Original Article

Adverse Geriatric Outcomes Secondary to Polypharmacy in a Mouse Model: The Influence of Aging

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Received December 17, 2014; Accepted March 28, 2015

Decision Editor: Placido Navas, PhD

Abstract

We aimed to develop a mouse model of polypharmacy, primarily to establish whether short-term exposure to polypharmacy causes adverse geriatric outcomes. We also investigated whether old age increased susceptibility to any adverse geriatric outcomes of polypharmacy. Young (n = 10) and old (n = 21) male C57BL/6 mice were administered control diet or polypharmacy diet containing therapeutic doses of five commonly used medicines (simvastatin, metoprolol, omeprazole, acetaminophen, and citalopram). Mice were assessed before and after the 2- to 4-week intervention. Over the intervention period, we observed no mortality and no change in food intake, body weight, or serum biochemistry in any age or treatment group. In old mice, polypharmacy caused significant declines in locomotor activity (pre minus postintervention values in control 2 ± 13 counts, polypharmacy 32 ± 7 counts, p < .05) and front paw wire holding impulse (control −2.45 ± 1.02 N s, polypharmacy −1.99 ± 1.19 N s, p < .05), loss of improvement in rotarod latency (control −59 ± 11 s, polypharmacy −1.7 ± 17 s, p < .05), and lowered blood pressure (control −0.2 ± 3 mmHg, polypharmacy 11 ± 4 mmHg, p < .05). In young mice, changes in outcomes over the intervention period did not differ between control and polypharmacy groups. This novel model of polypharmacy is feasible. Even short-term polypharmacy impairs mobility, balance, and strength in old male mice.

Polypharmacy (use of five or more different medicines) is prevalent in older adults (1,2). In population-based studies, 52% of people older than 80 years in Scotland (2) and 66% of those aged older than 75 years in Australia (3) use five or more medicines, and in nursing homes in the United States, 40% of residents use nine or more medicines (4). This is a consequence of the increase in multimorbidity with aging, for which multiple medicines may be prescribed (2,5). In observational studies, polypharmacy is associated with increased risks of adverse outcomes and geriatric syndromes in older people including hospitalization, institutionalization, falls, incontinence, cognitive impairment, and even death (6). In a population of Australian community-dwelling older men, we found that polypharmacy at baseline more than doubled the risk of incident frailty after 2 years (7). Some studies suggest that these associations are due to confounding by the indications for the medicines (8,9). Others suggest that the type of medicines rather than the number of medicines drives the association with adverse outcomes such as falls, functional impairment, and frailty (7,10,11). As the number of medicines taken increases, the risk of both predictable and unpredictable adverse outcomes increases combinatorially.

Not only are older adults at increased risk of receiving polypharmacy, but the biophysiological changes of old age alter susceptibility to the effects of medicines (12–14). Changes in body composition and hepatic and renal clearance impact on pharmacokinetics, increasing exposure to most drugs and altering the metabolites formed, with impacts on toxicity (13). For example, in old age, changes in drug disposition and metabolism of acetaminophen result in less hepatotoxicity and more nephrotoxicity (15–17). Age-related changes in specific receptors as well as changes in physiological reserve affect pharmacodynamics (18). Polypharmacy, especially given these age-dependent changes in pharmacokinetics and pharmacodynamics, dramatically increases the risk of drug interactions (19), yet most
drug interaction studies focus only on two medicines. Therefore it remains uncertain whether polypharmacy per se is harmful, and if polypharmacy is harmful, what the size of that harm might be in different age groups.

It is not ethical or feasible to use randomized controlled trials to rigorously test the effects of multiple concurrent medicines on geriatric outcomes in highly heterogeneous populations of older people. Observational studies are limited by the wide range of exposures to different combinations of medicines and doses, by residual confounding by different types and severity of comorbidities, and by confounding by environmental factors like diet and exercise (20).

Mouse studies are a routine part of the preclinical testing of the toxicity of individual medicines where the risk and degree of harm of a medicine can be ascertained in the absence of underlying disease. This type of approach could also be utilized to investigate the causality and mechanisms for the association between polypharmacy and adverse geriatric outcomes: frailty, physical and cognitive function, and survival. Preclinical testing of polypharmacy is now possible with the development of animal models of geriatric outcomes, for example, a Mouse Clinical Frailty Index that is comparable to the Frailty Index in humans (21–23) can be used to assess frailty; locomotor activity in an open field is used to measure physical activity; the rotorod test is used to measure gait speed and falls risk (24); and a front paw wire hang is used as a surrogate test for grip strength (25). We recently evaluated the effects of a range of anti-aging interventions, including the drug resveratrol, on Mouse Clinical Frailty Index in aging mice (26). The other tests of physical function have been used to evaluate the effects of some single drugs in toxicity studies in young animals (eg (27–29)), and in longevity studies in aged animals (30,31). To our knowledge, there are no previous animal studies evaluating the effects of polypharmacy on geriatric outcomes.

