Reduced Postburn Hypertrophic Scarring and Improved Physical Recovery With Yearlong Administration of Oxandrolone and Propranolol

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Background: Massive burns induce a hypermetabolic response that leads to total body wasting and impaired physical and psychosocial recovery. The administration of propranolol or oxandrolone positively affects postburn metabolism and growth. The combined administration of oxandrolone and propranolol (OxProp) for 1 year restores growth in children with large burns. Here, we investigated whether the combined administration of OxProp for 1 year would reduce scarring and improve quality of life compared with control.

Study Design: Children with large burns (n = 480) were enrolled into this institutional review board-approved study; patients were randomized to control (n = 226) or administration of OxProp (n = 126) for 1 year postburn. Assessments were conducted at discharge and 6, 12, and 24 months postburn. Scar biopsies were obtained for histology. Physical scar assessments and patient reported outcome measures of physical and psychosocial function were obtained.

Results: Reductions in cellularity, vascular structures, inflammation, and abnormal collagen ($P < 0.05$) occurred in OxProp-treated scars. With OxProp, scar severity was attenuated and pliability increased (both $P < 0.05$). Analyses of patient-reported outcomes showed improved general and emotional health within the OxProp-treated group ($P < 0.05$).

Conclusions: Here, we have shown improvements in objective and subjective measures of scarring and an increase in overall patient-reported physical function. The combined administration of OxProp for up to a year after burn injury should be considered for the reduction of postburn scarring and improvement of long-term psychosocial outcomes in children with massive burns.

Keywords: adrenergic, androgen, children, glucocorticoid, hypermetabolism, inflammation, physical function, recovery, scar scale, wound healing

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Severe burn injuries covering ≥30% of the total body surface area (TBSA) significantly disrupt metabolism by inducing a hypermetabolic and catabolic state that leads to body mass wasting.[1] Mechanistically, this process is driven by hypercatecholaminemia,[2] an increase in cortisol and pro- and anti-inflammatory mediators, and futile cycling of substrates.[3] Significant loss of lean body mass accompanies severe burn injury, leading to debility and loss of physical function.[4] Despite significant progress in delineating the causes and consequences of the hypermetabolic response, this state remains incompletely understood.[5] However, studies suggest that modulation of postburn hypermetabolism both acutely and over the longer term improves outcomes.[6] In addition to numerous nonpharmacologic strategies that have been developed to support the burn-induced hypermetabolic response, the current leading experimental pharmacological interventions for blunting postburn hypermetabolism and catabolism include the administration of propranolol or oxandrolone.

Propranolol is a nonselective β_1 and β_2 adrenoreceptor antagonist that decreases heart rate and reduces hypertension in the general population. On the basis of the hypothesis that elevated catecholamines are responsible for the catabolic phenotype associated with burn injury,[7] research began into the use of propranolol to attenuate postburn hypermetabolism.[8] Since then, the benefits of postburn propranolol have included[9] decreasing myocardial oxygen demand,[10] cardiac work,[11–13] and peripheral lipolysis[12] without increasing the risk of sepsis[14] or exacerbating altered immunity.[15] Similarly, long-term propranolol administration decreased resting energy expenditure, heart rate, and central fat accretion while preventing bone loss and improving lean mass.[16] When combined with participation in an exercise program, propranolol increased lean mass and aerobic capacity.[17]

Oxandrolone is a synthetic anabolic steroid hormone that is a less virializing testosterone analog[18] and is used to modulate postburn loss of lean mass.[19] Although oxandrolone had been used for its anabolic effects in other wasting diseases,[20,21] use of this anabolic agent to curb the catabolism associated with burns began in the late 1990s.[22] Early results suggested that twice daily oral dosing of 10 mg of oxandrolone, in combination with a high protein diet, significantly accelerated weight gain in the recovery phase following severe burns.[22] Subsequent research showed that both younger and older

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TABLE 1. Demographics

Characteristic	Control (n = 226)	OxProp (n = 126)
Age, y	7.1 ± 0.4	7.4 ± 0.5
Sex (% Male)	63	72
Hispanic (%)	94	90
Type of burn (Scald: Flame: Electrical)	73: 134: 19	41: 70: 15
TBSA (%)	52 ± 1	50 ± 1
Third-degree TBSA (%)	37 ± 2	35 ± 2
Delay to admit, d (IQR)	3 (2.25)	3 (7.75)
Hospital length of stay, d (IQR)	25 (20.2)	25 (14.75)

Data presented as mean ± standard deviation.
IQR indicates interquartile range.

groups of adult burn patients experienced a significant effect of oxandrolone treatment.[23] Next, the salutary effects of oxandrolone on body composition following burn injury were found to persist for at least 6 months after drug discontinuation, suggesting an extended benefit of the therapy.[24] In the acute phase following burn, oxandrolone decreases length of stay for adults[25] and may enhance erythropoiesis in children.[26] Although the duration of oxandrolone administration in adults was short, in children, the long-term administration of oxandrolone for up to 1 year postburn improved lean mass, lung function, and both bone mineral density and bone mineral content.[19,27–29] The benefits of long-term oxandrolone administration persisted well beyond the duration of therapy in severely burned children as well.[30,31]

Patients with large burns are at risk for developing pathologic scarring that is pruritic and inflamed and that limits movement and function; postburn hypertrophic scars affect 70% to 90% of these patients.[32] These scars continue to be a source of pain and discomfort and negatively impact quality of life for up to 2 years postburn.[33] Therapies such as laser or surgical revision can decrease the severity of burn scar.[32] However, there is a dearth of pharmacologic approaches that successfully reduce the effects of burn scars on function and quality of life after massive burns. One of the greatest risk factors for hypertrophic scarring following burn injury is delayed wound healing.[32] We have hypothesized that therapies that reduce wound healing time following a large burn, such as propranolol[34] or oxandrolone,[35] may also decrease hypertrophic scarring.

With declining mortality from burns, attention has naturally shifted to improving long-term quality of life.[36] To improve quality of life, we now focus on reducing the impact of scar and increasing physical activity.[32,37] The chronic stress, inflammation, and catabolism in the first year following burn injury are likely to underlie negative outcomes.[2] Therefore, modulation of these chronic stressors would be reasonably expected to improve long-term outcomes in burn survivors. Previously, we demonstrated that the coadministration of oxandrolone and propranolol (OxProp) for 1 year attenuated the burn-induced growth arrest in children with large burns.[38] On the basis of the promising results of singly and coadministered OxProp, we hypothesized that the yearlong OxProp administration to severely burned children would attenuate the development of hypertrophic scar, objectively measured in vivo scar characteristics, and overall patient-reported physical function.

METHODS

One thousand one hundred eighty-six eligible patients were admitted to Shriners Hospitals for Children—Galveston between 2003 and 2015 (Fig. 1). Patients were between the ages of 6 months and 18 years at the time of burn, with burns over ≥30% of TBSA, and required surgery for skin grafting after scald, flame, or electrical burn (Table 1). This study, which is part of an ongoing evaluation of the long-term administration of anti-adrenergic, anti-catabolic, or anabolic agents,[8,11–14,17,19,27–31,34,38–57] was approved by the University of Texas Medical Branch Institutional Review Board (ClinicalTrials.gov number, NCT00675714). Patients, or their legal caregiver if younger than 18 years of age, consented to participate in this trial; children between the ages of 7 and 18 years assented to participate in the protocol as well. Patients were randomized to control (n = 226) or OxProp for 1 year following injury (n = 126); patients were assessed for up to 2 years postburn.

In the case of anoxic brain injury and/or when the decision was made to not treat because of injury severity, patients were excluded. Demographics, including age, sex, ethnicity, and burn size, were recorded at the time of admission. All patients received standard of care for inhalation injury, wound treatment, and nutrition as described previously.[39]

Patients allocated to Control were enrolled continuously from 2003 to 2017. Because of the balanced design of the randomization schedule for all patients participating in studies at Shriners Hospitals for Children—Galveston (Fig. 1), more patients were allocated to the Control group. Beginning in 2003, patients were randomized to OxProp; breaks in OxProp randomization were taken from 2005 to 2007 and 2008 to 2009 in order to randomize patients to the Ox or Prop groups. OxProp administration began within 4 ± 0.5 days following admission. Treatment group assignment was not revealed to the patients. Oxandrolone (BTG Pharmaceuticals, West Conshohocken, PA) was administered at a dose of 0.1 mg/kg every 12 hours for a minimum of 1 year; propranolol (Roxane Laboratories, Columbus, OH) was administered at a dose of 4.0 ± 0.2 mg/kg/day for a minimum of 1 year. The propranolol dose was titrated to decrease heart rate by 15%. Propranolol dosage was determined by a physician blinded to the patient's randomization.

