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Ro 31-8220 对纤维蛋白原降解产物诱导的平滑肌细胞增殖的影响

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关键词 Ro 31-8220；纤维蛋白原降解产物；蛋白激酶 C；血管平滑肌；培养的细胞；胸主动脉

目的：研究纤维蛋白原降解产物的致有丝分裂原活性及一种新型 PKC 抑制剂 Ro 31-8220 (Ro)的作用。方法：大鼠主动脉平滑肌细胞增殖采用结晶紫染色法测定。结果：纤维蛋白原降解产物促进大鼠主动脉平滑肌细胞的增殖，Ro (0.01 – 1 μmol·L^{-1})剂量依赖地抑制增殖。结论：Ro 抑制纤维蛋白原降解产物诱导的平滑肌细胞的增殖。

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Uptake of $^{99m}Tc^{5+}$-complexes in ischemic myocardial slices and their dissociable ability

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KEY WORDS drug stability; myocardial ischemia; technetium compounds; diphosphates; succimer; glucoheptonate

AIM: To find how some technetium-complexes to deliver the active species, TcO_4^{3-}, to the target tissue from a dissociable polynuclear Tc^{5+} species in preserved states *in vivo*. **METHODS**: Effect of dissociation ability of the polynuclear Tc^{5+} complexes on their accumulation in ischemic myocardium was tested. Ability of dissociation as having an appropriate conformation to become biologically functional after entering the blood circulation was tested using a simple dilution method by thin layer chromatography (TLC) analysis. Various degree of ischemic myocardium slices of rat were incubated with 1/100 diluted $^{99m}Tc^{5+}$-succimer, $^{99m}Tc^{5+}$-GH and $^{99m}Tc^{5+}$-PPi. **RESULTS**: The TLC patterns of $^{99m}Tc^{5+}$-GH and $^{99m}Tc^{5+}$-PPi showed the presence of a fast increasing of free Tc-species as dilution degree increased. The relative radioactivity of peak of free pertechnetate ($R_f = 0.85 – 1.0$) with 1:500 dilution was: $^{99m}Tc^{5+}$-succimer 0 %, $^{99m}Tc^{5+}$ – GH 28.1 % ± 1.3 %, and $^{99m}Tc^{5+}$-PPi 46.0 % ± 2.9 % respectively. The uptake of the myocardium after ischemia for 3 h was $^{99m}Tc^{5+}$-succimer 420 % ± 110 % dose/g tissue, $^{99m}Tc^{5+}$-GH 710 % ± 180 % dose/g tissue, and $^{99m}Tc^{5+}$-PPi 1295 % ± 390 % dose/g tissue respectively. **CONCLUSION**: The dissociation and myocardial uptake showed: $^{99m}Tc^{5+}$-succimer < $^{99m}Tc^{5+}$-GH < $^{99m}Tc^{5+}$-PPi, the uptake by the ischemic myocardium is positively correlated to their dissociation.

$^{99m}Tc^{5+}$-succimer as a tumor imaging agent has been developed[1]. It was perhaps a result of the similarity of the TcO_4^{3-} pentavalent core to the

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phosphate molecule which was avidly taken up by tumor cells. Thus the $^{99m}Tc^{5+}$-succimer, undergoing complex dissociation, accumulated in tumor cells[2]. To find how some Tc-complexes to deliver *in vivo* the active technetium species, TcO_4^{3-}, to the target tissue from a dissociable polynuclear technetium species in a pentavalent but preserved state, effect of dissociation ability of the polynuclear Tc^{5+}-complexes on their accumulation in ischemic myocardium, was tested.

$^{99m}Tc^{5+}$-glucoheptonate ($^{99m}Tc^{5+}$ – GH) and $^{99m}Tc^{5+}$-pyrophosphate ($^{99m}Tc^{5+}$-PPi) complexes are accumulated in infarcted areas[3,4]. $^{99m}Tc^{5+}$-succimer and other Tc-complexes as holding an similar polynuclear and Tc^{5+} core were also tested.