We aimed primarily to develop a mouse model of polypharmacy and to establish whether short-term exposure to polypharmacy causes adverse geriatric outcomes in young or in old mice. Our secondary aim was to investigate whether old age increased susceptibility to any adverse geriatric outcomes caused by polypharmacy.

Methods

Animals

The polypharmacy model was established in young (14.1 ± 0.0 weeks, n = 10) and old (99.2 ± 0.2 weeks, n = 21) C57BL/6 male mice. This strain is standard in drug development, preclinical toxicology (32), and in studies on ageing (25) and was also used to validate the Mouse Clinical Frailty Index (22). Healthy C57BL/6 male mice were obtained from and housed at the Kears Labs, Ferring Institute of Medical Research, Sydney, Australia. Animals were maintained on a 12-h light–dark cycle, housed in boxes of up to four (separated if fighting), given cardboard rolls for stimulation, and had ad libitum access to food (23% protein, 6% fat, 5% fiber, Rat and Mouse Premium Breeder Diet, Gordon’s Specialty Stockfeeds, Yanderra, NSW, Australia) and water. The study was approved by the Northern Sydney Local Health District Animal Care Ethics Committee, Sydney, Australia.

Drug Regimens

The medicines for the polypharmacy regimen were selected based on drug classes most commonly used by older Australians (3), which are not likely to be toxic when given alone to a healthy mouse based on previous animal studies (27–30,33,34), have similar pharmacokinetics and pharmacodynamics in mice and humans (35–39), and are not routinely dose-adjusted in old age (40). Many different combinations of medicines are seen in clinical practice and these exposures represent common treatments of common comorbidities in older people. Doses were taken from previous studies of long-term administration of these medicines as monotherapy to mice, based on an anticipated food intake of 0.18 ± 0.05 g food/g mouse/day (41).

Based on the above-mentioned principles, the polypharmacy regimen we designed was a lipid-lowering agent for prevention and treatment of cardiovascular disease (simvastatin 350 mg/kg/day) (42), a proton pump inhibitor for treatment of gastroesophageal reflux disease (omeprazole 10 mg/kg/day) (33), a simple analgesic (acetaminophen 100 mg/kg/day) (43), and a selective serotonin reuptake inhibitor antidepressant (citalopram 10 mg/kg/day) (44). Studies of pathology of aged C57BL/6 mice (45) suggest that the cohort of mice in this study was unlikely to have conditions that would benefit from these medicines. Control feeds and feeds containing all five drugs (polypharmacy) contained 20% protein, 4.8% fat, and 59.4% carbohydrate (Standard Meat Free Mouse and Rat Feed, Specialty Feeds, Western Australia, Australia) and were manufactured by Specialty Feeds to our specifications. First, all mice were administered control feed for at least 2 weeks. At the end of this period, baseline assessments were performed. Then mice were administered control feed or polypharmacy feed containing five drugs for 2 weeks to achieve steady state. Young mice were randomly assigned to treatment groups. Old mice were stratified by Mouse Clinical Frailty Index (22) at baseline to ensure similar frailty in each treatment group.

Assessment

Three times a week for each cage, food and mice were weighed and fresh food was given. Food intake (g food/g mouse/day) was calculated. Throughout treatment, mice were observed as per Animal Care Ethics Committee guidelines for signs of distress and for survival.

At baseline and after 12 and 14 days of treatment (for young and old cohorts respectively), mice were assessed for body weight, blood pressure, and physical function. Old mice were also assessed using the Mouse Clinical Frailty Index (22) by an assessor blinded to their treatment group. Mice were maintained on the assigned diet throughout the postdrug testing period, which lasted up to 2 weeks. Upon completion of testing, mice were euthanized (using ketamine and xylazine) and blood samples were taken. Serum liver function tests, creatinine, albumin, and calcium were measured using an Abbott Architect Clinical Chemistry Analyzer by a National Association of Testing Authorities–accredited hospital laboratory, PaLMS (Pacific Laboratory Medicine Services) at Royal North Shore Hospital (Sydney, Australia).

