. Recognizing the culture of giving birth, from both the obstetrician’s and the mother’s perspective, can lead the way forward in understanding notions of perceived experience and practice in the obstetric care setting. Public health efforts should continue to address issues of access to obstetric care because rural areas still suffer from greater infant mortality rates and a diminishing presence of obstetric care providers in addition to issues of access to care by minorities in both rural and urban settings. Because infant mortality is a sensitive measure of population health, it would be reductive to state that infant mortality disparities across demographic groups in Alabama are solely because of differences in quality and access to obstetric care. Infant mortality disparities are complexly related to social and environmental factors among many others and reflect the experience of minority communities over the course of their lives.

References


Evaluating the Large Magnetic Anisotropy of the Nitroso Group in Oriented Nitrosoarenes

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Here we report the preparation and \textsuperscript{1}H-NMR properties of three nitrosobenzene derivatives: o-nitrosobenzonitrile (7), o-nitrosocumene (10), and o-nitrosobiphenyl (13). The nitroso (-N=O) groups of these molecules are forced into a primary (majority) orientation in the molecule by the steric effect of the neighboring substituent, thus allowing the NMR analysis of the magnetic environment around an oriented -N=O group at room temperature, as needed to detect any change in the magnetic field as a function of position near the nitroso group. Nitrosobenzenes studied here have been synthesized from their aniline derivatives and their \textsuperscript{1}H-NMR signals recorded and assigned in order to evaluate the unique magnetic features of the nitroso group. NMR signals of H's syn to the -N=O group are shown to be strongly shielded (moved to lower ppm on the chemical shift axis), while H's anti to the -N=O group are strongly deshielded (moved to higher ppm) relative to 'normal' chemical shift positions for these H atoms as observed in model amino and nitro analogs of these nitrosoarenes.

\section*{Introduction}

The structure of an organic nitroso function (-N=O) is shown in Figure 1, along with the parent nitrosoarene, nitrosobenzene (1). The C-N=O linkage has a bond angle at nitrogen of about 120° and contains an sp\(^2\)-hybridized N-atom. The nitroso group and the arene ring conjugate and are coplanar, yielding a planar molecular structure for 1.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{The structures of a C-nitroso group (left) and nitrosobenzene (1) (right).}
\end{figure}

Nitrosoarenes have several unusual and interesting properties. One such unique property is their reversible dimerization to azodioxides. Scheme 1 shows this dimerization reaction for nitrosobenzene 1, which dynamically interconverts monomer structure 1 with its dimer (Z or E) azodioxide forms 2 [1]. The Dimerization of nitrosoarenes occurs by a non-least motion process in which the two molecules approach in perpendicular planes to ultimately give the planar dimer molecule 2. As solids, nitroso compounds generally exist in the colorless dimeric azodioxide form, while in solution the colored monomer predominates. The dimerization/monomerization equilibrium of nitrosoarenes is reversible at room temperature.

A special electronic feature of C-nitroso compounds is their green or blue color, which stems from a low energy HOMO (highest occupied molecular orbital) – LUMO (lowest unoccupied molecular orbital) n→π* electronic transition, which occurs with absorption of visible photons [11]. Another unusual feature of the nitroso group electronic structure concerns its highly directional (anisotropic) induced magnetic field as observed in the \textsuperscript{1}H-NMR spectroscopy of nitrosoarenes, the subject of this report. To detect the novel NMR-based magnetic anisotropy of the -N=O function, the nitroso group must have a fixed orientation in the molecule with respect to nearby hydrogen atoms so that the directional magnetic effects are not ‘averaged out.’ Low temperature can be used to orient the -N=O group [3, 4] or special structural features built in to the nitroso compound molecular structure can be employed to ‘fix’ the -N=O group orientation (the approach used in our study).

