A PRESENTATION ON

"*In Ovo* Mefloquine and 4-Aminopyridine administration inhibits chorioallantoic membrane (CAM) angiogenesis in chicken embryos through ion-channel modulation."

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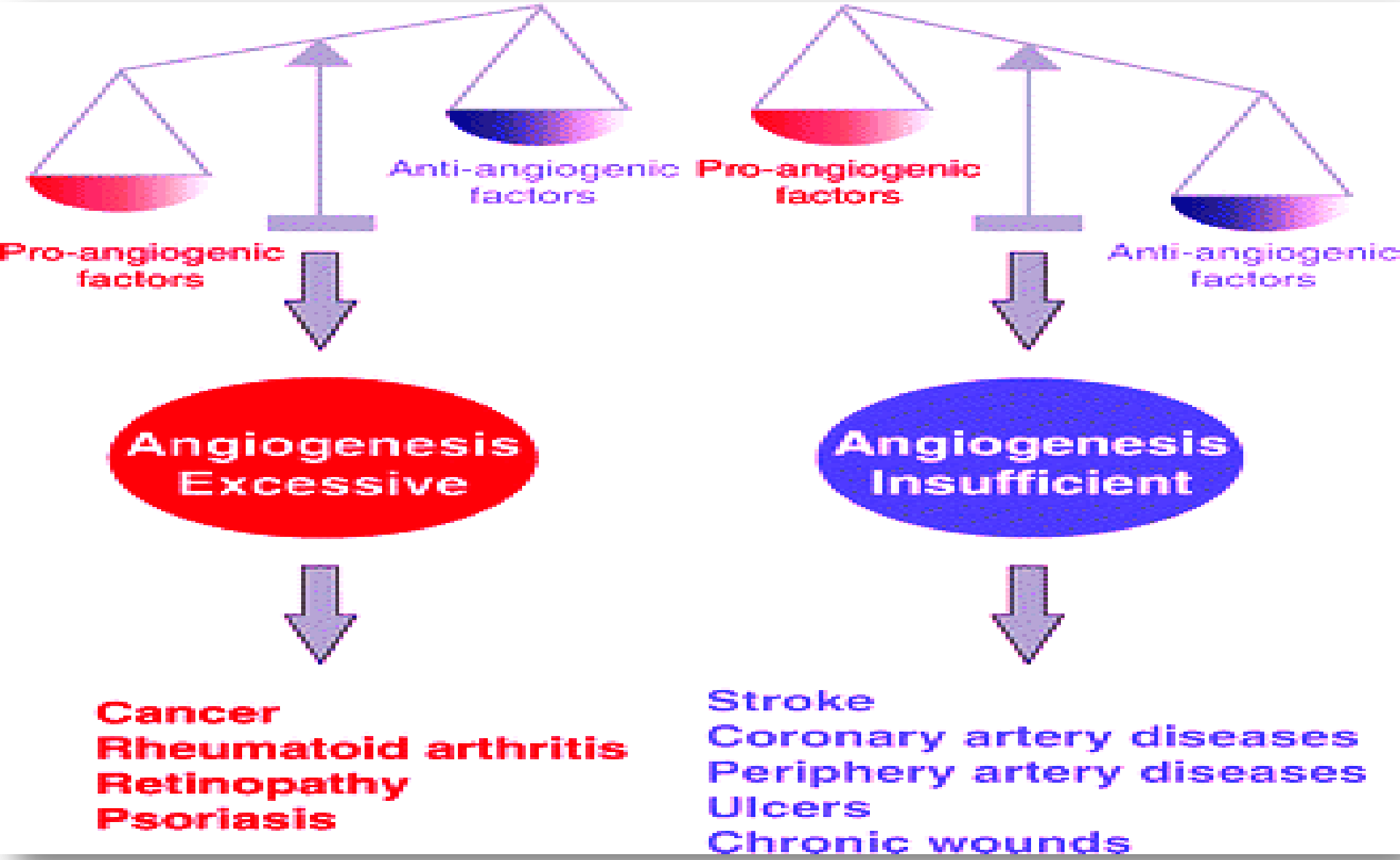
PRESENTATION OUTLINE

- INTRODUCTION
- OBJECTIVES FOR THE STUDY
- RATIONALE FOR THE STUDY
- MATERIALS AND METHODS
- RESULTS
- DISCUSSION & PROPOSED MECHANISMS
- FUTURE PROSPECTIVE
- REFERENCES