Blood pressure was measured by the tail cuff method using the CODA Non-invasive Blood Pressure System (Kent Scientific Corporation, CT, USA). Measurements were always carried out at the same time of the 12-h light cycle.

For rotorod latency, a measure of motor performance, all experiments were done under white light and mice were acclimatized to the room for 30 min before testing. The rotorod device gradually accelerated from 4 to 40 rpm over 300 s (Ugo Basile, Varese, Italy). Each test session consisted of three trials with a 30-min rest period between each trial. The first and second trials included a habituation phase for 60 and 20 s, respectively, during which the drum rotated at a fixed speed of
For the open field test, mice were acclimatized to the room for 30 min before testing. Each animal was placed in the middle of an open topped, perspex enclosure (50 x 50 x 50 cm) and activity was recorded with a camera situated above the arena (49). Activity of young mice was evaluated for 2 min and old mice, which are less ambulant, were assessed for 5 min, consistent with previous studies (50). The floor and walls of the arena were cleaned with 70% ethanol between each mouse. For analysis, the floor area was divided into a 4 x 4 grid (12 peripheral and four central squares) and the number of line crossings by the hind paws was counted. The number of times that a mouse reared up on its hind legs (on- and off-wall) was also counted. Locomotor activity of each mouse was assessed by the sum of line crossings and rearings (49).

The Mouse Clinical Frailty Index, as developed by Whitehead and colleagues (22) is a simple, noninvasive clinical assessment method for the quantification of frailty. It takes into consideration 31 parameters to give an overall score of frailty. In our study, frailty testing was carried out on old mice by an experienced, trained observer (AK). Old mice were allocated to each test group (control or polypharmacy) based on their baseline Frailty Index, with each group having a similar mean Frailty Index and a similar range of Frailty Indices at baseline.

Results
The polypharmacy regimen was tolerated by young and old mice, with no change in food intake or body weight, and no mortality observed over the study period for any age or treatment group (Table 1). Serum liver function tests, creatinine, and calcium were not significantly affected by the medication regimens. Mice in all groups consumed the lower end of the range of food anticipated, resulting in mean doses of approximately simvastatin 14 mg/kg/day, metoprolol 245 mg/kg/day, omeprazole 7 mg/kg/day, acetaminophen 70 mg/kg/day, and citalopram 7 mg/kg/day.

The change in outcome with treatment was calculated for each mouse and averaged within each age and treatment group to describe the effects of polypharmacy compared to control diet (Figure 1). Among old mice, compared to those treated with control diet, those treated with polypharmacy had a significantly greater impairment in performance on locomotor activity (Figure 1A), rotarod latency (Figure 1C), and front paw wire holding impulse (Figure 1E); no significant difference in change in Mouse Clinical Frailty Index (Figure 1I); and a significantly greater drop in blood pressure (Figure 1G). For young mice, the change in all outcomes over the treatment period did not differ between those receiving the control and polypharmacy treatments (Figure 1B, D, E, H). On two-way ANOVA, there was no statistically significant interaction between age and treatment for any outcome.

Figure 2 shows the mean results for locomotor activity, rotarod latency, and front paw wire holding impulse in each age and treatment group. Two-way mixed ANOVA analysis showed no significant interaction between time and treatment group for any outcome in the young mice (Figure 2A). In old mice, there was a significant interaction between time and treatment for mean front paw wire holding impulse (F[1,19] = 6.6, p = .019), rotarod latency (F[1,19] = 6.1, p = .023), mean locomotor activity in the open field (F[1,19] = 4.6, p = .045), but not mean Mouse Clinical Frailty Index. Plotting the mean values showed that in old mice polypharmacy reduced mean locomotor activity in the open field, but mean rotarod latency and mean front paw wire holding impulse increased over the study period in the old controls but not in the old polypharmacy group (Figure 2B). Over the intervention period, mean Mouse Clinical Frailty Index did not change significantly in the old control group (0.19 ± 0.03 pre, 0.18 ± 0.03 post) or in the old polypharmacy group (0.16 ± 0.01 pre, 0.19 ± 0.02 post), but there was a significant between-group difference in mean Mouse Clinical Frailty Index across the study period (F[1,19] = 6.6, p = .019).

Analysis
The study was powered to detect a significant difference in locomotor performance and rotarod latency in old mice after treatment with polypharmacy compared to control. We estimated that the size of a clinically relevant negative effect from polypharmacy would be similar to that of the positive effects on health span seen with metformin (31). If the variability was similar to this study, we would require a sample size of five per group to detect a difference of 0.008 m/min in locomotor performance and a difference of 30 s in rotarod latency with power of 80% and alpha of 0.05. In view of the greater interindividual variability in pharmacology of multiple drugs in old age, we estimated a sample size of 12 for the old animals treated with polypharmacy to detect a clinically relevant difference.