Fletcher and coworkers [3] have reported the low temperature \textsuperscript{1}H-NMR of nitrosobenzene 1, which showed, for the first time, the dramatic difference in the local magnetic field on the syn and anti sides of the nitroso structure 1 (Figure 2). Low temperature NMR is required to observe this effect because at ambient temperatures, C-NO rotation is fast on the NMR
timescale (msec), which averages the \( H_{\text{syn}} \) and \( H_{\text{anti}} \) magnetic environments (and their chemical shifts). The average chemical shift of the ortho Hs is 7.90 ppm, a typical value for aryl hydrogens located ortho to an electron withdrawing group on the ring (Figure 2, left). This dynamic averaging of the syn and anti \(^1\)H-NMR signals masks the large magnetic field difference between these locations. At low temperature (-100° C), the C-NO bond rotation is 'frozen out' on the NMR timescale, and discrete \( H_{\text{syn}} \) and \( H_{\text{anti}} \) values are observed at 6.28 ppm and 9.52 ppm, respectively (Figure 2, right) [3]. The large difference in \( \delta \)-values for the syn and anti hydrogens of 1 (\( \Delta \delta = 3.24 \) ppm) results from the extremely large magnetic field anisotropy for these nitrosoarenes. The average chemical shift of the ortho H's is 7.90 ppm, a typical value for aryl hydrogens located ortho to an electron withdrawing group on the ring.

Our approach to further investigate the NMR-based magnetic effects of the nitroso group has been to study nitrosoarenes, which possess only a single (or large majority) populations -N=O orientation in the molecule at room temperature. This is achieved synthetically by incorporation of bulky ortho groups to sterically favor a single nitrosoarene molecular conformation in which the -N=O orients away from the ortho substituent. We first observed this kind of steric control of the nitrosobenzene conformation in the course of converting o-bromoaniline (4) to o-bromonitrosobenzene (5) [2]. Figure 4 shows \(^1\)H-NMR spectra of aniline 4 and nitrosamine 5. The shielded doublet (ortho proton) at 6.75 ppm in the aniline structure becomes even more shielded in the nitroso structure, despite the e-density at that ortho position changing from electron rich (in the aniline due of the -NH$_2$ group e-donation) to electron poor (in the nitroso structure due to the -N=O group e-withdraw). The strong electron-withdrawing ability of the nitroso group (which is an even stronger electron withdrawing group than a nitro (-NO$_2$) group based on sigma values: \( \sigma_p=0.91 \) for NO and \( \sigma_p=0.78 \) for NO$_2$ [6]) is expected to give large deshielding of the ortho H, not shielding as observed. The only way to account for this ortho H chemical shift difference in these two structures is to realize the strong magnetic field shielding effect that occurs on the 'syn' side of the nitroso group.

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**Figure 2.** Facile C-NO rotation for 1 at 25° C yielding avg (syn and anti) \(^1\)H-NMR shifts at 25° C (left) and \(^1\)H-NMR shifts at -100° C where C-NO rotation is slow (left) [3]. NMR solvent is CD$_2$Cl$_2$.

**Figure 3.** Variable temperature \(^1\)H-NMR spectra of 1-chloro-4-nitrosobenzene (3) from -30 to -105° C [3].

**Figure 4.** \(^1\)H-NMR spectra of o-bromoaniline (4) and o-bromonitrosobenzene (5).

**Figure 5.** Target o-substituted nitrosobenzenes and their amine and nitro analogs.
The target nitrosoarenes of this study are shown in Figure 5 (compounds 7, 10, 13), along with their more reduced amine and more oxidized nitro analogs, whose \(^1\)H-NMR spectra are also of interest for comparison. Nitrosoarenes 7, 10, and 13 all possess a primary conformation with the \(-\text{N}=\text{O}\) group turned away from the ortho substituent, as illustrated in Figure 6. Thus, the H atoms ortho to the nitroso groups in 7, 10, and 13 are all positioned in the ‘syn’ location to the \(-\text{N}=\text{O}\) function, allowing us to observe the shielded side of the nitroso magnetic anisotropy effect. For 2-nitrosocumene (10), the preferred isopropyl group conformation will also provide a methine hydrogen NMR signal positioned on the ‘anti’ side of the oriented nitroso group as shown in Figure 6. In all cases, the nitrosoarene 1H-NMR signal chemical shifts can be compared to those for the analogous electron-rich structures (anilines 6, 9, and 12) and analogous electron-poor structures (nitroarenes 8, 11, and 14).