Bradycardia was treated by holding a single dose of propranolol and recommencing administration 16 hours later with one half of the original dose. Over the next 24 hours, the propranolol dose was re-escalated to the original dose.

Tissue Processing

Scar samples were obtained during surgical revisions from a total of 30 pediatric patients (15 control, 15 OxProp) between 6 and 12 months following injury. Tissues were fixed in 10% neutral-buffered formalin, processed through absolute alcohol, and embedded in paraffin before sectioning at 4 μm. Sections were then stained in hematoxylin & eosin (H&E) for histological scar assessments or processed for subsequent immunohistochemical (IHC) stains. Immunohistochemistry included staining for the beta-adrenergic and androgen receptors, through which propranolol and oxandrolone act, respectively. As previous studies have demonstrated that oxandrolone also inhibits the signaling downstream from the glucocorticoid receptor, staining was also performed for this receptor.[58]

Histological Scar Assessment and Scoring

Histology of scar H&E sections were evaluated using a scoring scale developed by a board-certified pediatric pathologist. Scarring parameters of circumscription, loss of rete ridges, epidermal thickening, inflammation, abnormal collagen distribution, and vascularity were assigned ordinal scores from 0 (none) to 4 (most severe). Tissue nodularity was assessed by percent of scar area for each section. Three trained observers in scar histopathology then scored each section under microscopic evaluation (Olympus BX41) of the tissue. H&E sections were randomized and blinded to histopathology scorers before analysis. Subsequently, each section was digitized at 400X absolute magnification and gridded with a reticle to

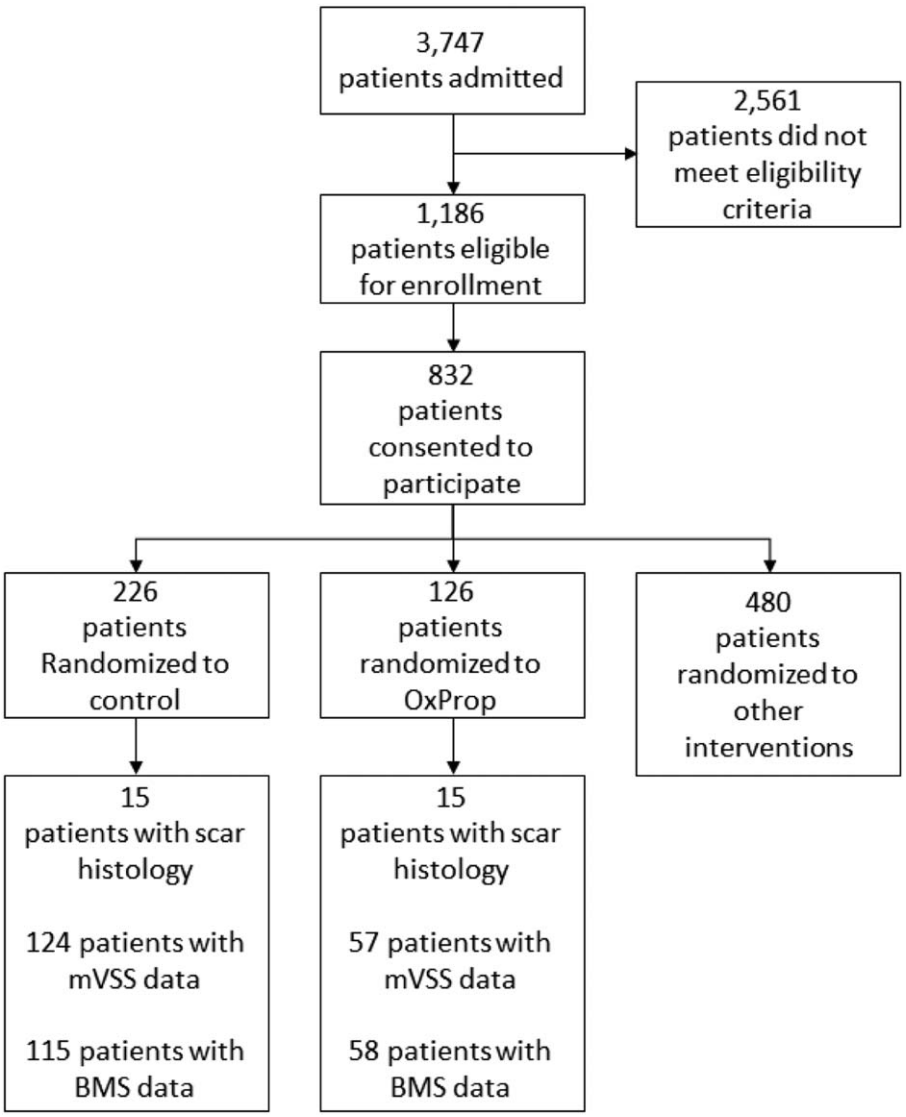

FIGURE 1. CONSORT diagram.

yield a 0.1 mm^2 area (version 1.12, Olympus DP22, cellSens software; Olympus Corp of the Americas, Waltham, MA). Tissue cellularity was quantified by counting positively stained nuclei in the papillary and mid dermis in 15 separate fields. For the scar assessment analysis, 6- and 12-month groups were combined.

Immunohistochemical Analyses

Tissues were processed as described above. Following sectioning, tissues were then deparaffinized and rehydrated. Antigen retrieval was performed in modified citrate buffer at pH 6.1 (DAKO target retrieval solution; Agilent, Santa Clara, CA) and heated to 95°C for 15 minutes. To quench endogenous peroxidase activity, sections were exposed to 2% hydrogen peroxide diluted in methanol for 30 minutes and then washed in tris-buffered saline with 0.1% tween (TBS-T). Sections were blocked in 3% horse serum in TBS-T for 1 hour and then incubated in primary antibody diluted in background-reducing antibody diluent (DAKO) at 4°C overnight. Negative controls received no primary antibody. Tissues were rinsed 3 times in TBS-T and then incubated in biotinylated secondary antibody for 1 hour at room temperature. After being washed 3 times in TBS-T, sections were further incubated in an avidin-biotinylated horseradish peroxidase (HRP) complex for 30 minutes and then exposed to hydrogen peroxide and diaminobenzidine (DAB) substrate (SK4105; Vector Labs, Burlingame CA) for 3 minutes.

The following primary antibodies were used for IHC: anti-CD31 (Abcam, ab199012, 1:250), anti-Ki67 (Abcam, ab15580, 1:1000), anti-glucocorticoid receptor (GCR) (Abcam, ab2768, 1:150), anti-alpha smooth muscle actin (α-SMA) (SigmaAldrich, A5691, 1:250), anti-beta2-adrenergic receptor (β2-AR) (Abcam, ab182136, 1:100), anti-androgen receptor (Abcam, ab108341, 1:250), anti-collagen 1 (Abcam, ab138492, 1:250), anti-collagen 3 (Abcam, ab6310, 1:250). Secondary antibodies corresponded to the primary antibody host: biotinylated anti-rabbit IgG (PK6101, 1:150, Vector Labs) and biotinylated anti-mouse IgG (PK6102, 1:150, Vector Labs). IHC analyses for each tissue section included quantification by blinded observers of microvascular structures per mm^2 by endothelial cell IHC for CD31, and $\alpha\text{-SMA}^+$ or $Ki67^+$ fibroblasts per mm^2, and scaled scores for tissue DAB intensity for β2-AR, Col-1, Col-3, GCR, and androgen receptor staining from 0 (none to lightly intense DAB) to 4 (highly intense DAB).

Tissue scores for H&E scar assessments and IHC sections were averaged for all blinded observers. After unblinding, scores for control and OxProp groups were compared by a 2-tailed Student *t* test using GraphPad Prism (version 7.03; GraphPad Software, La Jolla, CA). Unless otherwise indicated, data are presented as mean ± SEM; significant differences between groups were accepted at $P \leq 0.05$.

Modified Vancouver Scar Scale

A trained clinician completed the modified Vancouver Scar Scale (mVSS).[59] The mVSS is a validated tool that examines 6 parameters: pigmentation, vascularity, pliability, scar height, itch, and pain.[60] A total of 3267 individual records were gathered; matching the data for time postburn resulted in 124 control and 57 OxProp-treated patients from 6 months to 9 years postburn. mVSS records with null scores, nonmatched wounds, or unverified time points were excluded, resulting in 2792 records for analyses. Normally distributed data differences in total mVSS score and individual mVSS parameters between treatment groups were assessed and compared for each time point by 1-way analysis of variance (ANOVA). Data are represented as mean ± SEM.