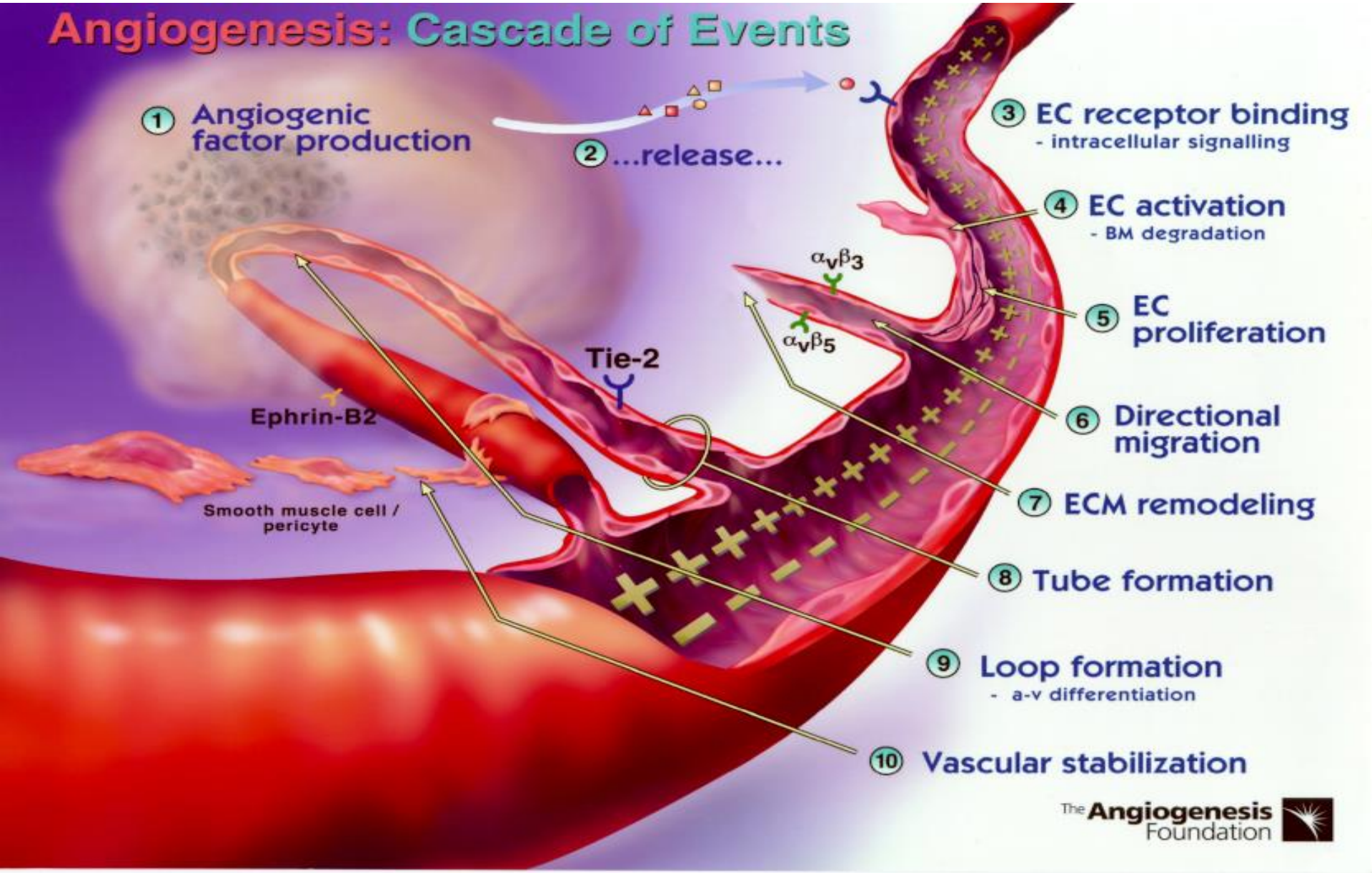
INTRODUCTION

- **Angiogenesis is the generation of new blood vessels from pre-existing vasculature.**
- **Angiogenesis has two faces, it plays vital role in maintenance of both health and diseases. Vessel growth could benefit in case of baldness, neurodegenerative ills, heart attack and could be helpful to bypass the clots in blood vessels (occlusion) as well as in tissue repair.**
- **Abundant ion channels are located on the endothelial cell surface. Ion channels have significant role in cell proliferation and thus in angiogenesis**

DISEASES INVOLVED IN ANGIOGENIC IMBALANCE



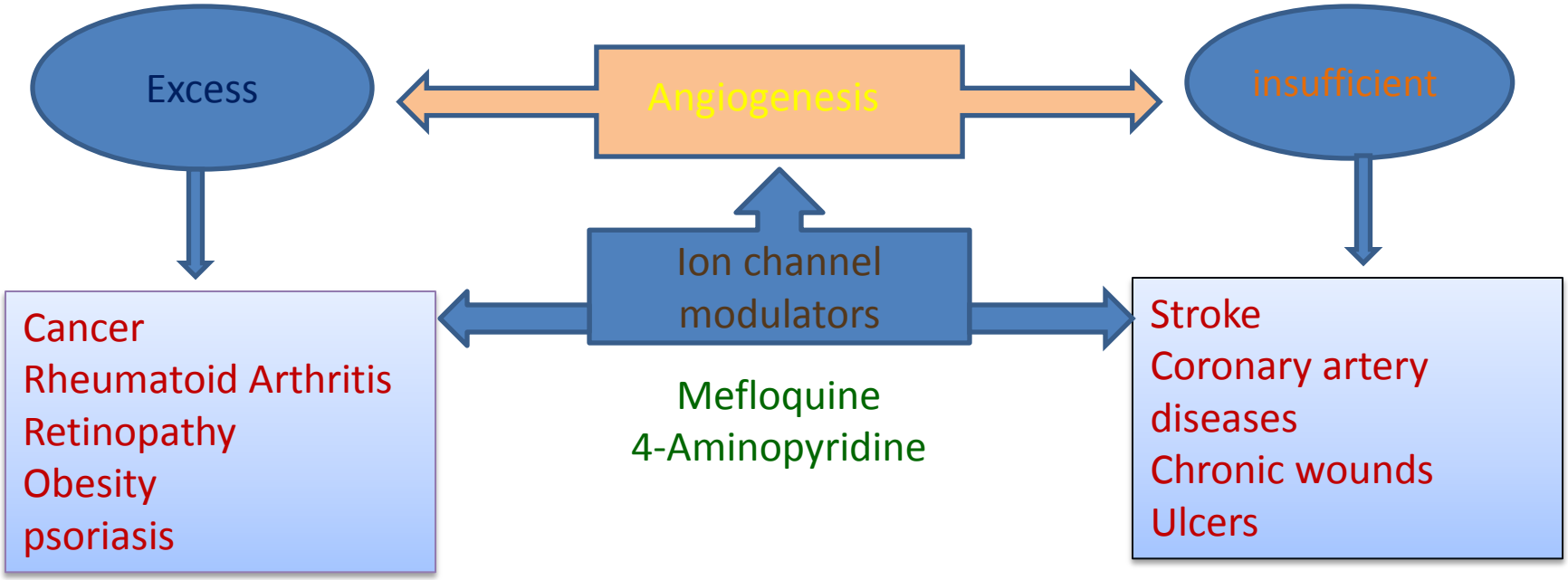
MECHANISM OF ANGIOGENESIS



OBJECTIVES OF THE WORK

- **The study aimed to investigate the effects of Mefloquine (Cl^- channel blocker) and 4-Aminopyridine (K^+ channel blocker) on angiogenesis**
- **At what concentration of the test drugs show maximum percentage inhibition of neovascularization..**
- **The number of branches formed in test drug treated groups in CAM assay and to compare with the control group.**

