



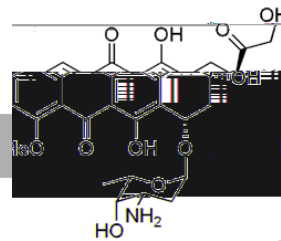
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إدارة كلية الطب

The concurrent effect of tumor and Doxorubicin chemotherapy on intracellular and interstitial nitric oxide concentrations in skeletal muscle

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Ph.D. candidate

There are no conflicts of interests to declare.

Doxorubicin



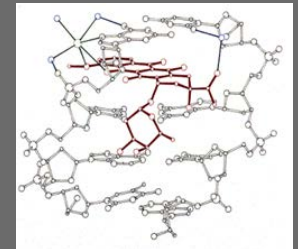
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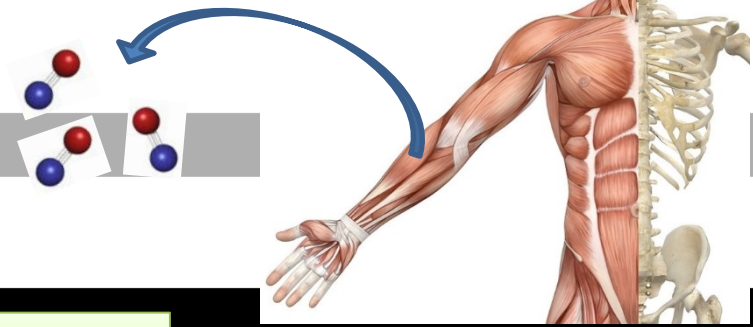
- Widely used chemotherapeutic agent for the treatment of solid tumors and hematological malignancies

- Cytotoxicity due to:
 - Inhibition of DNA replication and RNA transcription via DNA intercalation
 - Inhibits DNA repair by topoisomerase II inhibition



- Considered the most active agent in the treatment of breast cancer

Nitric Oxide



NADPH

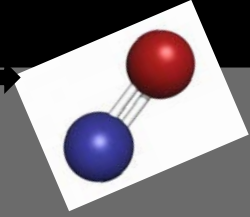
L-Arginine

O₂

eNOS

iNOS

nNOS



Nitric Oxide (NO):

- Potent vasodilatory molecule
- Stimulates GH production
- Reduces platelet aggregation
- Potent neurotransmitter
- In cardiomyocytes, DOX increases NOS expression which leads to a disruption in NO regulation

Does the administration of Doxorubicin affect nitric oxide production in skeletal muscle?

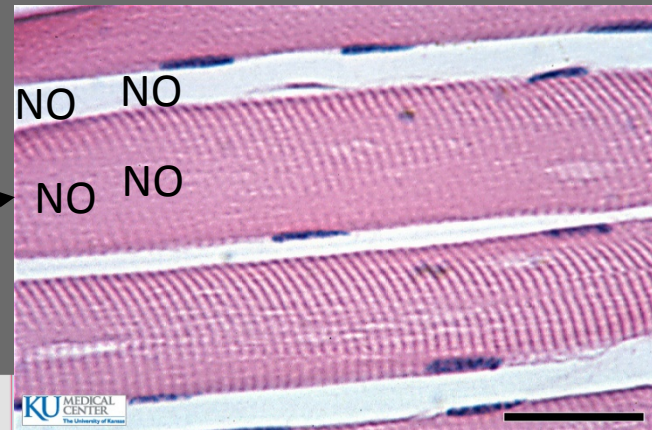
Purpose

Examine the concurrent effect of tumor and DOX on intracellular and interstitial NO concentrations in skeletal muscle

DOX

?

?



The University of Kansas Medical Center



Group 1

- Control
- Sham injection



Group 2

- DOX 10mg/kg ip



Group 3

- MCF-7 tumor
- DOX 10mg/kg ip

Xenograft group: female nu/nu

Day: 0 _____ 7 ... Tumor >200 mm³

- Estradiol pellet inserted subcutaneously.
- 5x10⁶ MCF-7 cells injected subcutaneously.
- Once the tumor size has achieved a size >200 mm³ the mouse is selected for experiments.