Patient and Caregiver Reported Outcome Measures

The Burn Model Systems (BMS) National Longitudinal Database is a National Institute on Disability, Independent Living, and Rehabilitation Research (NIDILRR)-funded, multicenter, longitudinal research project that includes patient reported outcome measures. These surveys assess emotional, social, and physical outcomes following burn injury, including measures of satisfaction with life, appearance, and scarring.[61–74] The patients completed self-reported outcome measures, including Community Integration Questionnaire, the Satisfaction with Appearance Scale, the Burn Specific Health Scale, and in children 14+ years of age, the Short Form 12 (SF-12), at discharge and 6, 12, and 24 months postburn. Statistical significance was assessed with the Wilcoxon Rank Sum test using R version 2.3.2.[75]

RESULTS

OxProp Decreases Cellularity, Vascular Structures, Inflammation, and Deposition of Abnormal Collagen in Burn Scars

No significant differences were detected between the groups for age, burn size, sex, or length of stay. Histologically, scars from patients in the control group were more cellular (12.65 ± 0.45 cells • 0.1 mm^{-2}) than those in OxProp-treated patients (9.27 ± 0.33 cells • 0.1 mm^{-2}) ($P < 0.001$), suggesting a more physiologically active scar (Fig. 2A). Significantly reduced Ki67 expression in OxProp-treated scars (20.33 ± 3.13 $Ki67^+$ fibroblasts • mm^{-2}) compared with control scars (38.89 ± 8.43 $Ki67^+$ fibroblasts • mm^{-2}, $P = 0.039$, Fig. 2B) confirmed that cellular proliferation was greater in the untreated scars. The number of αSMA^+ fibroblasts did not vary between treatment groups (data not shown).

Expression of CD31, a marker of vascular structures, was reduced ($P < 0.0001$) with OxProp treatment, indicating significantly fewer microvascular structures in OxProp scars (10.56 ± 0.79 per mm^2) than in control scars (16.58 ± 1.301 per mm^2) (Fig. 3B). This finding may correlate with a lower vascularity score in H&E sections (1.45 ± 0.13, control; 1.14 ± 0.12, OxProp, $P = 0.087$, Fig. 3A); however, examination of more samples is necessary to determine whether a difference exists.

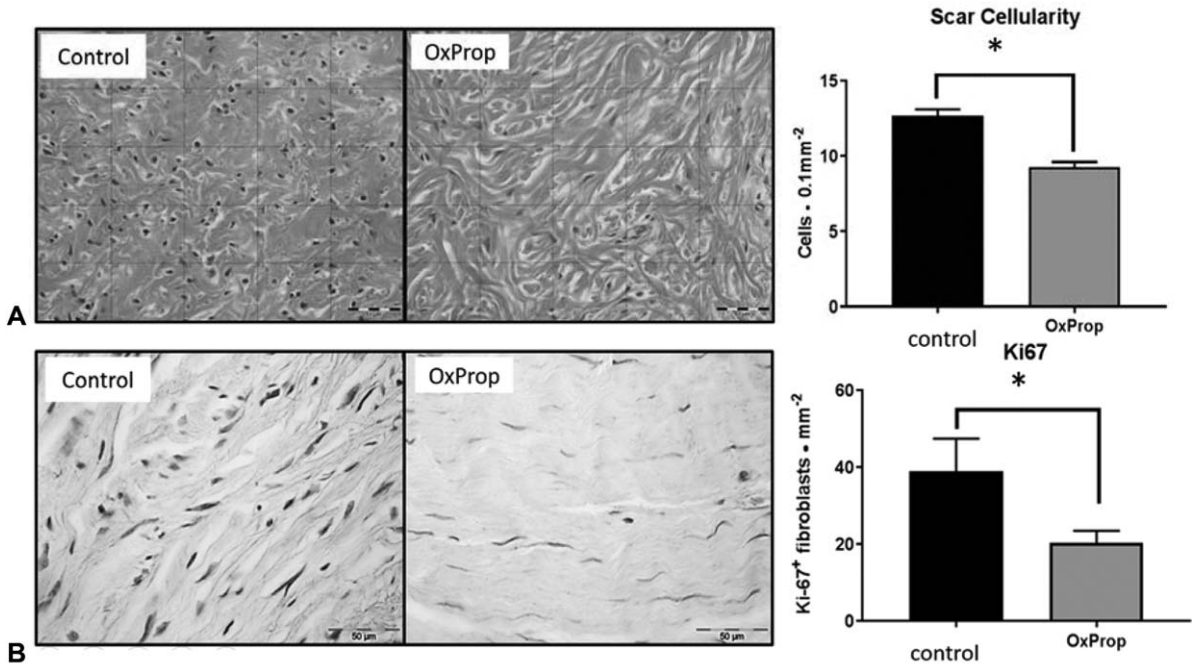

FIGURE 2. OxProp treatment reduces scar cellularity and dermal fibroblast proliferation. A, Representative images of H&E stained pediatric scar tissue after 12 months of control or OxProp treatment with associated quantification. When compared with control, scars from OxProp treated patient scars show significantly decreased dermal cellularity ($^*P < 0.001$). B, Ki67 immunostained sections of pediatric scar tissue 12 months postburn and accompanying quantification per mm^2. OxProp treatment significantly lessens the proliferative capacity of dermal fibroblasts ($^*P = 0.0392$) compared with control patients. Scale bars = 50 μm.

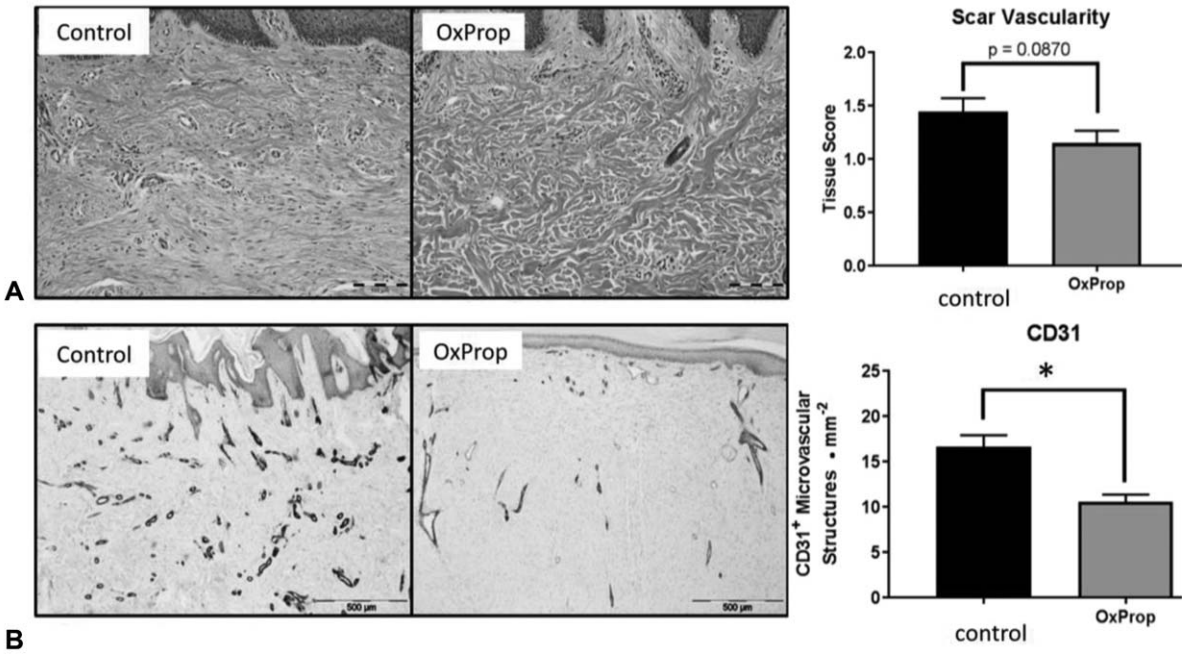

FIGURE 3. OxProp mitigates angiogenic potential in postburn scars. A, Representative H&E images of 12-month postburn treated scars with associated quantification. Scale bar = 100 μm. Tissue scores showed decreased vascularization in OxProp-treated patients' tissues compared with control ($P = 0.087$). B, DAB immunostained scar sections after 12 months of injury and treatment with accompanying quantification. Scale bar = 500 μm. CD31 expression is significantly decreased ($^{*}P < 0.0001$) in scars of patients treated with OxProp compared with control after 12 months indicating significant reduction in neovascularization during the postburn wound healing process.

Dermal inflammation was reduced with OxProp treatment (scar score: 1.02 ± 0.09; $P = 0.0019$) compared with controls (scar score: 1.60 ± 0.16) in the papillary and mid-dermal regions (Fig. 4A). In addition, percent nodularity decreased ($P < 0.0001$) with OxProp treatment (control: $36.22\% \pm 4.41$; OxProp: $12.22\% \pm 3.33$) (Fig. 4B). Here, we show that OxProp substantially diminishes ($P < 0.0001$) tissue histopathology scores for abnormal collagen deposition (1.28 ± 0.14) when compared with controls (2.25 ± 0.19, Fig. 4C). Expression of collagen I and collagen III, however, was similar between treatment groups (data not shown).