The change in each outcome over the intervention period was measured for each animal. The mean change in each outcome was calculated for each age and treatment group. To investigate the primary aim, whether polypharmacy affected geriatric outcomes in old or in young mice, the mean change in each outcome was compared between old control and old polypharmacy-treated mice and between young control and young polypharmacy-treated mice using the Student’s t test. To determine the relative susceptibility of young and old mice, the change in each outcome over the study period was compared between both age and treatment groups using two-way ANOVA to measure the significance of the interaction between age and treatment. Open field results were normalized to 2 min for both age groups for this two-way ANOVA. To further describe the outcome data, descriptive statistics were used to characterize the mean values for each age and treatment group, before and after treatment. A two-way mixed ANOVA analysis was used to determine the effect of time (within-subjects factor) and treatment (between subjects factor), within each age group. To determine the effect of age on outcomes pre- and post-treatments, a three-way mixed ANOVA was used to measure the significance of the interaction between age, treatment, and time. Open field results were normalized to 2 min for both age groups for the three-way mixed ANOVA. All data are presented as mean ± standard error of the mean.
Figure 1. Change in outcomes from baseline to after the intervention period with control or polypharmacy diet in old and young male C57BL/6 mice. (A) Locomotor activity old; (B) locomotor activity young; (C) rotarod latency old; (D) rotarod latency young; (E) front paw wire holding impulse old; (F) front paw wire holding impulse young; (G) systolic blood pressure old; (H) systolic blood pressure young; and (I) Mouse Clinical Frailty Index old. The difference between baseline and postintervention measures was calculated for each mouse and presented as the mean (±SEM) change (result at baseline minus result after intervention) for each age and treatment group. For locomotor activity, rotarod latency, and front paw wire holding impulse, higher values indicate better function and therefore a positive value for change indicates a decline in function over the intervention period. For Mouse Clinical Frailty Index, higher values indicate more severe frailty and therefore a negative value for change indicates a decline over the intervention period. Numbers in each group: old control, \( n = 8 \); old polypharmacy, \( n = 13 \); young control, \( n = 5 \); and young polypharmacy, \( n = 5 \). C, control diet; P, polypharmacy diet; * \( p < .05 \) for comparison of the change between baseline and postintervention measures of an outcome, within an age group.

Table 1. Characteristics of young and old mice administered control and polypharmacy diets

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control diet</th>
<th>Polypharmacy diet</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 2 weeks</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>Young</td>
</tr>
<tr>
<td>Number of mice</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Food consumption (g/kg/day)</td>
<td>(0.131 ± 0.004)</td>
<td>(0.114 ± 0.009)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>(25.4 ± 0.4)</td>
<td>(26.3 ± 0.6)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>(150 ± 3)</td>
<td>(153 ± 5)</td>
</tr>
<tr>
<td>Serum alanine aminotransferase (U/L)</td>
<td>N/A</td>
<td>(40.6 ± 7.6)</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>N/A</td>
<td>(26.8 ± 0.4)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>N/A</td>
<td>(34.0 ± 1.1)</td>
</tr>
<tr>
<td>Serum calcium corrected (mmol/L)</td>
<td>N/A</td>
<td>(2.6 ± 0.0)</td>
</tr>
</tbody>
</table>

*Serum calcium results were obtained for four control and nine polypharmacy old mice. BP, blood pressure; N/A, not applicable.
On three-way mixed ANOVA, there was no statistically significant interaction between time, age, and treatment for any outcome.

**Discussion**

In this study, we developed a mouse model of polypharmacy to establish whether short-term exposure to polypharmacy causes adverse geriatric outcomes. The polypharmacy regimen for the intervention period was tolerated by young and old mice, without standard signs of toxicity such as mortality or abnormal serum measures of hepatic or renal function (Table 1). In old mice, 2–4 weeks of polypharmacy resulted in significant declines in locomotor activity, rotarod latency, and front paw wire holding impulse, as well as lower blood pressure (Figure 1). In young mice, outcomes were not significantly affected by polypharmacy, with similar changes in outcomes observed in the control and polypharmacy groups over the treatment period (Figure 1). In this small sample, there was no statistically significant interaction between age and treatment on geriatric outcomes.