Treatment Timeline



Initiate experiments

Days: 0 3 7

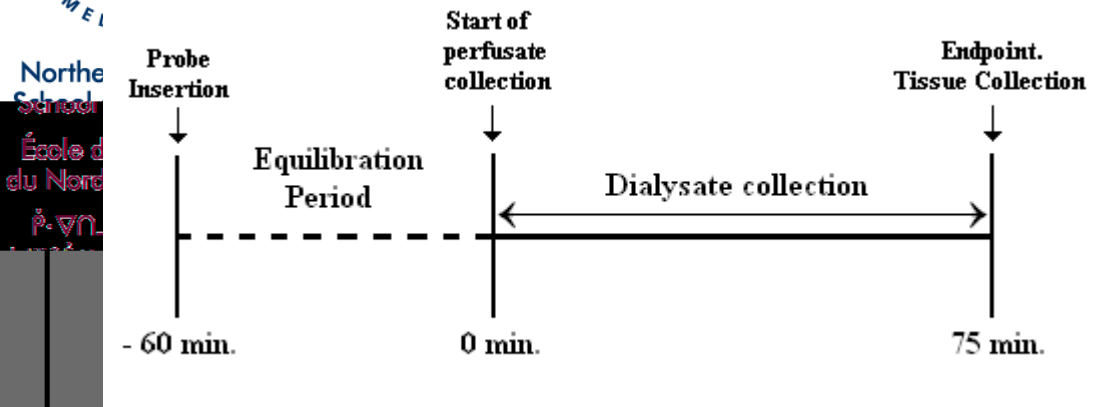
Group 1 (control): saline injection ip

Group 2 (DOX): 10 mg/kg ip

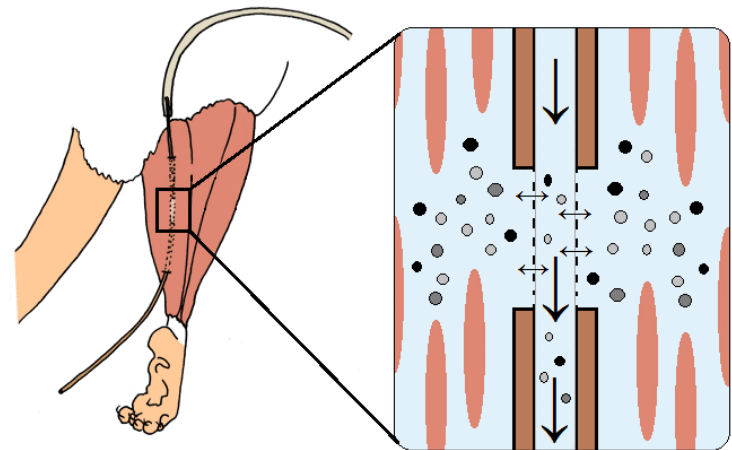
Group 3 (DOX+Tum): 10 mg/kg ip

Experimental timepoints at day 3 and 7

Experimental protocol



- Animals are anaesthetized
- Microdialysis probes are inserted into the hind limbs
- Probes are perfused with Ringer's solution at 3 mL/min



- Quadriceps and Gastrocnemius are excised, flash frozen

Sample analysis

Muscle samples:

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پیشرفت و پیشرفت

- Freeze dried, powdered

- 2 mg was added to PCA, homogenized and centrifuged

- Supernatant used for analysis

Dialysate samples:

- Represent an ultrapure filtrate

- Does not require pre-analysis preparation

Nitric Oxide Analyzer

- NO concentration determined by chemoluminescence

- Converts nitrate and nitrite (NO_x) to NO

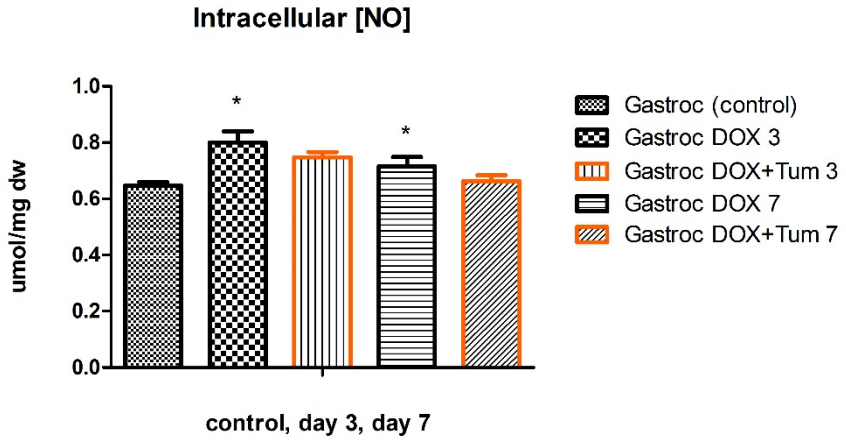
- Samples analyzed in duplicate

- Concentrations determined by comparison with a standard curve



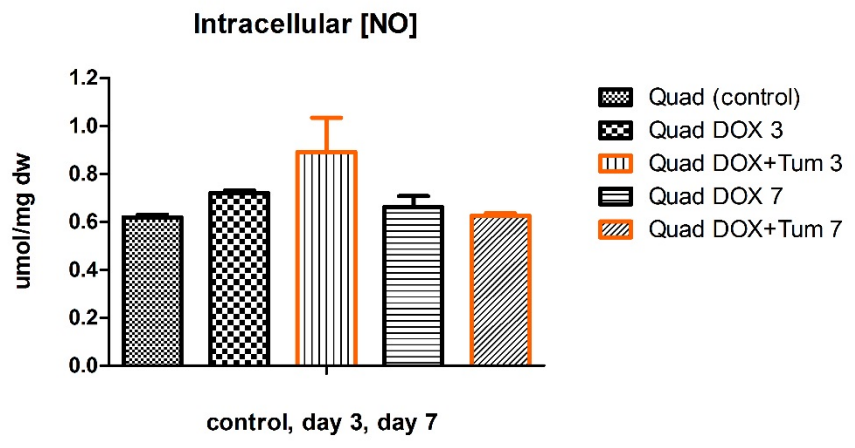
Results (P<0.05, SEM)

No
Sci
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du N
p.



Gastrocnemius

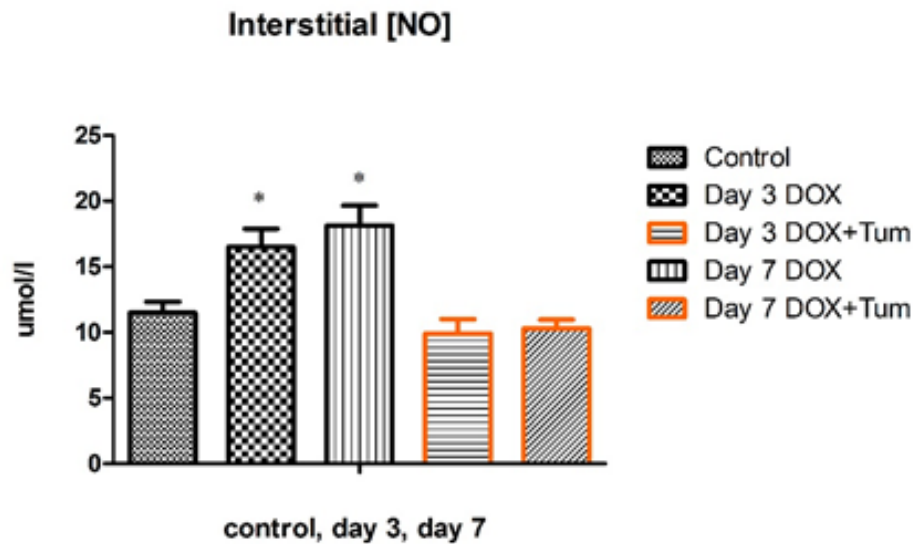
presence of a tumor



Quadriceps

- No significant difference

Results (P<0.05, SEM)



Interstitial space

- DOX alone increased NO
- NO stable in the presence of a tumor

Discussion

A steady elevation in NO concentrations...

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پیشرفت در علوم

- Peroxynitrites decreases mRNA levels of myoblast determination protein (MyoD)
- Increase in NO production via increased iNOS expression due to DOX has shown to inhibit transcription factor Jun-D which in tern decreases muscle creatine kinase (MCK) expression (Buck and Chojkier 1996)

No changes in NO production with a tumor...

- Increased metabolism of the drug?

Conclusion

- It has been recognized that during chemotherapeutic treatment, disruption of healthy skeletal muscle is an indicator of poor treatment results and increased patient mortality (Prado et al. 2009; Antoun et al. 2010).
- These findings provide a good step in identifying the underlying mechanisms involved in SM dysfunction associated with DOX therapy



Thank you..

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Dr. MacLean

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