OxProp treatment significantly increased glucocorticoid receptor expression in scar (Fig. 5). Scars from control patients expressed less glucocorticoid receptors in dermal fibroblasts (tissue score: 1.43 ± 0.20) than those from OxProp-treated patients (tissue score: 2.20 ± 0.22) ($P = 0.043$). Differences were not found in expression of the β2-AR or the androgen receptor (data not shown).

OxProp Reduces Severity of Hypertrophic Scarring in Severely Burned Patients

Mean mVSS total scores were reduced in OxProp-treated patients over time, most significantly at between 6 and 12 months postburn ($P < 0.05$) compared with the control patients. In OxProp-treated patients, mVSS scores were reduced from 8.8 ± 0.2 to 6.4 ± 1.0 compared with control patients, 8.5 ± 0.3 to 7.5 ± 0.5. OxProp showed the most significant reduction in total mean mVSS from 6 to 12 months (-2.4 ± 0.5, $P = 0.02$) compared with control (-0.99 ± 0.3) (Fig. 6). One individual mVSS parameter, pliability, was significantly greater with OxProp ($P < 0.0001$, Fig. 7). No other individual mVSS scar parameters changed significantly.

Patient-reported Physical Function is Improved with OxProp

We evaluated answers to questions on the questionnaires that addressed activity, overall health, and extent of physical or emotional limitations (Table 2). The control group contained 115 patients; the OxProp group contained 58 patients. There were no significant differences with respect to age, percent TBSA burned, percent third-degree TBSA burned, or length of stay. When asked "How often do you perform leisure activities (movies, sports, restaurants)?" patients treated with OxProp reported significantly greater participation in activities at 2 years ($P = 0.007$). Patients completing the SF-12 reported better general health at 2 years ($P = 0.049$) compared with controls. When asked how often participants accomplished less than they would like because of emotional problems, the OxProp group reported that the frequency of emotional limitations was reduced significantly ($P = 0.018$) 2 years postburn. Finally, OxProp patients reported significantly better overall mental health as measured by the SF-12 Mental Health composite score at 2 years when compared with control ($P = 0.0012$).

DISCUSSION

In this prospective, randomized, double-blind, controlled study of the yearlong coadministration of OxProp following severe burn injury in children, we have shown improvements in both objective and subjective measures of scarring and an increase in overall patient-reported physical function. These results demonstrate benefits of the long-term, combined administration of propranolol and oxandrolone. The present findings add to the already established benefits of the individual administration of OxProp, in addition to the improved growth with the combined therapies.[8,10,12–15,17,19,26,27–31,34,38,40–44,46,47,49,11,76,77]

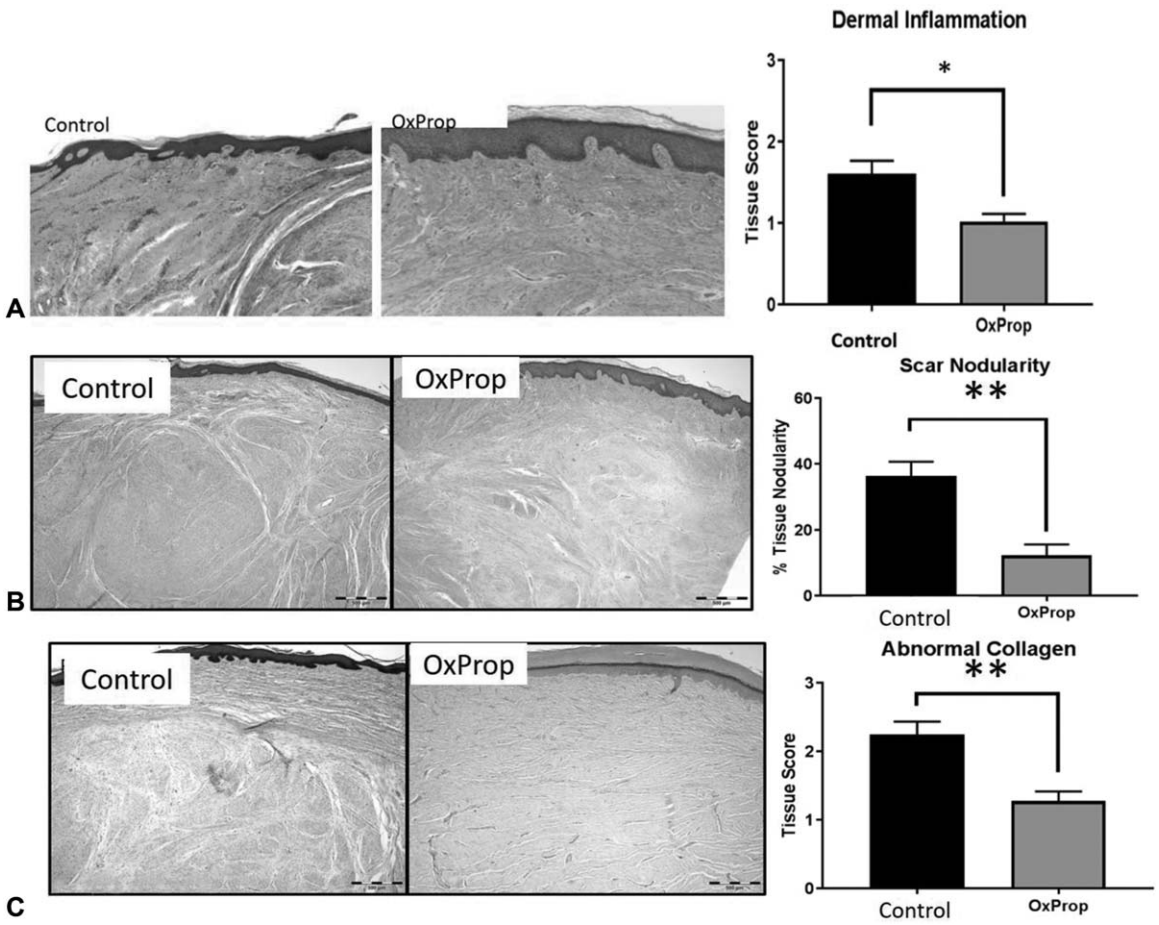

FIGURE 4. OxProp significantly reduces dermal inflammation, nodularity, and abnormal collagen deposition in postburn scars. (A) Dermal inflammation, (B) nodularity, and (C) abnormal collagen deposition in postburn scars are significantly reduced following OxProp year-long administration compared with control ($^{*}P < 0.05$; $^{**}P < 0.0001$). Scale bars = 500 μm.

From our histological analysis, we hypothesize that OxProp reduces postburn scarring via several potential mechanisms. First, dermal fibroproliferative capacity is reduced with OxProp treatment, as shown by the decrease in overall cellularity of the scar tissue treated with OxProp. The persistent decrease in Ki-67–positive cells at 12 months indicates that the proliferative capacity of deep dermal fibroblasts is reduced longitudinally with OxProp treatment. We previously demonstrated that propranolol changes β-AR expression

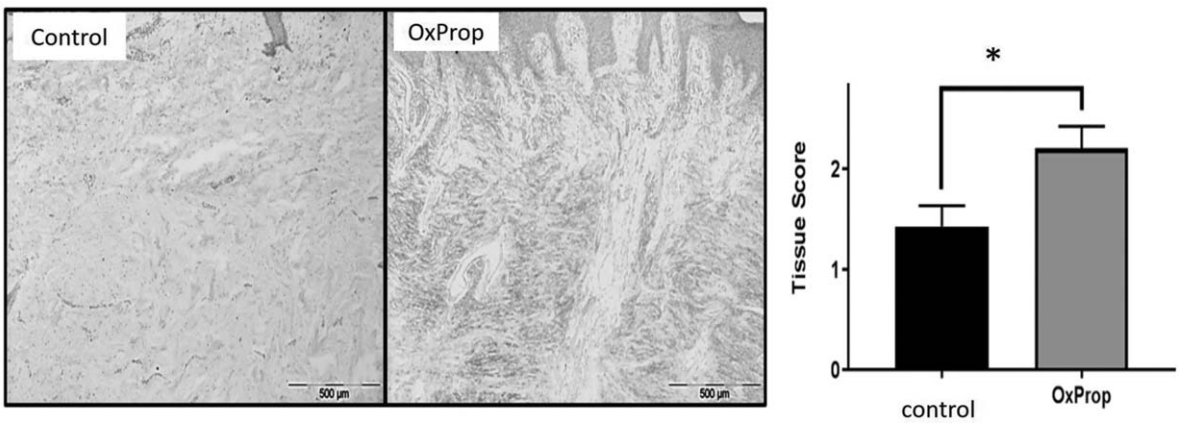

FIGURE 5. OxProp increases glucocorticoid receptor expression compared with control. ($^{*}P < 0.0426$).