MATERIALS AND METHODS

Labeling method Succimer, GH, PPi were labeled with $^{99m}Tc^{5+}$[1] (Tab 1).

Tab 1. Labeling condition.

Ligand	Succimer	GH	PPi
mmol·L^{-1}	1	10	56
Buffer (pH)	$NaHCO_3$ (8.28)	HAc (6.2)	–
$SnCl_2$	0.2 μmol	0.2 μmol	2 g·L^{-1}

The $^{99m}Tc^{5+}$-PPi complex was labeled using kit (Daiich RI Laboratories, Japan).

Ability of dissociation Ability of dissociation of these polynuclear chelates as having an appropriate conformation to become biologically functional after entering the blood circulation was tested using a simple dilution method by thin layer chromatography (TLC) analysis. Original sample, as well as the samples diluted 10, 100, and 500 times were tested by TLC (Merck silica gel) using acetone as the developing solvent. Ability of dissociation was expressed as the % dose of radioactivity at each peak in TLC over its total radioactivity.

Ischemic myocardium slices and sample uptake Various degree of ischemic myocardium slices of rat[5] were incubated with 1/100 diluted sample.

The uptake of $^{99m}Tc^{5+}$-complexes at various degree of dilution in the myocardial slices after ischemia for 3 h was calculated by (the radioactivity per g dry tissue)/(total radioactivity administered).

RESULTS AND DISCUSSION

Ability to dissociate The TLC revealed that $^{99m}Tc^{5+}$-succimer was less dissociable than $^{99m}Tc^{5+}$-GH, since it showed lack of the fast moving species and the single peak (only at R_f = 0.00, labeling complex) from nondiluted sample to samples diluted 10 – 500 times (Fig 1).

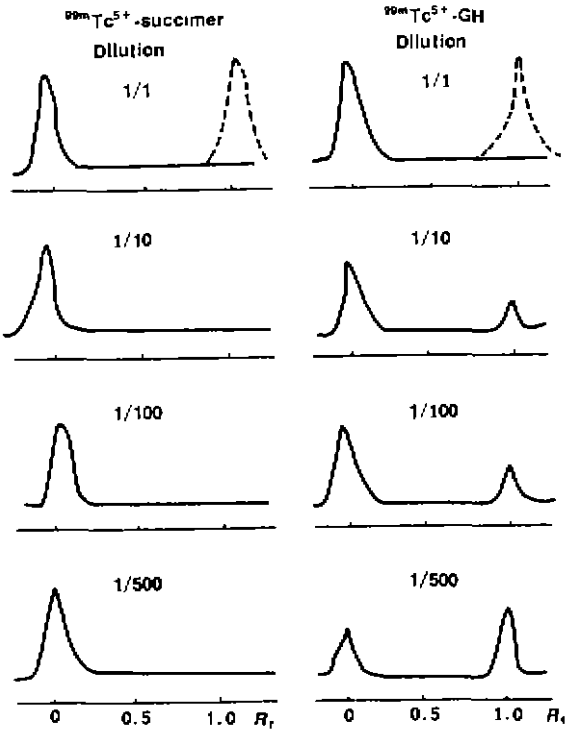

Fig 1. TLC analysis. R_f = 0: Tc-complex. R_f = 1.0: free pertechnetate.

As for $^{99m}Tc^{5+}$-GH, the peak of free pertechnetate (R_f = 0.85 – 1.0) appeared from 1/100 to 1/500 dilutions (Tab 2).

The TLC of $^{99m}Tc^{5+}$-PPi showed a behavior similar to the $^{99m}Tc^{5+}$ – GH, and the peak of free pertechnetate presented at each dilution from original solution to 1:500 dilution (Tab 2). The TLC showed also the presence of a faster increase of free Tc-species as dilution proceeded.

This is an indication of the $^{99m}Tc^{5+}$-PPi as being a more dissociable $^{99m}Tc^{5+}$-complex than $^{99m}Tc^{5+}$-GH and $^{99m}Tc^{5+}$-succimer.