Figure 6. (a) The nitroso conformational preference for ortho-substituted nitrosobenzenes. (b) the preferred conformation of 2-nitrosocumene giving H atoms syn and anti to the NO group.

Materials and Methods

The nitrosoarenes 7, 10, and 13 have been synthesized by potassium monopersulfate (KHSO\(_5\)) oxidation of the corresponding aniline using OXONE as depicted in Scheme 2 for the preparation of 7 as a representative example. Aniline precursors 2-aminobenzonitrile (6), 2-aminocumene (9), and 2-aminobiphenyl (12) are all commercially available from sources listed below and were used as received. The nitro compounds 2-nitrobenzonitrile (8) and 2-nitrosobiphenyl (14) were purchased from Matrix Scientific and TCI America, respectively. NMR spectra were recorded on Bruker 360 MHz and 500 MHz spectrometers and spectra were referenced to residual proton signals of the deuterated solvent employed.

Scheme 2. OXONE oxidation of 2-aminobenzonitrile (6) to prepare nitrosoarene 7.

2-nitrosobenzonitrile (7)[7]

2-aminobenzonitrile (6) (0.5810 g, 5.0 mmol, Matrix Scientific) was dissolved in DCM (12.50 mL). OXONE (6.155 g, 10.0 mmol) was dissolved in water (20.00 mL) and added to the 2-aminobenzonitrile solution via pipette. The reaction ran for 4 hours and 10 minutes at room temperature under vigorous stirring. TLC analysis (1:3 Hexanes:DCM) was used to monitor the reaction. The aqueous layer was extracted with DCM (3x3.00 mL) and the combined organic layers were dried with MgSO\(_4\), filtered, and concentrated. The crude product was purified through a plug of silica gel (1:2 DCM:Hexane) to yield pure 7 (0.2000 g, 27\%) as a light yellow solid, \(^1\)H NMR (360 MHz, CDCl\(_3\), 25 \(^\circ\)C): \(\delta\) (ppm) 8.06 (d, \(J = 8.1\) Hz, 1H), 7.85 (t, \(J = 7.3\) Hz, 1H), 7.76 (t, \(J = 7.9\) Hz, 1H), 6.99 (d, \(J = 8.2\) Hz, 1H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\), 25 \(^\circ\)C): \(\delta\) (ppm) 161.7, 135.3, 134.4, 133.4, 121.2, 116.6, 113.5, 112.0. \(^1\)H-NMR, \(^{13}\)C-NMR, COSY, and HSQC spectral analyses were performed to fully characterize 7 and assign its \(^1\)H-NMR signals.

2-nitrosocumene (10) [10]

2-aminocumene (0.1350 g, 1.0 mmol, TCI America) was dissolved in DCM (2.00 mL). OXONE (1.231 g, 2.0 mmol) was dissolved in water (4.00 mL) and added to the 2-aminocumene solution via pipette. The reaction ran for 1 hour at room temperature under vigorous stirring. TLC analysis (1:2 DCM:Hexane) was used to monitor the reaction. The aqueous layer was extracted with DCM (3x3.00 mL) and the combined organic layers were dried with MgSO\(_4\), filtered, and concentrated. The crude product was purified through a plug of silica gel (1:2 DCM:Hexane) to yield pure 10 (0.1000 g, 67\%) as a blue oil, \(^1\)H NMR (360 MHz, CDCl\(_3\), 25 \(^\circ\)C): \(\delta\) (ppm) 7.73 (d, \(J = 14.6\) Hz, 1H), 7.69 (t, \(J = 15.8\) Hz, 1H), 7.14 (t, \(J = 7.7\) Hz, 1H), 6.11 (d, \(J = 8.0\) Hz, 1H), 5.31 (septet, \(J = 6.9\) Hz, 1H), 1.59 (s, 6H). \(^{13}\)C NMR (125 MHz, CDCl\(_3\), 25 \(^\circ\)C): \(\delta\) (ppm) 163.8, 152.7, 136.7, 128.5, 125.3, 105.4, 27.2, 24.3. \(^1\)H-NMR, \(^{13}\)C-NMR, COSY, and HSQC spectral analyses were performed to fully characterize 10 and assign its \(^1\)H-NMR signals.