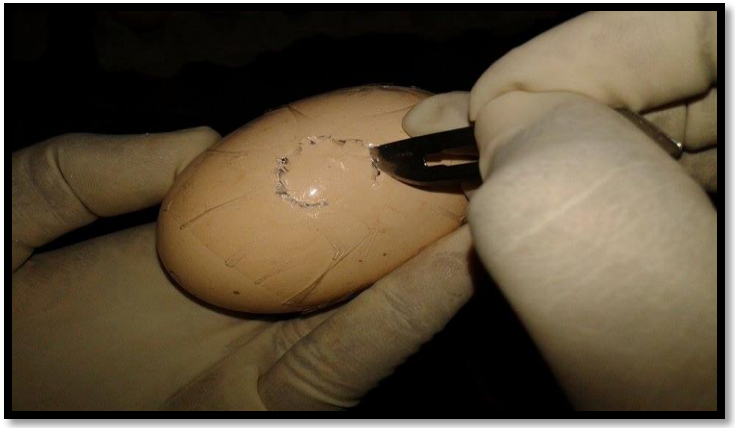
RATIONALE BEHIND THE STUDY



Ion channels ⟹ Cell proliferation

Endithelial cells contain ion channels

CAM assay



Chick choreoallantoic membrane assay:

Fertilized, white leghorn chicken eggs
(on 0 day of incubation)

↓

Incubated at 37^0C

↓

3^{rd} day
Narrow end was drilled to withdraw albumin

↓

7^{th} day
Small square window was opened to implant drugs

↓

Resealed and reincubated

↓

On 15^{th} day number of branching points were quantified

Dosing schedule:

GROUP NO.	TREATMENT (CAM Assay)	Dose (µg/egg)
I	-	-
II	Mefloquine	10
III	Mefloquine	50
IV	Mefloquine	100
V	4-Aminopyridine	100
VI	4-Aminopyridine	250
VII	4-Aminopyridine	500

Statistics

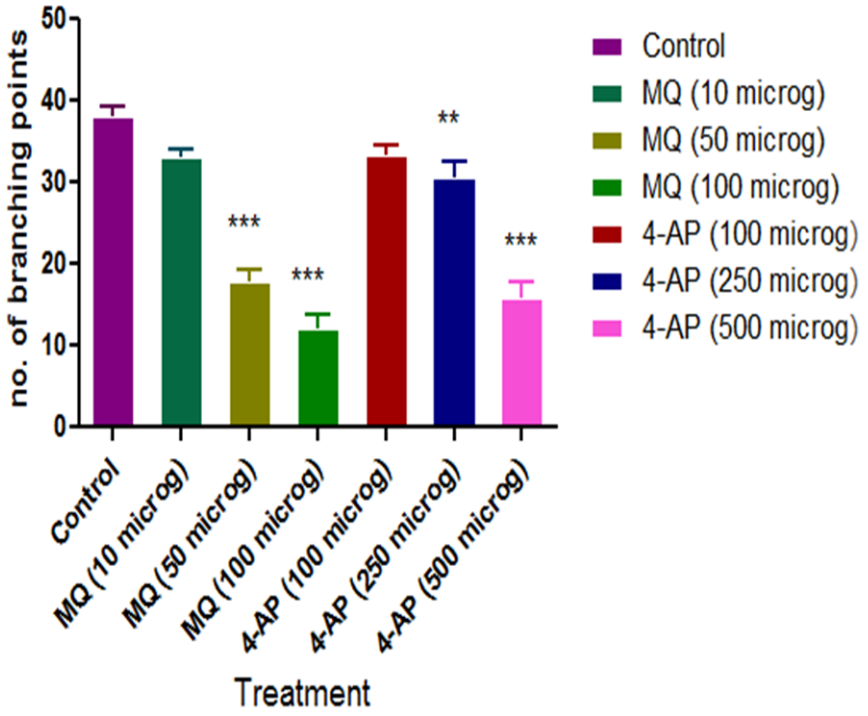
The data was analyzed by **one-way ANOVA** followed by **Dunnett test** using **Graph pad prism5.0 software.** Values are significant at *** p<0.05**. Comparison of the test groups were done with disease control group. Values are expressed as **mean ± SEM**

Results

CAM Assay

Number of branching points

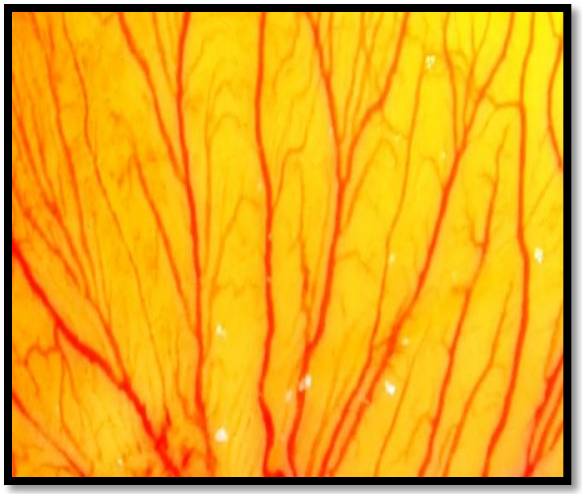
Group No	*Treatment (dose/egg)*	*No. of branching points*
1.	Control	38.16±1.138
2.	Mefloquine (10 µg)	33.16±0.792
3.	Mefloquine (50 µg)	18±1.414 ***
4.	Mefloquine (100 µg)	12.33±1.542 ***
5.	4-AP (100µg)	33.5±0.991
6.	4-AP (250µg)	30.83±1.662 **
7.	4-AP (500µg)	16±1.862 ***