Their dissociable ability was detected with the more dissociable $^{99m}Tc^{5+}$-complexes, in the order of $^{99m}Tc^{5+}$-PPi > $^{99m}Tc^{5+}$-GH > $^{99m}Tc^{5+}$-succimer.

Uptake by ischemic myocardial slices The uptake of $^{99m}Tc^{5+}$-PPi was the highest, and that of

$^{99m}Tc^{5+}$-succimer was the lowest (Tab 3).

Tab 2. Relative radioactivity at each peak in the TLC. $n=6$ samples, $\bar{x} \pm s$.

		Relative radioactivity	
	Dilution	Chelate R_f 0.00	Free pertechnetate R_f 0.85 – 0.10
$^{99m}Tc^{5+}$-PPi	1/1	95.9 ± 3.4	3.5 ± 0.7
	1/10	91.6 ± 2.6	7.7 ± 1.1
	1/100	71.7 ± 2.9	23.0 ± 5.2
	1/500	51.3 ± 4.5	46.0 ± 2.9
$^{99m}Tc^{5+}$-GH	1/1	99.2 ± 3.2	0.3 ± 0.1
	1/10	98.6 ± 4.8	0.9 ± 0.1
	1/100	91.8 ± 3.1	6.8 ± 0.4
	1/500	70.9 ± 1.1	28.1 ± 1.3

Tab 3. Uptake of $^{99m}Tc^{5+}$-complexes in ischemic myocardial slices. $n=6$, $\bar{x} \pm s$.

Sample	Radioactivity % dose/g tissue
$^{99m}Tc^{5+}$-succimer	420 ± 110
$^{99m}Tc^{5+}$-GH	710 ± 138
$^{99m}Tc^{5+}$-PPi	1 295 ± 390

Hence dissociation and myocardial uptake: $^{99m}Tc^{5+}$ – succimer < $^{99m}Tc^{5+}$-GH < $^{99m}Tc^{5+}$-PPi. The uptake by the ischemic myocardium for every one of the three compounds in the various degree of dilution is positively correlated to its dissociation ($r = 0.78$, $n = 6$).

For many hormones and pharmaceuticals are transported *in vivo* by binding to the plasma proteins, dissociating and functioning in the target organ, we think the moderate stability and dissociable ability of polynuclear Tc^{5+} complex are related to their organ's uptake.

Though $^{99m}Tc^{5+}$-PPi suffers from being highly uptaken by bone tissue, the effect of the $^{99m}Tc^{5+}$-PPi as a myocardial infarction detecting agent was darken, it has been put to practical use in clinic[3].

If some modalities can be found to deliver *in vivo* the active TcO_4^{3-} to the target tissue from a dissociable polynuclear Tc^{5+} species in a preserved state, detection of ischemia might as well become possible.

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五价锝标记化合物在缺血性心肌中的摄取与其离解性

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关键词 药物稳定性；心肌缺血；锝化合物；二磷酸类；琥珀酸；葡庚糖酸

目的：研究在机体内具有一定的稳定性而又能离解出可到达靶组织的活性形式 TcO_4^{3-} 的多核锝标记化合物。**方法**：模仿锝标记化合物进入血循环后的状况，采用稀释的方法，用硅胶板薄层层析法来测量其离解能力的大小。**结果**：500 倍稀释液的离解物峰的相对放射性百分数为：$^{99m}Tc^{5+}$-succimer 为 0，$^{99m}Tc^{5+}$-GH 为 28.1 % ±1.3 %，$^{99m}Tc^{5+}$-PPi为46.0 % ±2.9 %，而缺血 3 h 的心肌中的摄取为：$^{99m}Tc^{5+}$-succimer是420 % ±110 % dose/g tissue，$^{99m}Tc^{5+}$-GH是710 % ±180 % dose/g tissue，$^{99m}Tc^{5+}$-PPi 是 1295 % ±390 % dose/g tissue。**结论**：以上几种多核螯合物的离解性与其在缺血性心肌中的摄取成正相关关系。