2-nitrocumene (11) [10]

A solution of amine 9 (0.2704 g, 2.0 mmol, TCI America), potassium iodide (0.0166 g, 0.10 mmol), and CH\(_3\)CN (6.0 mL) was added to a rbf and allowed to heat to 80 \(^\circ\)C. 70\% aqueous tert-butyl hydroperoxide (TBHP, 0.73 mL, 7.6 mmol) was added in small portions over 30 minutes. A reflux condenser was then attached to the reaction and allowed to stir overnight. The mixture was then cooled to room temperature and quenched with 10\% KOH, washed with brine, and extracted with ethyl acetate. The organic layer was then dried with MgSO\(_4\) and concentrated \textit{in vacuo}. The crude product was purified by column chro-
matography using 1:4 ethyl acetate/hexane mixture to provide pure product (0.0020 g, 6.1 %) as a dark brown oil, 1H-NMR (360 MHz, CDCl₃): δ (ppm) 7.69 (d, J = 8.2 Hz, 1H), 7.51 (d, J = 18.6 Hz, 1H), 7.49 (t, J = 15.1 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 3.14 (septet, J = 6.9 Hz, 1H), 1.30 (d, J = 6.9 Hz, 6H).

2-nitrosobiphenyl (13) [12]

2-aminobiphenyl (0.1690 g, 1.0 mmol, Acros Organics) was dissolved in DCM (3.00 mL), OXONE (1.846 g, 3.0 mmol) was dissolved in water (13.00 mL) and added to the 2-aminobiphenyl solution via pipette. The reaction ran for 23 hours at room temperature under vigorous stirring. 1H-NMR was used to monitor the reaction. The aqueous layer was extracted with DCM (3x3.00 mL) and the combined organic layers were dried with MgSO₄, filtered, and concentrated. The crude product was purified by recrystallization with hexanes to yield pure 8 (0.1530 g, 84%) as a yellow solid, 1H-NMR (360 MHz, CDCl₃): δ (ppm) 7.78 (d, J = 14.7 Hz, 1H), 7.78 (t, J = 15.1, 1H), 7.70 (d, J = 7.7 Hz, 2H), 7.54 (3H, m), 7.36 (t, J = 7.7 Hz, 1H), 6.28 (d, J = 8.1 Hz, 1H). 13C-NMR (125 MHz, CDCl₃): δ (ppm) 163.3, 146.1, 134.2, 135.8, 132.2, 131.5, 128.3, 128.1, 127.1, 106.0. 1H-NMR, 13C-NMR, COSY, and HSQC spectral analyses were performed to fully characterize 13 and assign its 1H-NMR signals.

Results and Discussion

To elucidate the 1H-NMR signal assignments of all the H atoms of the nitrosoarenes 7, 10, and 13, a complete set of 1D (1H-NMR and 13C-NMR) and 2D (COSY, HSQC, and HMBC) [8] NMR experiments have been performed on these structures. The traditional 1H-NMR and 13C-NMR data give NMR signals for all chemically different sets of H's and C's in the structure, along with splitting and integration information which helps make the signal assignments to the atom positions in the structures. To prove the bonded connectivity of the atoms giving rise to the signals, 2D (correlated) NMR experiments are performed. An example of these data are provided for nitrosoarene 7 in Figures 7 (COSY spectrum), 8 (HMOC spectrum) and 9 (HMBC spectrum). The COSY spectrum of Figure 7 shows 3-bond couplings (J values) which provides information about H's on adjacent C atoms, i.e. those which are 3 bonds apart. Compound 7 has 4 H's (a-d). The circled cross peaks in Figure 7 indicate H atom signals for H atoms on adjacent (bonded) CH groups in 7. Hα shows a cross peak with Hb, indicating that they are on adjacent C's and so forth. Further analysis of the COSY spectrum shows that the H positions around the ring are have the sequence of either a,b,c,d or d,c,b,a.