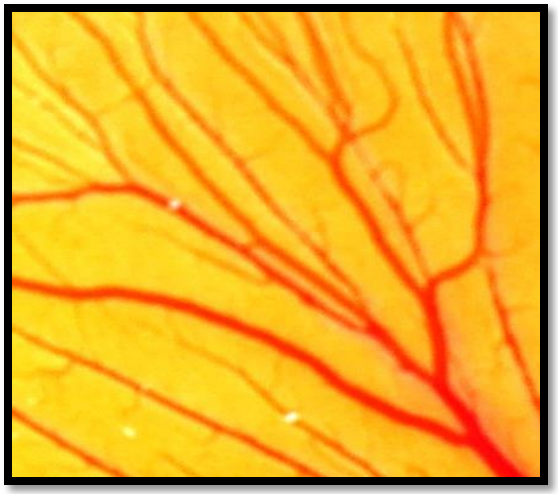
Results

CAM Assay

Images were captured from **Trinocular Microscope** at magnification of **40X**.



Control

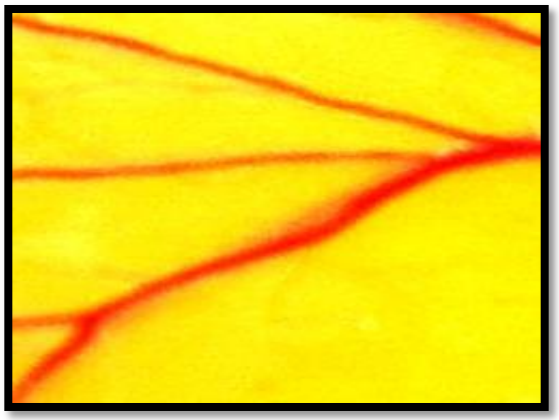


Mefloquine (10 µg)

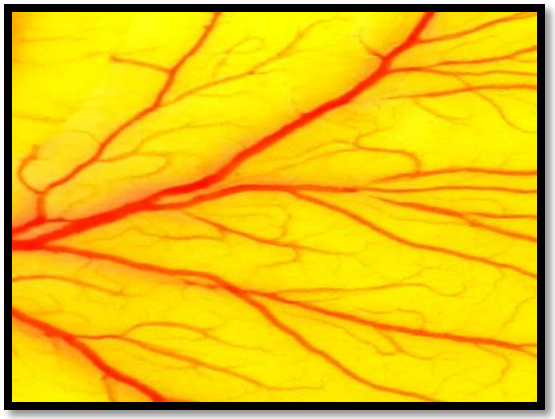
Mefloquine (50 µg)

Results

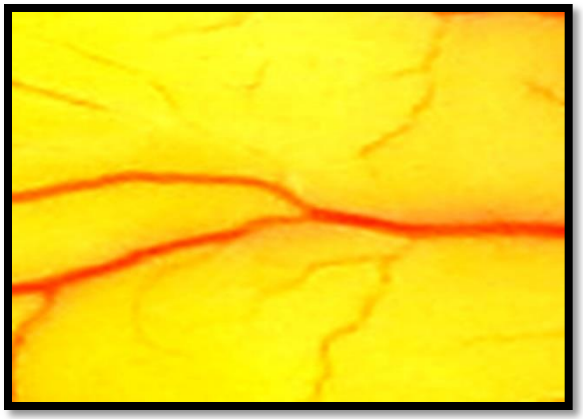
CAM Assay



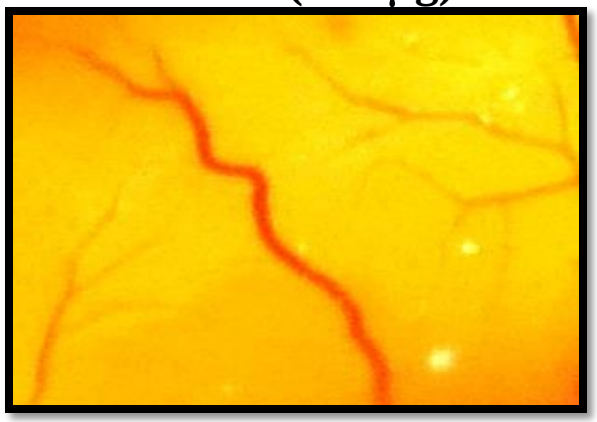
Mefloquine (100μg)



4-AP (100 μg)



4-AP (250 μg)