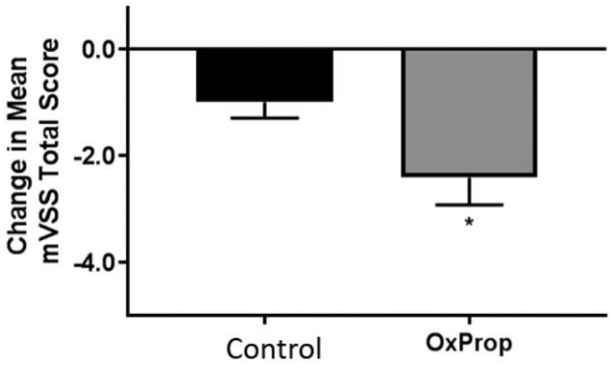

FIGURE 6. Severity of postburn hypertrophic scarring is reduced with OxProp following 1-year treatment. Total mVSS is significantly reduced in OxProp-treated patients between 6 and 12 months following burn injury. $^{*}P < 0.05$.

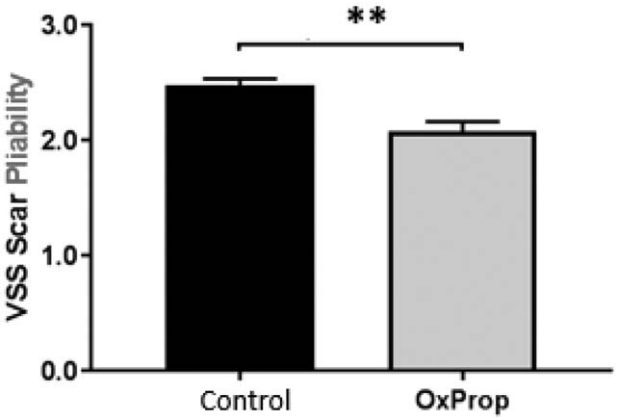

FIGURE 7. OxProp makes scar more pliable. Data shown are combined analyses means for all patients over all time points ± SEM. $^{**}P < 0.0001$.

and trafficking in scar dermal fibroblasts, providing a precedent supporting direct action of propranolol on this cell population.[78] Taken together, these findings may indicate that the scar matures faster with OxProp such that the scar is less physiologically active at the time of assessment. Next, we identified an overall reduction in blood vessels as detected via CD31 expression, suggesting that OxProp decreases angiogenic potential in postburn scars. These data also support the contention that OxProp results in a less physiologically active scar, as fewer blood vessels correlate with reduced metabolic activity. Currently, there is a dearth of investigations into oxandrolone's effect on angiogenic potential; however, several studies show that propranolol suppresses neovascularization.[79–81] Therefore, the anti-angiogenic effect shown in this study may be primarily induced by propranolol, although more investigations into the mechanism of action of oxandrolone are needed. The histological findings from our study have suggested mechanisms that may be responsible for the reduction in hypertrophic scarring with OxProp. In light of the changes in angiogenesis as detected by histology, we are now using laser speckle to assess blood flow through the scar to reduce the subjective nature of our assessments of blood flow/vascularity in burn scar.

We have also found that OxProp reduces dermal inflammation, nodularity, and abnormal collagen deposition. Twelve months following burn injury, OxProp diminished the severity of inflammatory cell infiltration within scars. Previous studies have concluded that both oxandrolone and propranolol alone can reduce collagen production through androgen receptor signaling perturbation[77] and β-AR antagonism, reducing vascular endothelial growth factor expression.[82,83] This further supports the hypothesis that OxProp treatment may improve wound healing and reduce scarring following burns. Collagen I or III presence was not different as assessed by immunohistochemistry. Future analyses will be focused on the expression of other extracellular matrix proteins or activity of proteins that modify or breakdown collagen. In addition, we found that OxProp increases glucocorticoid receptor expression, which may be a compensatory response to glucocorticoid antagonism through an androgen receptor-dependent manner.[58] Further study into the relationship among adrenergic blockade, androgen supplementation, and glucocorticoids is warranted.

The decrease in mean mVSS scores showed an overall reduction in scarring with OxProp administration. The change in pliability with OxProp indicated reduced stiffness of the tissue so that the scar was more flexible and moveable. This finding correlates well with our histological findings that abnormal collagen deposition was reduced.

From our analyses of patient-reported outcome measures, we conclude that the long-term coadministration of OxProp improves recovery from burn injury. The data show that patients treated with OxProp believed that their health was better and engaged in leisure activities more frequently than their counterparts in the control group. By 2 years postinjury, significantly more patients treated with OxProp were participating in activities. Patients on OxProp self-reported less frequent emotional limitations at 2 years postinjury than did the controls. The patients in the treatment group were also found to have greater improvement in mental health. These findings may result from improved healing with OxProp—these patients have resumed growing[38] and have more lean mass and muscle strength[27]; this would translate directly into an easier ability to function, allowing the patient to resume more daily activity and improving mental outlook.

Although we have suggested several hypotheses as to the mechanisms underlying the ability of coadministration of propranolol and oxandrolone to improve hypertrophic scarring and physical function, several alternative possibilities deserve mention. First, amelioration of the long-term adrenergic stress associated with burns improves anthropometric measures, specifically lean mass,[16] as does treatment with oxandrolone.[31] This increase in lean mass, when contrasted with the muscle catabolism of untreated patients, contributes to an overall increase in functional strength,[27] thereby increasing

TABLE 2. Quality of Life Assessments

Assessment	*P*	Interpretation	Time Point
How often do you perform leisure activities (movies, sports, restaurants)?	0.007	More activities performed with OxProp	2 y
How often do you accomplish less than you would like because of emotional problems?	0.018	Accomplishing more with OxProp	2 y
In general, would you say your health is excellent, very good, good, fair, or poor?	0.049	Perceived overall health is better with OxProp	2 y
Mental health composite score (based on results of SF12)	0.0012	Perceived mental health is better with OxProp	2 y

physical function.[84] As patients treated with the combination of OxProp reported improved general health, perhaps the increase in activity level creates a beneficial effect on scar tissue, such as the scar being stretched more because of increased activity. Alternatively, given that dermal fibroblasts proliferate in response to catecholamines, the excessive adrenergic stimulation after burn injury could lead to hyperproliferation within the developing scar.[85] Therefore, modulation of the persistent adrenergic stress associated with burn could decrease the activation of cutaneous fibroblasts, leading to a decrease in scar tissue formation.

CONCLUSION

Given the increasing evidence for the salutary effects of combined beta-blockade of burn-related hypermetabolism and the use of anabolic agents to blunt catabolism and increase anabolism, we conclude that the long-term administration of oxandrolone with propranolol should be considered for the treatment of pediatric burn survivors. The benefits of this combined treatment strategy, including resumed growth, increased muscle mass, greater strength, and now reduction in scar and greater activity, suggest that OxProp may be the most efficacious modulator of the hypermetabolic response that we have tested in burned children. Although long-term treatment of burned adults with oxandrolone has not been trialed, our reports of beneficial effects of OxProp suggest that this combination should be trialed in adults as well.