Figure 7. COSY spectrum for 7 including crosspeaks of interest.

An HSQC analysis is a 1H,13C inverse NMR experiment that allows identification of the carbon directly attached to the protons already assigned from the COSY spectrum. The HMOC spectrum (Figure 8) correlates the one-bond coupled (1J) 13C atoms and attached H's, in this case identifying the four CH groups of nitrosoarene 7. From the Figure 8 data, we can assign the C's (A-D) bearing the associated H atoms (a-d).

Figure 8. 13C-HSQC spectrum of 7 showing important cross-peaks.

To complete the assignment, another heteronuclear 2D correlation spectrum is recorded, the HMBC, which show 3 bond -C.H couplings. What remains to be established is the identity of the three quaternary C's (Cα, Cβ, and Cγ) and their ordering with respect to the CH-CH-CH(CH) segment. The Figure 9 HMBC data shows a single J coupling between Cα, and Hα, which proves the connectivity of all carbon atoms of the ortho-disubstituted benzene 7 as labeled in Figures 7-9. Thus, the cyclic connection around the benzene ring of compound 7 is shown unequivocally to be (-C(NO)-C(CN)-CHd-CHc-CHb-CHA-). The H atom signal assignment for 7 now allows for an analysis of the nitroso magnetic effect in this structure.
Figure 9. HMBC spectrum for Compound 7.

Figure 10 compares the $^1$H-NMR spectra for the ortho-cyano aniline (6), ortho-cyano nitrobenzene (8), and the target of interest ortho-cyano nitrosobenzene (7). The $H_a$ proton (Ha) for nitrosobenzene 7 is the most shielded of the group appearing at 7.0 ppm as compared to the other electron deficient nitroarene 8, for which this same Ha signal is the most deshielded (8.4 ppm) due to its electron deficiency. Clearly the syn environment of the $-N=O$ group has shielded the Ha proton by at least 1.4 ppm.

A corresponding comparison of amino, nitro, and nitroso-substituted cumenes (compounds 9, 11, and 10,) is shown in Figure 11. In this case, one can gauge the magnetic environment of both the syn and anti locations relative to the $-N=O$ group by monitoring the chemical shifts of Ha (syn) and He (anti) in this series. The methane He signal moves to higher ppm (is deshielded) by 0.5 ppm upon change of the e-donor amino group of 9 to the e-acceptor nitro group of 11, as expected because the ring is becoming more electron deficient. What is especially dramatic is the further deshielding shift of the He signal by +1.9 ppm (from 3.4 to 5.4 ppm) when the similarly e-acceptor nitroso group effect in 10 as compared to the electronically similar nitro structure 11. At the syn position, a strong shielding effect on Ha of -1.6 ppm (from 7.7 to 6.1) is observed when comparing nitro compound 11 to nitroso compound 10. Thus the spectra of Figure 11 offer a dramatic illustration of large magnetic shielding syn to the oriented nitroso group and strong magnetic deshielding anti to nitroso group.

Finally, the $^1$H-NMR spectra of the amine, nitro, and nitroso biphenyl structures 12, 14, and 13, respectively, are displayed in Figure 12. The syn proton Ha of the nitro biphenyl 14 appears at $\delta = 7.9$ and is strongly shielded in the corresponding nitroso biphenyl 13, appearing at $\delta = 6.25$ ppm (+1.65 ppm shift).
In summary, we have shown that by employing nitrosobenzene structures with ortho substituents, it is possible to orient the -N=O group so as to distinguish the syn and anti environments near this strongly magnetically anisotropic moiety. By comparing H syn and Hanti chemical shift values to the electronically similar nitroarene structures, one observes large shielding effects syn to the nitroso group and large deshielding effects at the location anti to the nitroso group. This is consistent with the low temperature NMR report by Fletcher but in these cases, room temperature NMR experiments may be used to evaluate degree and direction of the magnetic anisotropy of the nitroso group in the nitrosoarenes of this study.

References


"Where are you from? Where did you go to school? Why did you choose your major? How did you end up at UA?"