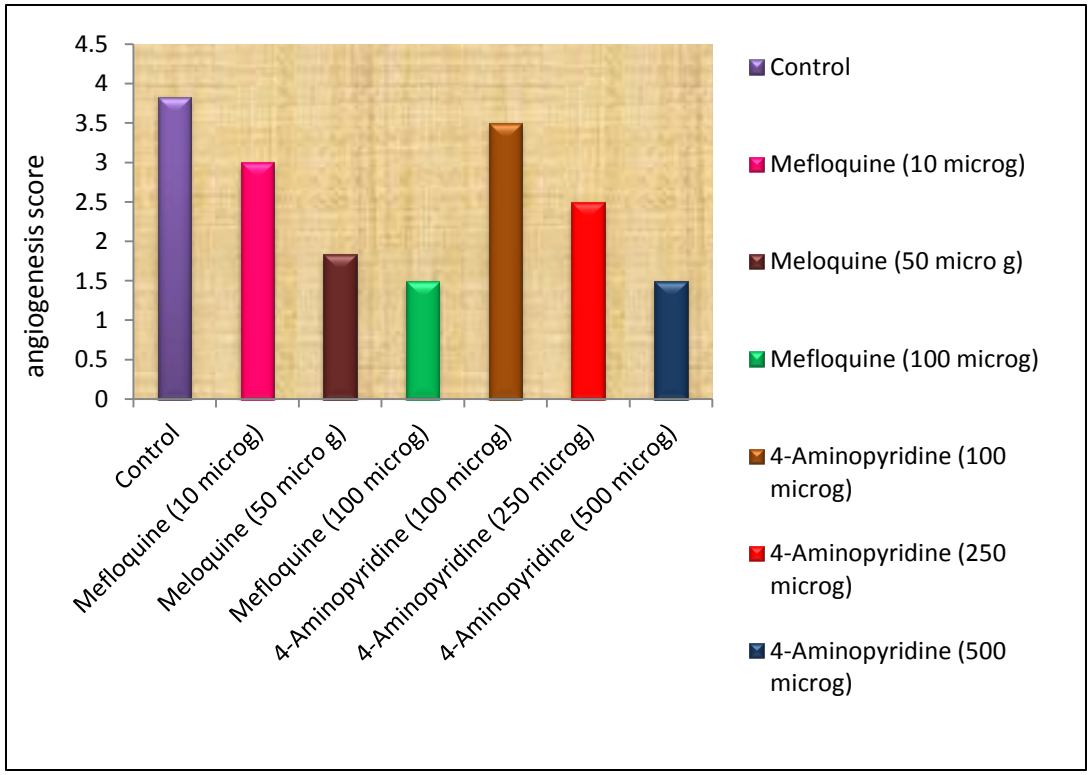


4-AP (500 μM)

Results

CAM Assay

Angiogenesis score

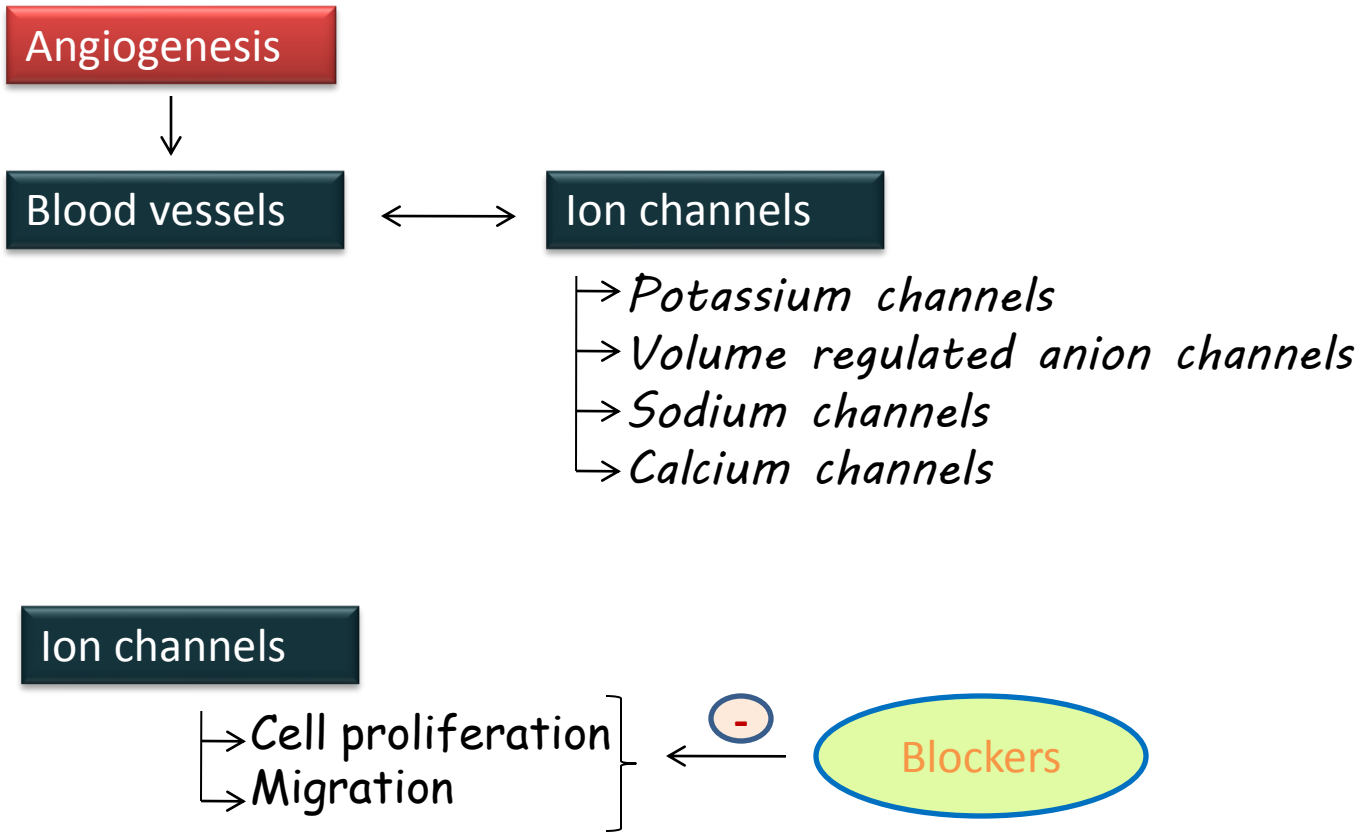


No. of branching points	Angiogenesis score
≥35	4
25-34	3
15-24	2
<15	1

Significance values of the results obtained

S.No.	Experimental method	Parameter	Treatment	Significance		
				Low dose	Medium dose	High dose
1.	CAM Assay	Number of branches	Mefloquine	NS	***	***
			4-AP	NS	**	***
		Angiogenesis score	Mefloquine	-	-	-
			4-AP	-	-	-

Discussion


Angiogenesis
Blood vessels
Ion channels
Potassium channels
Volume regulated anion channels
Sodium channels
Calcium channels
Ion channels
Cell proliferation
Migration
-
Blockers