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REFERENCES

1. Porter C, Tompkins RG, Finnerty CC, et al. The metabolic stress response to burn trauma: current understanding and therapies. *Lancet*. 2016;388:1417–1426.
2. Jeschke MG, Gauglitz GG, Kulp GA, et al. Long-term persistance of the pathophysiologic response to severe burn injury. *PLoS One*. 2011;6:e21245.
3. Wolfe RR, Herndon DN, Jahoor F, et al. Effect of severe burn injury on substrate cycling by glucose and fatty acids. *N Engl J Med*. 1987;317:403–408.
4. Hart DW, Wolf SE, Mlcak R, et al. Persistence of muscle catabolism after severe burn. *Surgery*. 2000;128:312–319.
5. Sidossis L, Porter C, Saraf M, et al. Browning of subcutaneous white adipose tissue in humans after severe adrenergic stress. *Cell Metab*. 2015;22:219–227.
6. Herndon DN, Tompkins RG. Support of the metabolic response to burn injury. *Lancet*. 2004;363:1895–1902.
7. Wilmore DW, Long JM, Mason ADJ, et al. Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg*. 1974;180:653–669.
8. Herndon DN, Barrow RE, Rutan TC, et al. Effect of propranolol administration on hemodynamic and metabolic responses of burned pediatric patients. *Ann Surg*. 1988;208:484–492.
9. Finnerty CC, Herndon DN. Is propranolol of benefit in pediatric burn patients? *Adv Surg*. 2013;47:177–197.
10. Minifee PK, Barrow RE, Abston S, et al. Improved myocardial oxygen utilization following propranolol infusion in adolescents with postburn hypermetabolism. *J Pediatr Surg*. 1989;24:806–811.
11. Williams FN, Herndon DN, Kulp GA, et al. Propranolol decreases cardiac work in a dose-dependent manner in severely burned children. *Surgery*. 2011;149:231–239.
12. Baron PW, Barrow RE, Pierre EJ, et al. Prolonged use of propranolol safely decreases cardiac work in burned children. *J Burn Care Rehabil*. 1997;18:223–227.
13. Wurzer P, Branski LK, Clayton RP, et al. Propranolol reduces cardiac index but does not adversely affect peripheral perfusion in severely burned children. *Shock*. 2016;46:486–491.
14. Jeschke MG, Norbury WB, Finnerty CC, et al. Propranolol does not increase inflammation, sepsis, or infectious episodes in severely burned children. *J Trauma Acute Care Surg*. 2007;62:676–681.
15. Kobayashi M, Jeschke MG, Asai A, et al. Propranolol as a modulator of M2b monocytes in severely burned patients. *J Leukoc Biol*. 2011;89:797–803.
16. Herndon DN, Rodriguez NA, Diaz EC, et al. Long-term propranolol use in severely burned pediatric patients: a randomized controlled study. *Ann Surg*. 2012;256:402–411.
17. Porro LJ, Al-Mousawi AM, Williams F, et al. Effects of propranolol and exercise training in children with severe burns. *J Pediatr*. 2013;162:799–803. e791.
18. Kicman AT. Pharmacology of anabolic steroids. *Br J Pharmacol*. 2008;154:502–521.
19. Murphy KD, Thomas S, Mlcak RP, et al. Effects of long-term oxandrolone administration in severely burned children. *Surgery*. 2004;136:219–224.
20. Jekot WF, Purdy DW. Treating HIV/AIDS patients with anabolic steroids: a retrospective study. *AIDS Pat Care*. 1993;7:68–74.
21. Mendenhall CL, Anderson S, Garcia-Pont P, et al. Short-term and long-term survival in patients with alcoholic hepatitis treated with oxandrolone and prednisolone. *N Engl J Med*. 1984;311:1464–1470.
22. Demling RH, DeSanti L, Oxandrolone. Oxandrolone, an anabolic steroid, significantly increases the rate of weight gain in the recovery phase after major burns. *J Trauma*. 1997;43:47–51.
23. Demling RH, DeSanti L. The rate of restoration of body weight after burn injury, using the anabolic agent oxandrolone, is not age dependent. *Burns*. 2001;27:46–51.
24. Demling RH, DeSanti L. Oxandrolone induced lean mass gain during recovery from severe burns is maintained after discontinuation of the anabolic steroid. *Burns*. 2003;29:793–797.
25. Wolf SE, Edelman LS, Kemalyan N, et al. Effects of oxandrolone on outcome measures in the severely burned: a multicenter prospective randomized double-blind trial. *J Burn Care Res*. 2006;27:131–139.
26. Capek KD, Andersen CD, Watson CR, et al. Oxandrolone enhances erythropoiesis in pediatric burns. *Shock*. 2017;47:66–67.
27. Chao T, Porter C, Herndon DN, et al. Propranolol and oxandrolone therapy accelerated muscle recovery in burned children. *Med Sci Sports Exerc*. 2018;50:427–435.
28. Tuvdendorj D, Chinkes DL, Zhang XJ, et al. Long-term oxandrolone treatment increases muscle protein net deposition via improving amino acid utilization in pediatric patients 6 months after burn injury. *Surgery*. 2011;149:645–653.
29. Sousse LE, Herndon DN, Mlcak RP, et al. Long-term administration of oxandrolone improves lung function in pediatric burned patients. *J Burn Care Res*. 2016;37:273–277.
30. Reeves PT, Herndon DN, Tanksley JD, et al. Five-year outcomes after long-term oxandrolone administration in severely burned children: a randomized clinical trial. *Shock*. 2016;45:367–374.
31. Porro LJ, Herndon DN, Rodriguez NA, et al. Five-year outcomes after oxandrolone administration in severely burned children: a randomized clinical trial of safety and efficacy. *J Am Coll Surg*. 2012;214:489–502. discussion 502–484.
32. Finnerty CC, Jeschke MG, Branski LK, et al. Hypertrophic scarring: the greatest unmet challenge after burn injury. *Lancet*. 2016;388:1427–1436.
33. Wurzer P, Forbes AA, Hundeshagen G, et al. Two-year follow-up of outcomes related to scarring and distress in children with severe burns. *Disabil Rehabil*. 2017;39:1639–1643.
34. Ali A, Herndon DN, Mamachen A, et al. Propranolol attenuates hemorrhage and accelerates wound healing in severely burned adults. *Crit Care*. 2015;19:217.
35. Demling RH, Orgill DP. The anticatabolic and wound healing effects of the testosterone analog oxandrolone after severe burn injury. *J Crit Care*. 2000;15:12–17.
36. Pereira C, Murphy K, Herndon D. Outcome measures in burn care: is mortality dead? *Burns*. 2004;30:761–771.
37. Pallua N, Künsebeck HW, Noah EM. Psychosocial adjustments 5 years after burn injury. Burns 2003; 29:143–152.
38. Herndon DN, Voigt CD, Capek KD, et al. Reversal of growth arrest with the combined administration of oxandrolone and propranolol in severely burned children. *Ann Surg*. 2016;264:421–428.
39. Guillory AN, Herndon DN, Silva MB, et al. Oxandrolone coadministration does not alter plasma propranolol concentrations in severely burned pediatric patients. *J Burn Care Res*. 2017;38:243–250.
40. Hart DW, Wolf SE, Ramzy PI, et al. Anabolic effects of oxandrolone after severe burn. *Ann Surg*. 2001;233:556–564.