I am originally from Poland. I obtained my PhD in pharmaceutical sciences at the Medical University of Lublin in Poland. I did my post-doctoral training at the National Institute on Aging in Baltimore, where I did research in Drug Discovery Section of the Laboratory of Clinical Investigation.

I’ve been interested in natural products and natural medicines since high-school and dreamt of becoming the Potions Professor at the Hogwarts School of Witchcraft and Wizardry. I successfully applied for the position of an assistant professor of pharmacology and natural product discovery at the University of Alabama. I have my own lab at the UA where together with my students we are looking for natural compounds that can be potentially used for the prevention and treatment of aging-associated diseases.

What interests you about plant metabolism-based drugs? What do you hope to achieve in your research?

This may be a surprise for many of the readers that approximately 70% of currently approved drugs were first identified in natural samples, just to mention: aspirin (acetylsalicylic acid), morphine, codeine, paclitaxel, and many others. 2015 Nobel Prize in Medicine or Physiology was awarded for the discovery of natural compounds: artemisinin and ivermectin. Plants produce numerous secondary metabolites that they use to protect themselves from different forms of abiotic and biotic stresses. Plants can’t escape from herbivores but they can produce compounds which will prevent herbivores from eating these plants. Secondary metabolites usually impact evolutionary conserved cellular signaling pathways, that humans share with insects attacking the plants. Therefore, secondary plant metabolites impact numerous biological processes in human body and constitute perfect source of potentially useful drug templates.

In my lab we focus on the identification of compounds that can be useful in the prevention and treatment of neurodegenerative diseases. The big goal of my lab is to develop a library of natural compounds that can be potentially used as new drug leads.

What are some pharmacological procedures/ tests that you regularly run in your lab?

Identification of pharmacologically active compounds in plant extracts is a very challenging task. It is very difficult to “fish out” biologically active molecules from hundreds or thousands of secondary metabolites present in natural mixtures. In my lab we design and develop our own bioassays that help us to identify new potential drugs. For example, we are able to immobilize fully functional transmembrane proteins and create affinity chromatography columns, that we further use to screen natural mixtures for compounds binding to these transmembrane targets. Just recently, together with Dr. Yuping Bao, from the Department of Biological and Chemical Engineering we are trying to develop a new way of discovering new potential drugs from plant extracts, that involves the use of nanoparticles.
Where do you see you and your research headed towards in the future?

I would like to develop a strong and successful drug discovery program here at the University of Alabama. As previously mentioned the big goal of my research program is to develop a library of natural compounds that could be potentially used as new drug leads in the prevention and treatment of neurodegenerative diseases. I hope this program attracts many ambitious and talented students willing to work with me in my “potion lab”. I wish one day my team discovers compounds that will help millions of people suffering from debilitating diseases like Alzheimer’s or Parkinson’s disease.

What has been most rewarding in your research experience?

I think that one of the most rewarding experiences is to see your students growing to become independent researchers. I love sharing my passion for science with others and nothing makes me happier than seeing students eager to solve scientific problems. I jokingly call this process “scientific inception”. Maybe one day they have a brilliant idea that might have been initiated by discussion that we had in the lab. So I will be indirectly involved in possible solutions that these guys may come up with. I have mentored several students in my career so far and sharing with them their first “Eureka” moments is definitely one of the most rewarding experiences in my career as a researcher.

What do you find most undervalued about herbal medicine? Do we not appreciate natural remedies in the 21st century?

I think that many people do not really fully understand the concept of modern natural medicines, that we study in my lab. Unfortunately, not many health professionals get training in the area of drug discovery so many consider natural compounds as the alternative approach to modern western medicine. First of all, we do not look for alternative solutions to what is known as western medicine. We focus on identification of individual, natural compounds that can be potentially used as drugs. As I previously mentioned, many of the currently approved drugs were first identified in nature. Herbs constitute an invaluable source of pharmacologically active compounds. Secondary plant metabolites evolved as pharmacologically active molecules to impact various biological processes in herbivores and pollinators. Therefore, these metabolites are nature-designed medicines that influence evolutionary conserved mechanisms in human bodies.

What advice would you give a student interested in pursuing this kind of medicine?