To our knowledge, none of the drugs in the polypharmacy regimen has been shown to impair functional outcomes when used as monotherapy at the administered doses in rodents, although there is limited data from old mice. Lifelong simvastatin has been shown to have no effect on functional outcomes in old mice (30). Acetaminophen at doses from 25 to 300 mg/kg has no effect on rotarod performance in young rats with neuropathic pain (29) and its effects on function in old rodents are unknown. Metoprolol administered for 28 days to young rats does not result in central nervous system depression at doses from 300 to 750 mg/kg, but does cause mild depression at 1000 mg/kg, which is four times higher than the dose used in the polypharmacy diet (28), with no reports of its effects in old mice. In young mice, cilostazol does not affect rotarod performance at the 10-mg/kg dose used in our polypharmacy regimen (27), but does impair performance on the rotarod at a much higher dose (100 mg/kg) (27), and its effects on physical function have not been evaluated in old mice. The effects of omeprazole on physical function in rodents are not well documented in any age group. The results of this study demonstrate clearly that polypharmacy with drug doses previously shown to have no effect on performance individually in young mice impairs physical function in old but not in young mice.

The strengths of this study are the selection of clinically relevant treatment regimens and rigorous collection of validated outcomes that are standard to studies of pharmacology, toxicology, and aging (22,25,32). The geriatric outcomes can be translated to comparable measures in humans (22,25). Furthermore, the physical impairments seen with polypharmacy in old mice are consistent with results of observational studies in older adults (6), such as the association of polypharmacy with reduced 6-min walk distance (51) and impaired balance (52).

Further studies should assess both pharmacokinetic and pharmacodynamic factors to determine the mechanisms of the observed impairments in physical function.
effects of polypharmacy on functional outcomes in old age. It is possible that the intervention period (2 weeks) may have been too short in order to observe the full effects of drug therapy on outcomes. The Mouse Clinical Frailty Index (22) contains many items that may take longer than 2–4 weeks to change, such as those relating to the integument and musculoskeletal systems. Larger studies may be required to more precisely describe the effects of polypharmacy and to determine whether there is a significant interaction between age and treatment effect. Although we did not detect an interaction between age and treatment, if these studies had only been performed on young and not on old animals, it would have appeared that there was no effect of polypharmacy on geriatric outcomes. This finding supports the need to study exposures of the medicines that are used in older people in old animals (14). Future experiments should also investigate the effects of polypharmacy in middle age (12 months) as well as in the very old (30 months) to determine changes in susceptibility through the lifespan (53).

Studies of long-term treatment from middle to old age will be better able to determine the functional risk of chronic polypharmacy in old age.

Future studies are needed to investigate the generalizability of this study. The results reported here may be specific to the medicines selected. Further studies are required to elicit the effects of polypharmacy regimens containing varying classes and doses of medicines. Although our results are consistent with those of observational studies in male and female older adults, our experiments used only male C57BL/6 mice. Studies in different sexes, strains, and species may differ due to strain and species differences in pharmacology in old age (32,54) and in aging itself (53,55).

In humans, it is important to evaluate the risks of a medication against the potential benefits of therapy, on geriatric and other outcomes. This assessment is complicated by multiple factors such as age, sex, multimorbidity, and polypharmacy. In this study, we present a model for polypharmacy in healthy young and old mice. The toxicological information gained from studies in healthy animals provides valuable translational data to inform clinicians of the nature and magnitude of the impairments in geriatric outcomes caused by polypharmacy.

In conclusion, in this study we present a novel model of polypharmacy, which is feasible and shows that short-term treatment significantly impairs mobility, balance, and strength in old C57BL/6 male mice. This preclinical model can be used to inform clinicians of the adverse effects of polypharmacy and to test the effects of new medicines on geriatric and other outcomes in the setting of polypharmacy, which is feasible and shows that short-term treatment significantly impairs mobility, balance, and strength in old C57BL/6 male mice. This preclinical model can be used to inform clinicians of the adverse effects of polypharmacy and to test the effects of new medicines on geriatric and other outcomes in the setting of polypharmacy, which is the condition under which medicines are commonly used in older adults.

Funding
This study was funded by the Penney Ageing Research Unit, Royal North Shore Hospital, Australia. Rafael de Cabo and Sarah Mitchell are supported by the National Institute on Aging, National Institutes of Health.

Acknowledgments
We thank Dr. Chris Vaughn for advice and assistance in measurement of the rotarod latency and locomotor activity in open field and Ms. Jillian Patterson for providing statistical advice.

Conflicts of Interest
The authors do not have any conflicts of interest relevant to this paper.

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