41. Jeschke MG, Finnerty CC, Suman OE, et al. The effect of oxandrolone on the endocrinologic, inflammatory, and hypermetabolic responses during the acute phase postburn. *Ann Surg*. 2007;246:351–360. discussion 360–352.
42. Aarsland A, Chinkes D, Wolfe RR, et al. Beta-blockade lowers peripheral lipolysis in burn patients receiving growth hormone. Rate of hepatic very low density lipoprotein triglyceride secretion remains unchanged. *Ann Surg*. 1996;223:777–787. discussion 787–779.
43. Barrow RE, Wolfe RR, Dasu MR, et al. The use of beta-adrenergic blockade in preventing trauma-induced hepatomegaly. *Ann Surg*. 2006;243:115–120.
44. Guillory AN, Herndon DN, Silva MB, et al. Propranolol kinetics in plasma from severely burned adults. *Burns*. 2017;43:1168–1174.
45. Hart DW, Wolf SE, Chinkes DL, et al. Beta-blockade and growth hormone after burn. *Ann Surg*. 2002;236:450–456. discussion 456–457.
46. Herndon D, Rodriguez N, Diaz E, et al. Long-term propranolol use in severely burned pediatric patients: a randomized controlled study. *Ann Surg*. 2012;256:402–411.
47. Herndon DN, Hart DW, Wolf SE, et al. Reversal of catabolism by beta-blockade after severe burns. *N Engl J Med*. 2001;345:1223–1229.
48. Jeschke MG, Finnerty CC, Kulp GA, et al. Combination of recombinant human growth hormone and propranolol decreases hypermetabolism and inflammation in severely burned children. *Pediatr Crit Care Med*. 2008;9:209–216.
49. Olah G, Finnerty CC, Sbrana E, et al. Increased poly(ADP-ribosyl)ation in skeletal muscle tissue of pediatric patients with severe burn injury: prevention by propranolol treatment. *Shock*. 2011;36:18–23.
50. Branski LK, Herndon DN, Barrow RE, et al. Randomized controlled trial to determine the efficacy of long-term growth hormone treatment in severely burned children. *Ann Surg*. 2009;250:514–523.
51. Cree MG, Zwetsloot JJ, Herndon DN, et al. Insulin sensitivity and mitochondrial function are improved in children with burn injury during a randomized controlled trial of fenofibrate. *Ann Surg*. 2007;245:214–221.
52. Finnerty CC, Ali A, McLean J, et al. Impact of stress-induced diabetes on outcomes in severely burned children. *J Am Coll Surg*. 2014;218:783–795.
53. Gauglitz GG, Herndon DN, Kulp GA, et al. Abnormal insulin sensitivity persists up to three years in pediatric patients post-burn. *J Clin Endocrinol Metab*. 2009;94:1656–1664.
54. Herndon DN, Ramzy PI, DebRoy MA, et al. Muscle protein catabolism after severe burn: effects of IGF-1/IGFBP-3 treatment. *Ann Surg*. 1999;229:713–720. discussion 720–712.
55. Jeschke MG, Finnerty CC, Herndon DN, et al. Severe injury is associated with insulin resistance, endoplasmic reticulum stress response, and unfolded protein response. *Ann Surg*. 2012;255:370–378.
56. Jeschke MG, Kulp GA, Kraft R, et al. Intensive insulin therapy in severely burned pediatric patients: a prospective randomized trial. *Am J Respir Crit Care Med*. 2010;182:351–359.
57. Tuvdendorj D, Zhang XJ, Chinkes DL, et al. Intensive insulin treatment increases donor site wound protein synthesis in burn patients. *Surgery*. 2011;149:512–518.
58. Zhao J, Bauman WA, Huang R, et al. Oxandrolone blocks glucocorticoid signaling in an androgen receptor-dependent manner. *Steroids*. 2004;69:357–366.
59. Nedelec B, Shankowsky HA, Tredget EE. Rating the resolving hypertrophic scar: comparison of the Vancouver Scar Scale and scar volume. *J Burn Care Rehabil*. 2000;21:205–212.
60. Sullivan Ta, Smith J, Kermode J, et al. Rating the burn scar. *J Burn Care Rehab*. 1990;11:256–260.
61. Amtmann D, Gibran NS, Herndon DN, et al. Letter to the editor #2: description of the Burn Model System National Database sample. *Burns*. 2016;42:704–705.
62. Amtmann D, McMullen K, Bamer A, et al. National Institute on Disability, Independent Living, and Rehabilitation Research Burn Model System: review of program and database. *Arch Phys Med Rehabil*. 2017 [Epub ahead of print].
63. Amtmann D. Project Directors of the National Institute on Disability IL, Rehabilitation Research Burn Model System Centers P, et al. BMS letter to the editor #1: introduction to the Burn Model System Centers Program. *Burns*. 2016;42:944–946.
64. Gerrard P, Kazis LE, Ryan CM, et al. Validation of the Community Integration Questionnaire in the adult burn injury population. *Qual Life Res*. 2015;24:2651–2655.
65. Goverman J, Mathews K, Goldstein R, et al. Adult contractures in burn injury: a Burn Model System National Database Study. *J Burn Care Res*. 2017;38:e328–e336.
66. Goverman J, Mathews K, Goldstein R, et al. Pediatric contractures in burn injury: a Burn Model System National Database Study. *J Burn Care Res*. 2017;38:e192–e199.
67. Goverman J, Mathews K, Holavanahalli RK, et al. The National Institute on Disability, Independent Living, and Rehabilitation Research Burn Model System: twenty years of contributions to clinical service and research. *J Burn Care Res*. 2017;38:e240–e253.
68. Goverman J, Mathews K, Nadler D, et al. Satisfaction with life after burn: a Burn Model System National Database Study. *Burns*. 2016;42:1067–1073.
69. Kazis LE, Lee AF, Rose M, et al. Recovery curves for pediatric burn survivors: advances in patient-oriented outcomes. *JAMA Pediatr*. 2016;170:534–542.
70. Kazis LE, Sheridan RL, Shapiro GD, et al. Development of clinical process measures for pediatric burn care: understanding variation in practice patterns. *J Trauma Acute Care Surg*. 2018;84:620–627.
71. Levi B, Jayakumar P, Giladi A, et al. Risk factors for the development of heterotopic ossification in seriously burned adults: a National Institute on Disability, Independent Living and Rehabilitation Research burn model system database analysis. *J Trauma Acute Care Surg*. 2015;79:870–876.
72. Schneider JC, Nadler DL, Herndon DN, et al. Pruritus in pediatric burn survivors: defining the clinical course. *J Burn Care Res*. 2015;36:151–158.
73. Schneider JC, Simko LC, Goldstein R, et al. Predicting heterotopic ossification early after burn injuries: a risk scoring system. *Ann Surg*. 2017;266:179–184.
74. Simko LC, Espinoza LF, McMullen K, et al. Fatigue following burn injury: a burn model system national database study. *J Burn Care Res*. 2018;39:450–456.
75. RCT. R: A Language and Environment for Statistical Computing. 2015. Available at: https://www.R-project.org/. Accessed February 10, 2016.
76. Pham TN, Klein MB, Gibran NS, et al. Impact of oxandrolone treatment on acute outcomes after severe burn injury. *J Burn Care Res*. 2008;29:902–906.
77. Xiang L, Wiren K, Xiao-Wei Z, et al. The effect of oxandrolone treatment on human osteoblastic cells. *J Burns Wounds*. 2007;6:53–64.
78. El Ayadi A, Prasai A, Wang Y, et al. beta-Adrenergic receptor trafficking, degradation, and cell-surface expression are altered in dermal fibroblasts from hypertrophic scars. *J Invest Dermatol*. 2018 [Epub ahead of print].
79. Lamy S, Lachambre M, Lord-Dufour S, et al. Propranolol suppresses angiogenesis in vitro: inhibition of proliferation, migration, and differentiation of endothelial cell. *Vascul Pharmacol*. 2010;53:200–208.
80. Pasquier E, Ciccolini J, Carre M, et al. Propranolol potentiates the anti-angiogenic effects and anti-tumor efficacy of chemotherapy agents: implication in breast cancer treatment. *Oncotarget*. 2011;2:797–809.
81. Zhang L, Mai HM, Zheng J, et al. Propranolol inhibits angiogenesis via down-regulating the expression of vascular endothelial growth factor in hemangioma derived stem cell. *Int J Clin Exp Pathol*. 2014;7:48–55.
82. Hajighasemi F, Mirshafiey A. Propranolol effect on proliferation and vascular endothelial growth factor secretion in human immunocompetent cells. *J Clin Immunol Immunopathol Res*. 2010;2:22–27.
83. Romana-Souza B, Nascimento AP, Monte-Alto-Costa A. Low-dose propranolol improves cutaneous wound healing of burn-injured rats. *Plast Reconstr Surg*. 2008;122:1690–1699.
84. Lee JO, Herndon DN, Andersen C, et al. Effect of exercise training on the frequency of contracture-release surgeries in burned children. *Ann Plast Surg*. 2017;79:346–349.
85. Pullar CE, Isseroff RR. The β2-adrenergic receptor activates pro-migratory and pro-proliferative pathways in dermal fibroblasts via divergent mechanisms. *J Cell Sci*. 2006;119:592–602.

DISCUSSANTS

Dr Tina L. Palmieri (Sacramento, CA):

This paper adds to the previous fine work by Dr Herndon's group on the use of propranolol in major burn injury. This study extends the work to oxandrolone. I will refer to the combination of propranolol and oxandrolone as OxProp.

The study has 2 components: one, a basic science evaluation of the actual scar, and the second an evaluation of the patient's view on their quality of life.

The scar portion of this study contains both a histologic evaluation of the scar and a Vancouver Scale scoring. The histological evaluation occurred in 15 patients per group and was a mixture of scars obtained at the 6- and 12-month intervals. In this portion of the study, markers of inflammation and dermo-cellularity were improved in the OxProp group. In the scar study portion of the paper, there was decreased pliability and increased vascularity in the scar.

The second portion of the study, which examined patient-reported outcomes, reported overall superior results in the OxProp group.

This study epitomizes the elegant bench work and dedication of Dr Herndon's group to identifying the mechanisms underlying outcomes after the administration of propanolol and now combined OxProp. It is unfortunate that there is no comparison of isolated propranolol or isolated oxandrolone administration to act as further controls to help to identify the impact of each agent on scar formation.

I have several questions to this fine study.

The linchpin on scar evaluation is obtaining equivalent scar specimens. How did you assure parity in the biopsy with respect to scar location on the body, wound healing, and type/timing of graft received by the patient? Given that scars vary markedly over time in the first 2 years, was there an equal number of 6- and 12-month samples between groups that were analyzed?

Second, your CONSORT diagram, which is wonderfully executed, has 3 major categories, and over 400 patients were not included in this study. It would be interesting for us to know what other studies these patients were in. If the patients had administration of isolated propranolol or oxandrolone, they could be used as controls and help elucidate my questions.

Third, these agents all have specific side effect profiles, and combining them increases the risk for the side effects that include cardiac events, decreased seizure potential, and elevated liver functions. What was the incidence of these complications?

Finally, on a practical level, it is challenging for a mother to give a 4-year-old 1 drug several times a day. Administering 2 drugs multiple times a day is a tour de force. Do we really need to give both of these drugs to obtain the reported effects?

Thank you for a wonderfully presented paper and further science on the use of propranolol.

Response from David Herndon:

Thank you very much, Dr Palmieri. I appreciate the opportunity to clarify the use of OxProp for scar reduction – which is beyond the original application of these therapeutics.