I would definitely encourage students, interested in medicine or drug discovery to potentially focus their career on natural compounds. My advice would be to follow the example of Dr. Youyou Tu, who studied ancient Traditional Chinese Medicine manuscripts in her search for a potent antimalarial drug. Dr. Tu was awarded the Nobel Prize in Physiology or Medicine for the discovery of artemisinin in 2015. Studying ethnobotanical literature as a means to identify possible sources of new drug leads may be a good start in the identification of potential sources of new medicines.

Dr. Lukasz Ciesla was interviewed by two of JOSHUA’s editors, Kylie Surgot and Michelle Tan.

Image via UA Department of Biological Sciences.
In Memoriam: David E. Nikles

Elaine Hatfield

David “Dave” Eugene Nikles, born July 3rd, 1954, passed away at home on Sunday, March 19th, 2018. He is survived by his wife Jackie, daughter Sarah, son Daniel, mother Lucille Nikles, and siblings Tim Nikles (Jeanne Nikles), Laura Nikles (Denise Bell), and Sue Nikles Moore (Tim Moore).

Dave attended the University of Akron, where he received his Bachelors of Science in Chemistry in 1977. He went on to attend Case Western Reserve University, earning his PhD in Inorganic Chemistry in 1982. After graduating, he became a staff scientist at Hoechst Celanese Research Division in Summit, New Jersey, where he worked from 1982 to 1990. In August of 1990, he joined the faculty of The University of Alabama, and remained active in education and research until his passing. During his tenure, he served as Associate Director for the Center for Materials for Information Technology and as Director of the Central Analytical Faculty. He had been a member of the American Chemical Society for 40 years, and an active member of the National Information Storage Consortium.

While Dave loved interacting with students at the University, one of his greatest delights was coaching youth soccer. This love grew from a need for coaches in the YMCA youth soccer program, in which his son Daniel played. After many years of coaching with the YMCA, he earned several coaching licenses, allowing him to coach travel club teams and JV soccer at Paul W Bryant High School, where both of his children attended and Daniel played. He ultimately joined the PARA Foundation’s developmental soccer league and had been coaching enthusiastic 8- and 9-year-old children for the past several years. Dave’s lasting impact is evident in the numerous students he mentored. A few of his current and former student offered their words on his effect on their lives:

"Dr. Nikles was an incredible mentor and friend who was the embodiment of perseverance and passion. He displayed unequivocal love for research and for the education of his students and community and has left a lasting impression on his lab and the University of Alabama as a whole."
- Sudarsan Murali (Pre-med, UA)
In Memoriam: David E. Nikles

“He was so amazingly patient with me when I started off and had no idea what was going on, and he wanted nothing more than to help me succeed, no matter what I desired to do. He believed in me even when I doubted myself and helped me to figure out what it was I passionate about in the chemical field. I am beyond grateful to have had the privilege to know him and to have worked alongside him!” – Mikaela Armstrong (Biochemistry, UA)

“Dr. Nikles was an incredible mentor, teacher, and friend. I couldn’t have chosen a better professor to research with for the past three years. He was one of the most selfless men I have ever known and was ready to help anybody at a moment's notice. I only wish that I could have had more time to spend with him; he will truly be missed.” – Mitch Clayton (Chemical Engineering, UA)

“Dr. Nikles loved soccer and listening to oldies music. Stepping into his office was the chance to step into his life. Carefully tended flowers perched under the window on a stack filled with material constants and tables. Soccer trophies sat proudly next to patents and academic awards. Row upon row of lab notebooks spoke to the years of time, work, and thought he devoted to science and research. But those notebooks were also filled with the names of the students he mentored, and the ideas and ambitions he fostered and encouraged. Dave Nikles was a brilliant scientist whose door was always open, and who generously shared his wisdom and experience with all of his students. Over the course of my three years working with him, he taught me to be an independent researcher and thinker. All of my success has stemmed from his dedication to teaching and research. Dr. Nikles will be dearly missed, but I know that his memory and hard-work will be continued by all his students.” – Rachel Kress (PhD student, RU)