Mechanisms

Mefloquine

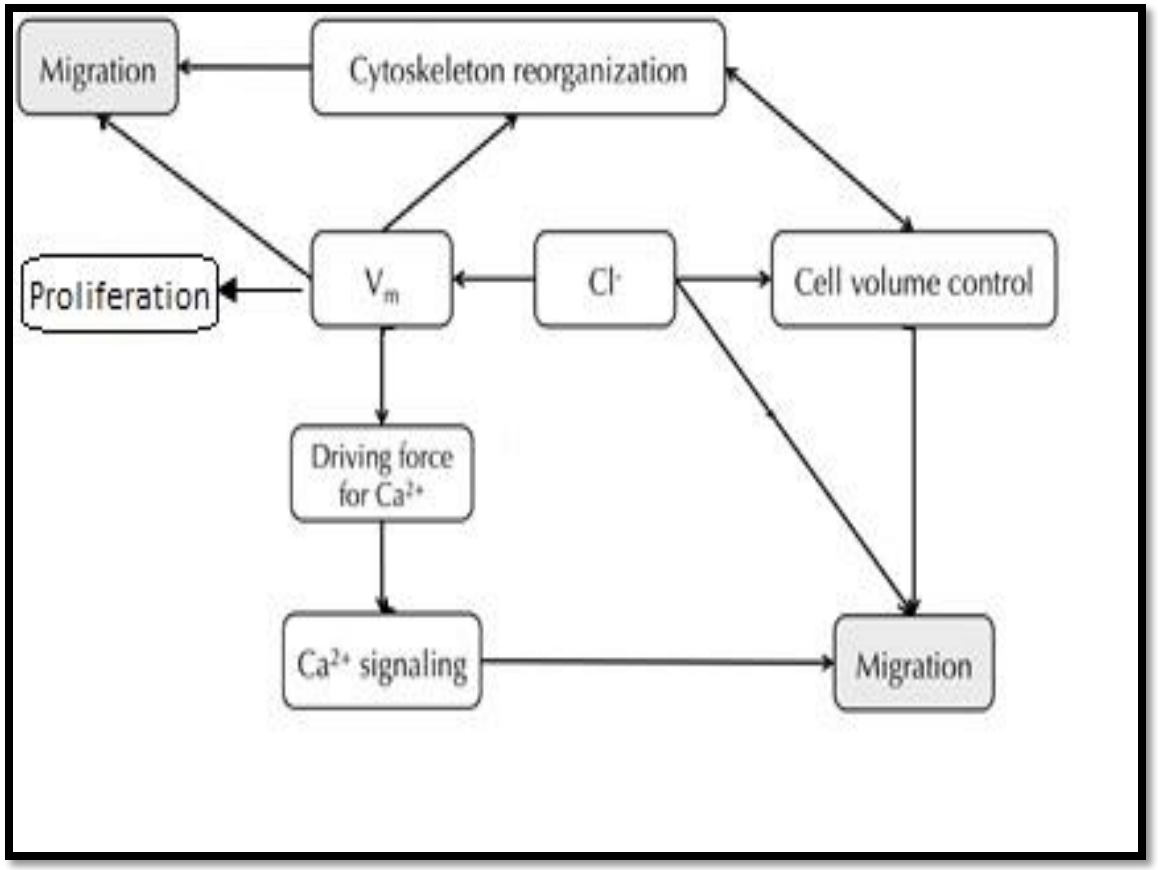
Maintains **membrane potential**

↓

Regulation of the **driving force for Ca^{2+}**

↓

that regulates **secondary messengers** for cell proliferation



Mechanisms

Mefloquine

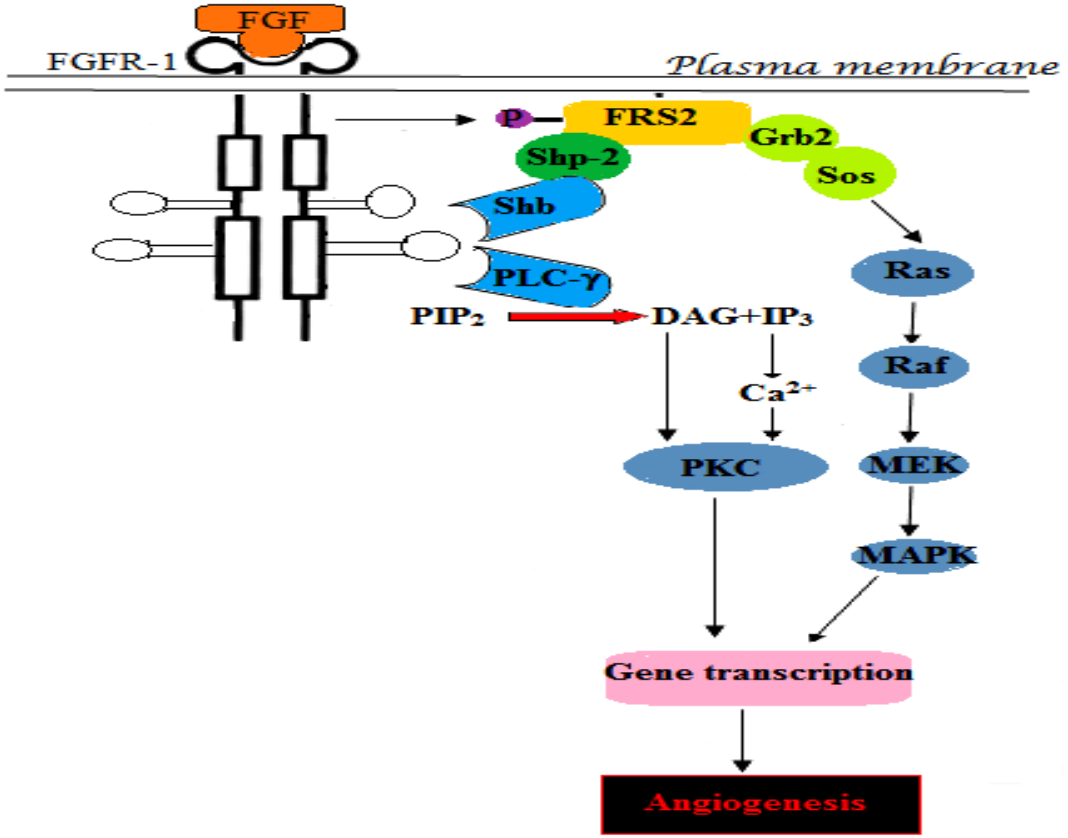
Chloride is needed for **the expression of Ras** (a small G-protein molecule)