The scar biopsies were obtained from sites undergoing surgical revision, from similar locations on the trunk in this patient population with massive injuries. The timing of the days postburn was controlled for in our analysis. There were an equal number of observations at 6 and 12 months. The mean observation period was 9 months for the scars. There were no significant differences in reconstructive surgeries and/or the number of operations during the acute burn period in this patient population.

A randomization schedule was established by our statistician using random allocation sequence software to distribute patients into each arm of the interventional study. The CONSORT diagram does include the 480 patients who were randomized to other interventions based on each study's inclusion and exclusion criteria, and the patients' willingness to participate in each study. A group of patients received oxandrolone, and another received propranolol alone. Those patients did achieve improvements in strength and decrease in tachycardia and blood pressure. Preliminary comparison of these treatment groups has shown that although propranolol or oxandrolone alone may reduce some aspects of scarring, the effects were greater in the patients who received the combined therapy. Given that the combined therapy reduces the effects of elevated catecholamines and cortisol, while supplementing androgen levels that are typically suppressed following burn injuries, it is not a surprise that effects on quality of life or even scarring are found. Oxandrolone is reported to heal wounds quicker and to allow patients to build more muscle, while propranolol decreases catecholamine signaling – potentially reducing anxiety while decreasing heart rate. Although 1 drug would impart benefit, we believe that by administering both, the benefits are greater.

The combined administration of OxProp is believed to stimulate protein synthesis and anabolism due to oxandrolone and temper hypermetabolic catabolism owing to propranolol. Our data suggest an additive effect that increases quality of life after severe burn injury. The mechanism of these agents in scar modulation is ripe for further investigation. These effects are probably mediated by signaling via the adrenergic, androgen, and glucocorticoid receptors; propranolol acts via the adrenergic receptors, while oxandrolone activates the androgen receptor while blocking glucocorticoid receptor signaling. Although these receptors signal independently, there is a cross-talk between these signaling pathways. Additional mechanistic studies would allow us to determine whether the observed effects are additive or synergistic.

In terms of side effects, propranolol occasionally causes bradycardia. We do have patients observed by their families and in hospital for bradycardia. If it does occur, the drug is held for 1 dose. However, generally, the tachycardia is so pronounced in this patient population that this side effect is quite rare. Liver function test abnormalities due to oxandrolone are vanishingly rare in this patient population and disappear over time. At the current time, we believe that a larger multicenter trial is needed in order to be able to generalize the results. There have been no long-term studies of administering these agents after discharge from the hospital in adults with burns, so studies to determine the safety and efficacy are needed.

We decreased the problem of compliance with drug administration by using extended-release propranolol tablets, which are given as a single daily dose, in the outpatient setting for 1 year postinjury. The oxandrolone was given twice per day. We have had very few complaints regarding this dosing regimen. It is a burden for the subjects in the control group to take placebos, of course, but this is part of the dedication to participating in an investigational study.

Dr William G. Cioffi (Providence, RI):

I rise to read into the record the comments of Dr Basil Pruitt who wished to discuss this paper, but at the last moment could not travel to the meeting.

Dr Pruitt wishes to compliment Dr Herndon and his colleagues on expanding our knowledge of the pathophysiology of injury and informing us of the therapeutic effectiveness of long-term treatment with OxProp on postburn hypertrophic scarring and physical recovery in severely injured children. The authors have added another chapter to their encyclopedia of burn injury and treatment that serves as a worldwide reference.

To address concerns and clarify the findings reported this morning, we will need additional information.

(1) In Table 2, you note that the OxProp group contained 32% more males than the control group. As males are likely to be more physically active and potentially less concerned about appearance, did the difference in sex influence your findings?
(2) In 2016, you reported that OxProp treatment reduced growth arrest in severely burned children by increasing the duration and length of their growth spurts. I believe that the effect was associated with grown spurts, so it would be interesting to know

whether there were a similar number of growth spurts in the study group and the control group.

(3) Other factors that have been reported to influence wound healing and scarring include exercise and diet. How were those variables controlled or did they differ between the 2 groups?

(4) The histologic examination of scar biopsy supports the significant anti-inflammatory effect of OxProp with reduction of abnormal collagen deposition but, interestingly, no effect on collagen I or III. Can you speculate about what accounts for this differential effect on collagen type?

As scar formation is to some extent body site dependent, how well matched were the scar biopsies in the 2 groups of patients? Is the expression of fewer glucocorticoid receptors in dermal fibroblasts of the control group associated with low scar tissue levels of glucocorticoids? Conversely, does the lack of difference in scar tissue expression of the beta-2 AR and the androgen receptor focus attention on Oxandrolone for improvement in overall physical function as assessed in the BMS questionnaire response?

Lastly, Figs. 6 and 7 and the timing of tissue sampling suggest that the treatment effects are most evident at 12 months and may speak for longer-term treatment of OxProp. An important question is whether these effects are dose related, and would bigger doses be better than those employed in this study?

I would like to compliment the authors on this impressive work and thank the Association for this ''in absentia'' privilege of the floor. Basil A. Pruitt, Jr.

And if I may add 2 additional questions.

Dr Herndon, you use the Vancouver Scale. It is a validated measure but it is somewhat subjective in nature. Did you evaluate the inter- and intrarater variability? And why not use more objective measures of scars such as cutometer or other such things?

Finally, there are also a variety of treatments which affect scars. These include pressure garments, silicone, steroids, lasers, massage therapy, etc. How did you control for these treatments?

Response from Dr David Herndon:

Thank you very much, Dr Pruitt, in absentia, and Dr Cioffi.

To answer Dr Pruitt's questions first, he noted that there were more boys in 1 group than another group. This difference was not statistically significant, and we have not seen a difference in sex distribution of these effects in these patients, albeit these patients are 7 to 9 years of age, before puberty, so the lack of a sex effect may not hold in later studies.

We previously published that OxProp significantly improved growth compared with oxandrolone or propranolol alone in prepubertal burned patients. Growth is better in the OxProp group than in the control group. In children, growth plate physiology remains a powerful indicator for both the systemic sequelae of burn injury and their reversal. In this study of scarring, there were a similar number of growth spurts in the control and study groups.

Both Drs Pruitt and Cioffi asked whether the patients received similar nutrition, exercise, and pressure therapy. All patients were treated according to our standard of care; these patients were in a standardized program and all received 3 months of exercise and pressure garments. In addition, dietary recall was not significantly different between the different individuals.

Dr Pruitt also asked if this might be an anti-inflammatory effect, and we do think that propranolol exerts anti-inflammatory effects, and oxandrolone may as well. We expect that the difference in collagen deposition may be an effect of OxProp on the expression and/or activity of enzymes that remodel the collagen after it is produced. Studies are ongoing now to determine whether matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are affected by oxandrolone and/or propranolol. Additional studies of the anti-inflammatory effects of propranolol are underway as well; preliminary evidence suggests that propranolol influences the macrophage populations within the wound and scar.

We did examine expression of the androgen receptor and found that there was no change in receptor expression. We also found that expression of the ß 2-AR was unchanged. This was surprising, as we have previously found that this receptor is upregulated in the scar of patients receiving propranolol; studies in cell culture have confirmed this finding and also showed that the administration of propranolol increases the degradation of the receptor. The increase in glucocorticoid receptor expression was intriguing because it is known that oxandrolone inhibits cortisol signaling not by preventing cortisol from binding to the glucocorticoid receptor, but rather by blocking transcription of cortisol-induced genes via cross-talk between the glucocorticoid and androgen receptors. We are now engaged in studies to determine why and how the combined administration of OxProp may alter the trafficking of the ß -ARs, the androgen receptor, and the glucocorticoid receptor.

We do not have any data regarding longer administration, or greater doses, of these agents. As the propranolol dose is determined by reduction of heart rate, giving a greater dose could induce bradycardia or other adverse events. The development of novel delivery methods to apply the drugs to the wound/scar may allow circumvention of this issue; similarly, the use of other beta-blocking agents may be efficacious. We will need to perform additional studies in order to determine if there is a better dosing schedule, amount, or therapeutic agent that would result in even greater effects.

Dr Cioffi asked about inter-rater variability of the Vancouver Scars Scale. We solved that by having 1 clinician rate all of the scars, eliminating inter-rater variability; the clinician was blinded to the drug randomization group. We have moved to using a variety of objective measures in our current studies of scarring, including assessment with the DermlabCombo – which allows measurement elasticity, transepidermal water loss, hydration, scar thickness, and color; the patient and observer scar assessment scale (POSAS); the Galveston photographic scar score; 3D photography to calculate surface area and volume, assessment of texture and color; and blood flow and thermoregulation measured by laser speckle and laser doppler techniques.

I thank the Association and the president for the privilege of presenting our study.