“I best remember Dr. Nikles not for his incredible research, but for his passion for sharing that research with students. During any given semester, and most summers, you would find his lab full of high school and undergraduate students being taught to perform their first experiments and conducting novel research. Dr. Nikles was the type of mentor that not only gave you room to explore and grow, but motivated you to do so. I was fortunate to work with Dr. Nikles over a period of seven years throughout high school and college. His positive effect on my career is exceeded only by his impact on how I see the world. There are people in everyone's lives who help shape who they are, and Dr. Nikles is one of those people for me. He will be missed greatly, and he leaves behind a space that will be difficult to fill.” -Adam Beg (Medical Student, UAB)

“Dr. Nikles was something of a scientific father to me. I will always remember his taking time away from his family to help me write the reports for the high school science competitions, and then taking me again under his wing during my undergraduate years. The greatest lesson he taught me was to be unafraid in trying new things, and that discovery does not often visit the timid at heart; it has served me well everywhere I have been. Most importantly than the professional aspects, however, was that he was my friend, and I always knew I could seek him out for life advice. Without Dr. Nikles’ mentorship, I can honestly say I would probably not be an engineer, much less sitting in this chair writing this at the University of South Alabama. I truly stand on the shoulders of a giant, and I will greatly miss him.” -Dr. Gregory Poole (Assistant Professor of Mechanical Engineering, USA)
In 1982, when Julia Tutwiler proposed to the University of Alabama’s board of trustees that women should be allowed admission into the university, she changed the course of UA’s history in a meaningful way. Now the university is taking advantage of the 125th anniversary of the acceptance of women, for which Tutwiler helped make possible, by recognizing the accomplishments of women that have come before us here at UA, and for all that is to come of our present and future graduates.

In 1893, the first year women were allowed admittance to the university, only two women were enrolled. Now 56% of our 38,563-person strong student body are women. What a difference the time has made. The women of our campus are making huge impacts within every reach of our community, bringing their touch to every college, department, and discipline as well as to other states and countries. The STEM fields have been historically male dominated throughout the entire US, and though women are still within the minority, their numbers within these fields are growing, and their influence is apparent.

Heather Willauer, a chemist who invented a method for synthesizing jet fuel from seawater, and Marillyn Hewson, the President and Chief Executive Officer of Lockheed Martin are just a couple of University of Alabama graduates that exemplify the influence of UA women on the STEM fields. Additionally, our current women students are increasingly present in research initiatives across campus. The Fulbright, a prestigious international scholarship program that is awarded to individuals who desire to teach or complete research abroad, was awarded to 11 UA students last year, over half of which were women. Furthermore, of the 21 students awarded the Randall Research award this year which recognizes the best research activity by undergraduates on campus, 11 were women.

Different student organizations on campus have been formed in order to support, empower, prepare, and educate the increasing number of women who want to enter different STEM fields. Some of these organizations include the Society of Women in Medicine, the Society of Women Engineers, Women in Technology, League of Women Coders, and Association for Women in Science. It is wonderful to see how women on UA’s campus are teaming up to help each other succeed in all of their goals, and their engagement, dedication and prowess will undoubtedly lead to the brightest of futures.
2019 JOSHUA Submission Guidelines

We accept articles from current undergraduate students at The University of Alabama (UA). If you are a graduate student or recent alumnus of UA, we will consider your article if the majority of your work was conducted while you were an undergraduate at UA. Undergraduate students from other institutions may submit; however, priority will be given to those who conducted their research at UA.

1. Your name, e-mail address, and phone number must be included.

2. Your submission must relate to science or health.

3. Your work must be sponsored by a faculty member.

4. The length of your submission must be between 2000 and 4500 words. We will accept longer submissions if the author can limit the submission to the required length for the publication, and any extra material is able to be published online.

5. Figures, charts, and graphs are allowed but not required. (Note: The final printed color will be mostly black and white.)

6. Your paper must contain an abstract.

7. Your citations must follow the guidelines listed on our website at JOSHUA.UA.UA.EDU.

8. The deadline for submission is in February 2019.

9. E-mail submissions to joshua.alabama@gmail.com
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