↓

that regulates a large number of **intracellular signaling pathways**

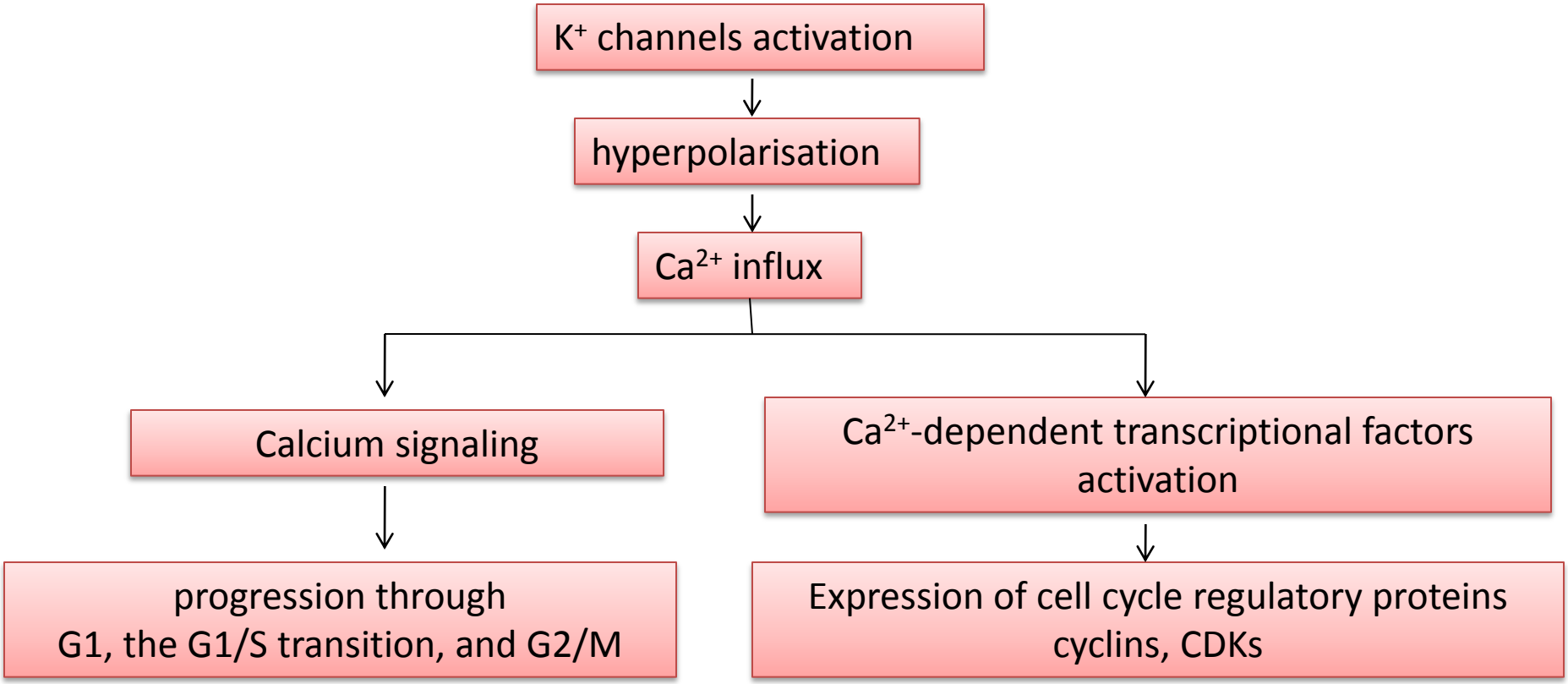
↓

affecting cell **proliferation and motility.**



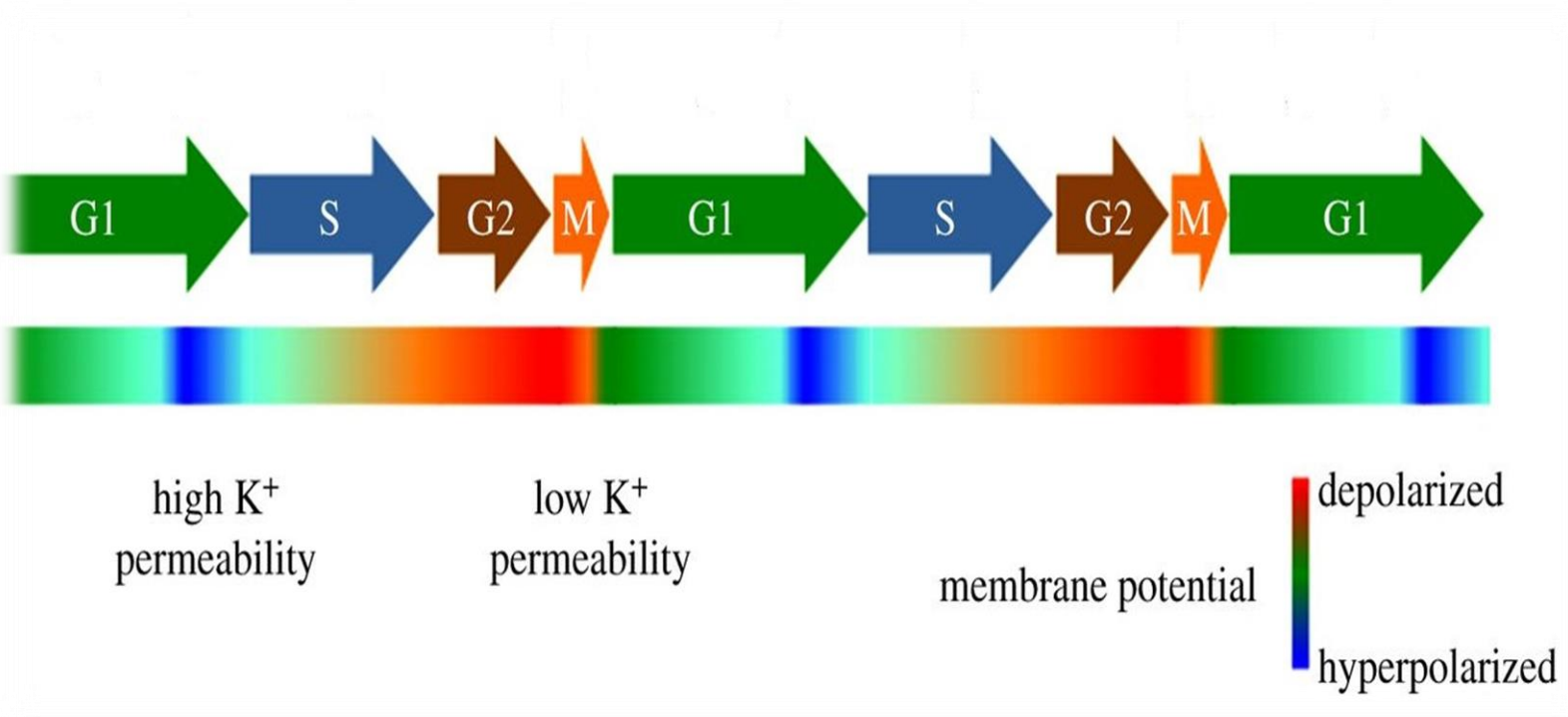
Mechanisms

4-Aminopyridine



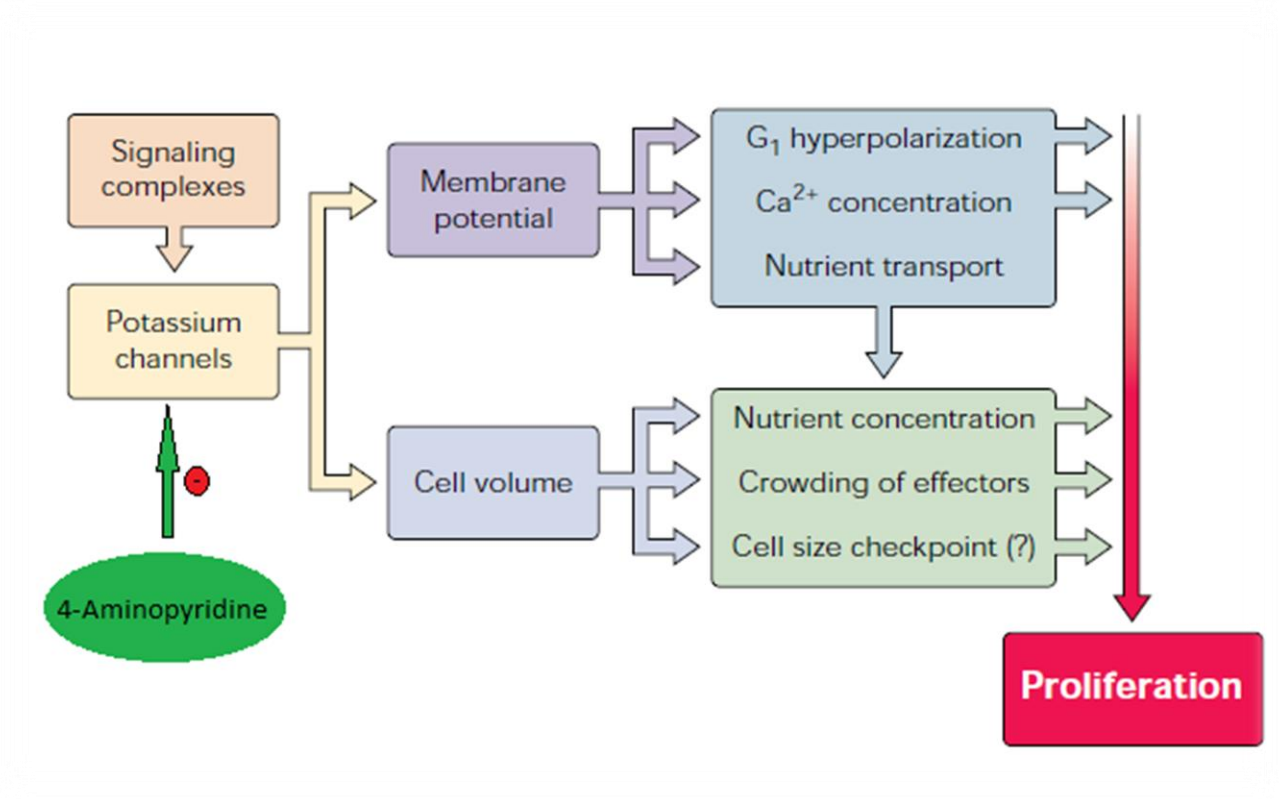
Mechanisms

4-Aminopyridine



Mechanisms

4-Aminopyridine



Conclusion

- We report here that VRAC blocker, Mefloquine and volume regulated K^+ channel blocker, 4-AP inhibit new vessel formation in all three models. These results suggest that Mefloquine and 4-AP may be useful in the therapy of angiogenesis-dependent tumor growth and other angiogenesis-dependent diseases